2 March-April 1982
Volume 47: Number 2

Coden: JFDAZ 47(2):347-694 (1982)
ISSN: 0022-1147

JOURNAL of FOOD SCIENCE

Oplied Science and Engineering

IFT

A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS

Please Note: Traditional Format Changed for IFT's 42nd Annual Meeting in Las Vegas

☐ The 42nd IFT Annual Meeting of June 22-25, 1982 will follow a Tuesday-through-Friday format rather than the traditional Sunday-through-Wednesday format.

As indicated in the calendar below, technical sessions and symposia will begin on Wednesday morning and conclude on Friday afternoon. The IFT FOOD EXPO will begin at 1:00 P.M. on Tuesday, and close at 12:30 p.m. Friday.

Other changes include the Basic Symposium and the Short Course. The Basic Symposium will be held Monday and Tuesday, June 21-22. The Short Course, which was usually held at the conclusion of the Annual Meeting, will also be held on Monday and Tuesday, June 21-22.

The IFT Executive Committee meeting, traditionally held on Saturday from 1:30 P.M. to 6:00 P.M. will be held at the same hours on Monday, June 21.

The IFT Council Meeting will be held on Tuesday, June 22, from 10:00 A.M. until approximately 4:30 P.M.

The Opening Session—usually held on Sunday evening—will begin at 7:00 P.M. on Tuesday, June 22. The Opening Mixer will follow the Opening Session at 9:00 P.M.

JUNE 1982

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

EXECUTIVE COMMITTEE

COUNCIL

BASIC SYMPOSIUM

SHORTCOURSE

IFT FOOD EXPO

TECHNICAL SESSIONS & SYMPOSIA

OPENING SESSION

MAY

						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

JULY

				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

Basic Research

Research Notes

NOTICE: Page Charges for Publication of Research Papers

A page charge of $50 per printed page is effective for papers published in the JOURNAL OF FOOD SCIENCE *and we sincerely hope that authors' sources of financial support share the prevailing view that publication costs are, in fact, part of the cost of doing research.*

Where solely personal funds are available for payment of page charges and such payment would be a hardship for the authors, application for "Waiver Under Hardship Clause" should be made—prior to publication—on a separate signed statement addressed to the Director of Publications, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601.

March-April 1982
Volume 47: Number 2

JOURNAL of FOOD SCIENCE

ISSN:0022-1147

- **MANUSCRIPTS** (3 copies) should be submitted to:

  Dr. Aaron E. Wasserman
  IFT Scientific Editor
  P.O. Box 6197
  Philadelphia, PA 19115

NO RESPONSIBILITY is assumed by the Institute of Food Technologists for statements and opinions expressed by the contributors to its publications.

MANUSCRIPTS should conform to the style used in *Journal of Food Science* and authors are urged to consult the "IFT Style Guide for Research Papers" (see next paragraph for availability). *Journal of Food Science* reserves the privilege of editing manuscripts to make them conform with the adopted style of the journal or returning them to authors for revision. Editing changes may be reviewed by authors before publication.

- **STYLE GUIDE:** The "IFT Style Guide for Research Papers" is published annually in *Journal of Food Science* (last date of publication: November-December 1981 *JFS,* Vol 46, Issue 6, pp. 2008-2011. Copies are also available from IFT's Director of Publications.

- **PAGE CHARGES for Publications.** The IFT Executive Committee has established a page charge of $50 per printed page for all papers published in *Journal of Food Science.* The page charge shall not constitute a bar to acceptance of research manuscripts because the author is unable to pay the charge.

- **SUBSCRIPTIONS:** All communications related to handling of subscriptions, including loss claims, change of address, and orders for back issues should be sent to:

  Subscription Department
  Institute of Food Technologists, Suite 2120
  221 N. LaSalle Street
  Chicago, IL 60601 USA

Member Subscriptions–$15 per year.

Non-Member Subscriptions–Accepted only on a calender year basis–no refunds. Rates include postage. Payment must accompany order. Domestic, Canada, and Mexico–$45, all other destinations–$50. Reduced rates for 2- and 3-year subscriptions.

Change of address notice, with old address label, is required 8 weeks before issue date.

Claims for lost copies are allowed only if received within one month after publication (3 months for foreign subscribers).

Single copies and available back issues, $10 each postpaid; remittance to accompany order.

- **REPRINTS:** Single reprints are not available from *Journal of Food Science.* Address requests for single reprints to authors, whose addresses are found with each article. Original size and/or microform copies of individual articles, or issues, or entire volumes may be purchased from University Microfilms International, 300 N. Zeeb Road, Ann Arbor, MI 48106, U.S.A., or Dept. F.A., 30-32 Mortimer St., Dept. P.R., London W1N 7RA. England.

  Quantity reprints can be ordered from IFT Publications Department–minimum of 100 copies. Price schedule available upon request.

AUTHOR INDEX

Volume 47: Number 2

Minced Fish Production From Capelin (*Mallotus villosus*). A New Method for Gutting, Skinning and Removal of Fat from Small Fatty Fish Species

OLA EIDE, TORGER BØRRESEN, and TERJE STRØM

ABSTRACT

A new method for the production of fish mince from a small fatty fish is presented. The method involves (a) cutting the fish into short pieces, (b) washing out the depot fat, dark pigments and viscera under acid (pH 4) or neutral conditions, and (c) bone separation. The resulting mince has a white appearance and a low fat content (approximately 7% of dry wt). The water-holding capacity of the acid mince is low, whereas neutral minces have values corresponding to cod mince. The fish oil can easily be recovered. Characterization of the lipids in capelin mince is presented.

INTRODUCTION

CAPELIN (*Mallotus villosus*) is a small Arctic and pelagic fish (12–20 cm, 20–45g). It is in quantity the most important fish for the Norwegian fishing industry. The annual catch of one to two million tons constitutes about 50% of the total amount of fish landed in Norway. Today 99% of the total capelin catch is processed to fish meal and fish oil. There is, however, a pronounced interest for increasing the utilization of capelin for food production. The fish is caught in great quantities in short peiods of time. This points towards a handling technique where the fish is converted to a mince before further processing. Most of the existing processes for mince production are developed for the white, lean fish species. Capelin is a small, fatty fish, and when conventional mincing technology is applied, great problems are encountered.

A major problem in the production of a homogeneous mince from capelin is the variation of the fat content from 2–3% at the end of the spawning period in April to 15–20% in the summer. This is largely attributed to the variation of the amount of the depot fat, whereas the amount of structure fat is fairly constant at 4–6% dry wt (Jangaard, 1974).

Mohr and Ormberg (1977) and Mohr et al. (1973, 1976) studied the distribution of lipids in the capelin and found most of the depot fat in fat cells in the peritoneum and underneath the skin, independent of the seasonal variations of the total fat content. However, the capelin muscular tissue has a fat content comparable to lean fishes.

The mechanical strength of the fat cells, as well as the connective tissues in the skin and in the dark colored peritoneum is determined to fibrous collagen (Mohr, 1971, 1977). This suggests a possibility of removing the lipids and the dark pigments from the fish by a method which affects the degradation of the collagen fibers. In cold water fish species the mechanical strength of the collagen fibers is determined by intermolecular aldimine crosslinks (Mohr, 1971). These crosslinks are stable at low temperatures under neutral conditions, but are broken at temperatures of 40–45°C, and/or by lowering the pH to pH 3–4, under which conditions fibrous collagen is converted to acid soluble collagen (Novak et al., 1977).

Gildberg and Raa (1979) have studied the solubility of muscle and skin from capelin at different pH's at 8°C. They found a great relative difference in solubility between the two at pH 4, where the solubility of the muscle was minimal.

This paper describes the development of a technology for the production of a fish mince from small, fatty fish species, applying low pH (4.0) and/or an elevated temperature (40°C).

Authors Eide, Børresen, and Strøm are with the Institute of Fishery Technology Research, Tromsø, Norway.

EXPERIMENTAL

CAPELIN used in experiments were caught by a purse seiner at the Barents Sea (N 75° 30', E 25° 31') in October 1978 (Summer capelin). Samples of fish (1 kg) were frozen at sea at −80°C, and stored at this temperature. In the laboratory the fish was thawed in air at 10°C for 12 hr.

Each fish was cut with a scalpel into 2–3 cm long segments perpendicular to the long axis. The fish segments (300g) were placed in a 1L round bottom flask, and acetic acid (9g) was added to a final pH of 4. A blank was prepared in the same manner without the addition of acetic acid (pH 6.8). The temperature was quickly adjusted (20–50°C) by adding pretempered water (300g). The fish segments were washed by incubating the mixture at the proper temperature on a shaker (130 rev/min). After a period of time (15–90 min) the liquid phase was decanted off. The bones were removed from the fish segments by dissection.

The resulting mince was analyzed for protein (Kjeldahl-N, AOAC, 1975), lipids (Bligh and Dyer, 1959), ash (540°C, 12 hr), and water (105°C, 12 hr).

The lipid phase was analyzed for the total amount of dry matter, and for lipids by a modified Soxhlet procedure. The lipids in the liquid phase were transferred to an ether phase using a conventional apparatus for continuous liquid; liquid extraction. The difference between the total amount of dry matter and the weight of the lipids is expressed as fat-free dry matter.

The water-holding capacity of the fish mince was measured by a centrifugation method. Sample holders and 50 ml centrifuge tubes as shown in Fig. 1 were used. Water removed during centrifugation drains through the polyester membrane in the sample holder, and is collected in the bottom of the centrifuge tube. The tubes were centrifuged in a MSE table centrifuge with swing out rotor. The conditions were: 2g sample; centrifugation time, 5 min at 1500 x *g*

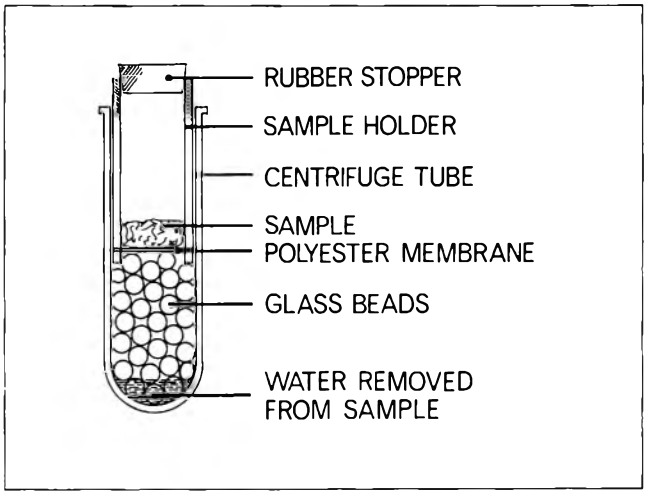


Fig. 1—Sample holder and centrifuge tube for measuring water-holding capacity in minced fish samples. The sample holder is made of acryl plastic. The centrifuge tube is made of polycarbonate and has a 50 ml volume.

(3600 rpm); at 10°C. The sample holder was weighed before and after centrifugation for determination of weight loss of the sample.

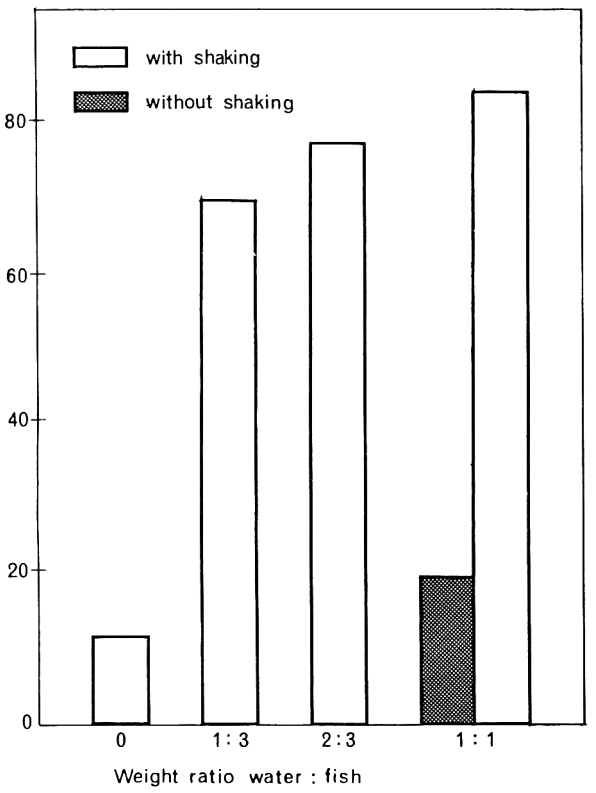


Fig. 2—Percent of lipids removed from capelin segments during washing for 60 minutes on a shaker (130 rev/min) with varying fish-water ratios (w/w). The experiment was done at pH 4 and 40°C.

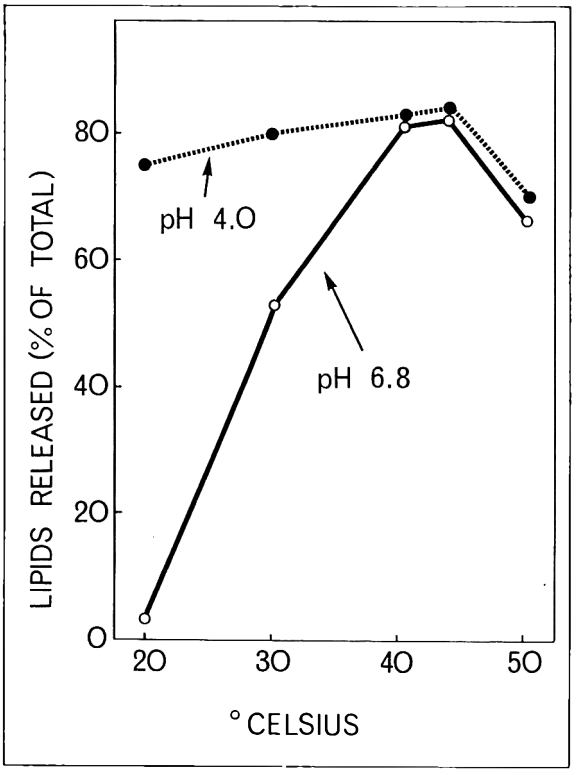


Fig. 3—Percent of lipids released vs temperature of the washing solution. Washing was carried out for 60 min on a shaker (130 rev/min), and the fish-water ratio was 1:1 (w/w).

The water-holding capacity is expressed as g water/g fat free dry matter in the sample after centrifugation.

Lipids extracted from the fish and the mince by the Bligh and Dyer method, and lipids recovered from the liquid phase by the Soxhlet method described above (ca 5g), were separated into neutral and polar groups by distribution between petroleum ether and 87% ethanol (Galanos and Kapoulas, 1962). The neutral lipids (0,25g) were subsequently separated by chromatography on a 7% (w/w) hydrated Florisil column (12g) into hydrocarbons, steryl-esters, triglycerides, sterols, diglycerides, monoglycerides and free fatty acids (Carroll, 1961, 1963, 1976).

The fatty acids in the polar lipids and in the triglycerides were methylated at room temperature by reaction with sodium methoxide in methanol (Glass and Christophers, 1969; Hood et al., 1972). Free fatty acids were methylated by reaction with 12% BF_3 in methanol for 5 min at 95°C (Lambertsen and Hansen, 1978). The methylated fatty acids were analyzed by GLC with a Carlo Erba Fractovap Linear Gas Chromatograph fitted with a flame ionization detector and a glass capillary column (20m) containing Carbowax 20M. The methyl esters were eluted with nitrogen (2.4 ml/min) between 170–220°C (1°C/min).

RESULTS

IN ORDER TO PREPARE a homogeneous mince from both the summer and the winter capelin it is necessary to remove most of the depot fat. Preliminary experiments showed the necessity of cutting the fish into small segments and to stir the segments in water to achieve separation of lipids from the fish (Fig. 2). In the laboratory the fish was cut with a scalpel. For practical purposes on a larger scale, mechanical equipment consisting of a set of rotating knives may be used. By stirring the fish segments in water (fish-water 1:1) at pH 4 the lipids are removed as illustrated in Fig. 3. Independent of the temperature (20–45°C), maximum release of lipids at pH 4 is obtained in approximately 60 min (Fig. 4). However, the same amount of lipids can also be removed in 60 min under neutral conditions if the temperature is adjusted to 40–45°C (Fig. 3, 4).

During stirring under optimal conditions both skin and black peritoneum are broken down and partly dissolved; and the intestines are completely washed off the fish segments. Thus, the dark pigments are removed from the fish into the liquid. The increase of fat-free dry matter in the liquid phase follows closely the release of fat from the fish (Fig. 4).

After washing out the depot fat, skin, viscera and the black pigments from the fish segments, the backbones were removed by dissection in the laboratory. This may be done on a larger scale by using a meat- and boneseparator, or applying other bulkhandling techniques. The resulting mince has a white appearance comparable to ordinary minces from white fishes like cod.

The water-holding capacity of the capelin mince was compared with that of capelin muscle and cod mince (Table 1). It is evident that acid capelin mince has a very low water-holding capacity, whereas the value for neutral capelin mince is very close to the value for cod mince. The same value for the water-holding capacity is obtained whether the capelin mince is produced under neutral conditions, or under acid conditions and subsequently adjusted to neutral pH.

The water-holding capacity for neutral capelin mince is lower than that for whole capelin muscle. This is thought to be due partly to the higher lipid content in the whole muscle preparation, and partly to a slight denaturation of muscle material during preparation of the mince.

Capelin mince prepared under optimal conditions has a fat content of approximately 7% of the dry material (Table 2). By comparing the fat content of the mince with the content of structure fat in the capelin (4–6% of dry material, Jangaard, 1974), and the high rate of fat separation during the washing step (80.5%), one can assume that the

lipids remaining in the mince are mostly structure fat. This is consistent with the high content of polar lipids and low content of triglycerides in the mince (Table 3). As can be seen from Table 4, the high content of polar lipids gives the mince a fairly high content of polyunsaturated fatty acids, the polar lipids containing 55–60% of $C_{20:5}$ and $C_{22:6}$.

The yield of the mince is 55% of fat-free dry material, or approximately 35% of wet weight whole capelin. This is 80% of theoretical yield (Sørensen, 1978).

As can be seen from Table 3, a fish oil with good quality can be produced from fresh raw material by applying the present procedure. The oil contains approximately 95% triglycerides, less than 3% free fatty acids and is free of phospholipids. The composition of the fatty acids in the triglycerides and the free fatty acids in the oil is nearly identical, approximately 50% monounsaturated, 20–25% polyunsaturated, and 20–25% saturated fatty acids (Table 4). In the mince, the free fatty acids have a composition intermediate to the polar lipids and the triglycerides; the content of mono- and polyunsaturated fatty acids both being approxiamtely 35–40% (Table 4). This observation is in agreement with results obtained by Lambertsen and Hansen (1978).

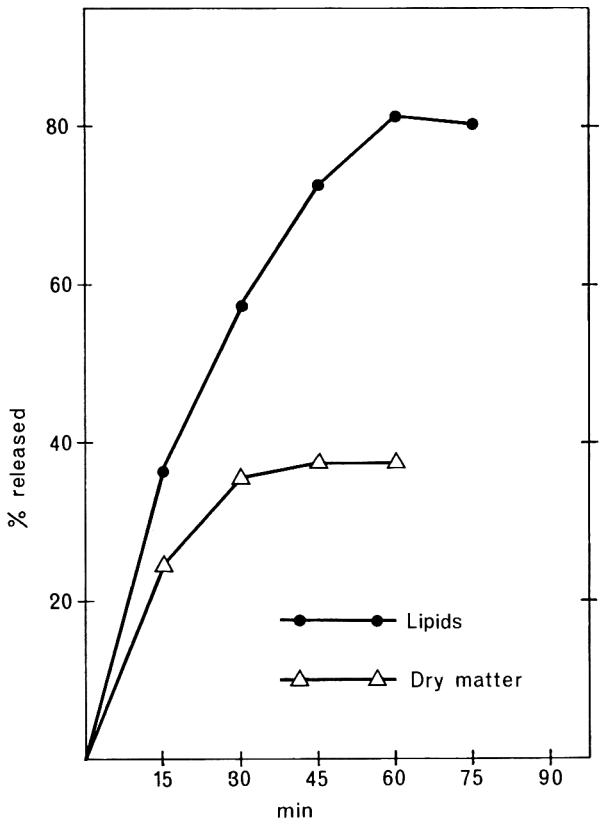


Fig. 4—Percent of lipids and percent of fat free dry matter released during washing vs washing time. The graphs apply for the acid process (pH 4) in the temperature range 20–45°C, and for the neutral process (pH 6.8) in the temperature range 40–45°C. Fish: water ratio was 1:1 (w/w), and the shaker was operating at 130 rev/min.

Table 1—Water-holding capacity of capelin, capelin minces, and cod mince[a]

	g water/g fat free dry wt
Capelin muscle	5.5
Capelin mince, pH 4.5	2.1
Capelin mince, pH 6.8	4.8
Cod mince	4.5

[a] The cod mince was prepared by passing a fillet through a meat grinder.

DISCUSSION

BY APPLYING CONDITIONS under which aldimine cross-links in collagen are unstable (pH 3–4; temperature 40–45°C) collagen rich tissues, i.e. skin, black belly lining, and fat cells, are broken down. The purpose of cutting the fish into short segments and stirring these segments in water is to improve the separation of lipids released from the fat cells of the fish muscle.

Three results are of special importance with regard to processing of minced fish from capelin: Firstly, most of the depot fat is separated from the capelin at temperatures

—Continued on page 354

Table 2—Composition of capelin and capelin mince[a]

	Summer capelin[b]			Capelin mince[b]		
Component	%WW	%DW	%FFDW	%WW	%DW	%FFDW
Protein	12.5	37.3	88.0	20.3	91.0	98.1
Lipids	18.3	54.6	–	1.6	7.1	–
Ash	1.7	5.1	12.0	0.4	1.8	1.9
Water	66.5	–	–	77.7	–	–
Yield	–	–	–	35	–	55

[a] The mince was prepared by washing fish segments at pH 4 and 40°C. Washing was carried out for 60 min on a shaker (130 rev/min) and the fish-water ratio was 1:1 (W/W).
[b] %WW—percent of wet weight; %DW—percent of dry weight; %FFDW—percent of fat free dry weight.

Table 3—Lipid composition of (1) summer capelin, (2) capelin mince made by washing fish segments at pH 4 and 40°C, and (3) fish oil released during washing

	% of Total lipids		
Components	Summer capelin	Capelin mince	Capelin oil
Neutral lipids	92.1	41.6	99.7
Polar lipids	7.9	58.4	0.3
Hydrocarbons	0.5	1.0	0.2
Sterylesters	1.1	2.5	0.2
Triglycerides	79.1	28.3	94.9
Sterols	2.5	2.3	0.6
Diglycerides	1.8	1.4	0.7
Monoglycerides	2.7	1.6	0.8
Free fatty acids	4.4	4.6	2.2

Table 4—Fatty acid composition of triglycerids, polar lipids and free fatty acids of (1) summer capelin, (2) capelin mince made by washing fish segments at pH 4 and 40°C, and (3) fish oil released during washing.

	% Total fatty acids						
	Summer capelin		Capelin mince			Capelin oil	
Fatty acids	Triglycerides	Polar lipids	Triglycerides	Polar lipids	Free fatty acids	Triglycerides	Free fatty acids
$C_{14:0}$	6.3	2.3	7.5	2.0	5.8	8.4	5.5
$C_{16:0}$	9.9	18.0	9.5	16.6	11.7	10.6	12.4
$C_{16:1}$	8.0	1.0	7.9	0.7	2.3	9.2	8.7
$C_{18:0}$	1.1	2.0	0.7	–	1.1	–	0.7
$C_{18:1}$	12.5	11.6	14.3	9.2	15.5	13.4	14.2
$C_{18:2}$	2.3	1.3	2.5	1.3	1.7	2.1	1.7
$C_{18:3}$	1.1	0.8	1.2	0.9	0.7	1.1	1.0
$C_{18:4}$	5.5	2.0	4.9	1.8	3.8	5.9	5.5
$C_{20:1}$	15.2	3.5	13.9	1.5	10.3	15.3	15.8
$C_{20:5}$	7.7	19.9	7.1	19.9	10.1	8.2	8.2
$C_{22:1}$	14.0	1.0	14.2	–	10.8	13.8	13.8
$C_{22:6}$	8.1	34.6	7.6	41.6	19.1	7.4	8.4

Effects of Various Kitchen Heat Treatments, Ultraviolet Light, and Gamma Irradiation on Mirex Insecticide Residues in Fish

DAVID A. CIN and MANFRED KROGER

ABSTRACT

Concentrations of the chlorinated hydrocarbon insecticide mirex ($C_{10}Cl_{12}$) were determined in brown trout from a defined contaminated area of Spring Creek, Centre County, PA, using electron-capture gas chromatography. Conventional heat treatments, namely, baking, frying, poaching, and baking without skin, did not cause significant decreases of the contaminant. Ultraviolet irradiation led to significant reductions ($p < 0.05$) in mirex concentration in muscle tissue. Exposures of 24, 48, and 72 hr led to degradations of 30.0%, 42.8%, and 45.6%, respectively, of the initial mirex concentration. Gamma irradiation also led to significant reductions ($p < 0.05$) in mirex concentration in muscle tissue. Following absorption of 1, 3, and 5 Mrad, degradations of 9.8%, 23.1%, and 37.5%, respectively, of the initial mirex concentration were observed.

INTRODUCTION

CASES OF WATER CONTAMINATION with organochlorine pesticides or industrial chemicals have been much in the news during the 1960's and 1970's. The result has often been an appearance of persistent contaminants in the exposed aquatic life. One such case of alleged environmental pollution occurred near State College in Centre County, PA, where a state government agency has identified and measured the insecticides Kepone and mirex in the water and fish of Spring Creek, presumably due to effluents from a nearby chemical manufacturing plant. Since the fish were shown to contain organochlorine contaminants above the current action levels (Food & Drug Administration, 1978), signs were posted along the popular trout-fishing creek admonishing anglers not to consume any fish caught. The current FDA action level for mirex in fish is 0.1 ppm.

First prepared in 1946 by dimerization of hexachlorocyclopentadiene, mirex was not used until it was shown to be effective against the fire ant (Solenopsis) which by 1960 had infested large areas in the Southeastern United States. During the 1970's various studies showed and confirmed the environmental persistence of mirex, its bioconcentration ability and its toxicity (Dorough et al., 1974; Gaines and Kimbrough, 1970).

Having an abundant supply of samples with biologically incorporated mirex near the laboratory, specific processing methods were investigated as means of degrading that environmental contaminant in fish.

The Pennsylvania Fish Commission cooperated with the procurement of trout samples. Mirex was studied because of suitable available analytical methods for this compound and because no other insecticide, except DDT, has inspired such controversy and toxicological study.

MATERIALS & METHODS

Samples

Brown trout (*Salmo trutto*) were caught by electro-stunning, placed on ice, identified, weighed and measured and then stored at −20°C. Additionally, 20 brown trout were obtained from Erie County, PA. They were known to be uncontaminated.

Authors Cin and Kroger are with the Dept. of Food Science, The Pennsylvania State Univ., University Park, PA 16802.

Standard

A mirex standard, 98% pure, was supplied by Applied Science, State College, PA. It served as the means of gas chromatographic identification and quantification.

Reagents

Water, ethyl ether, petroleum ether, and methanol were distilled in all-glass systems. Florisil (Fisher Scientific, Pittsburgh, PA) was activated at 125°C. Benzene (Pesticide Grade), sodium chloride and anhydrous sodium sulfate were also obtained from Fisher. Other items were acetonitrile (Baker's Resi-Analyzed) and hexane (Mallinckrodt's Nanograde).

Analytical method

Specific fish tissue samples, while still frozen were homogenized using the dry ice technique of Benville and Tindle (1970). The result was a very fine frozen powder.

The technique used for mirex extraction was obtained from the Pennsylvania Department of Environmental Resources. Harrisburg, PA (Kefford, 1979) supplied to it, in turn, by a cooperative government agency in Virginia, where it was used to quantify mirex in fish from the James River.

About 10g homogenized fish tissue were placed in a 400-ml Omni mixer cup and 200 ml of 20% water in acetonitrile were added. After blending for 5 min and filtration through paper into a 2-liter separatory funnel, 600 ml water, 10g sodium chloride, and 200 ml of a 50% ethyl ether/petroleum ether mixture were added to the filtrate. This was shaken vigorously for 3 min and allowed to separate. The water-acetonitrile layer was drained into a 1-liter separatory funnel and re-extracted with 100 ml of the ether mixture. The bottom layer was then discarded and the solvent layers, containing the nonpolar mirex, were combined, washed twice with 100 ml portions of water containing 2% NaCl, passed through a 2-inch column of anhydrous sodium sulfate and evaporated not quite to dryness.

A chromatographic column was prepared from a 50-ml buret sawed off about 10 inches above the stopcock. On top of a glass wool plug were poured 1.6g Florisil and 1.6g anhydrous sodium sulfate. The column was pre-wetted with 5 ml of Solvent M (1% methanol and 4% benzene in hexane). The elution rate was adjusted to 5 ml/minute.

The extract was taken up in 10 ml petroleum ether and a 1 ml portion (representing 1.0g of fish) was placed on the column. Then 10 ml of Solvent M were added. Eluates were concentrated to 0.4 ml in a water bath under a gentle stream of air. They were then brought to 1.0 ml with a 10% benzene-in-hexane solution and used in the gas chromatographic determinative step with injections of 2–5 μl.

Standard concentrations of mirex in benzene were injected after the first sample of the day and again after every two or three sample injections. Peak area was determined by multiplying peak height by its width at half-height. Injected amounts of the sample were varied so as to obtain a peak close in size to the standard peak it was being compared with, about 50–75% on the scale of the recorder chart paper. The sample peak was quantified by setting up a ratio between sample peak and standard peak area. A representative chromatogram of extracted mirex is shown in Fig. 1. A linear relationship existed between quantity of mirex injected and peak area.

The details of gas chromatography were as follows. A Hewlett Packard 5730A Gas Chromatograph fitted with a Hewlett Packard 18173A Linear Electron-Capture Detector (14 mCi of ^{63}Ni) was

used. Pyrex columns, 1/8 inch i.d., 6 ft length, 100/120, mesh Supelcoport (Supelco Inc., Bellefonte, PA) were coated with: (i) 4% SE-30/6% SP-2401 on (ii) 10% SP-2100. The carrier gas was methane with 5% argon, flow rate 40 ml/min; and the temperatures were: injection port, 250°C; column, 220°C; detector, 300°C. All quantification of mirex was done on column (i). The identity of mirex was confirmed with gas chromatographic column (ii). Additional, although indirect, confirmatory information was supplied by the Pennsylvania Department of Environmental Resources through mass spectrometry in brown trout caught at the same location (Kefford, 1979).

When a blank (all steps in the analysis without the fish sample) was carried through the procedure, no peaks were found. No peaks were obtained with brown trout from Erie County, PA. When these control fish were spiked with mirex (5 μg/10g of fish) in a benzene solution, recoveries were about 85%.

Heat treatment

Since all heat treatments were ineffective in reducing mirex concentrations, there is no need here to describe in detail the conditions of the experiments. They were not considered critical information. It is now known that mirex degradation does not occur below 525°C (Holloman et al., 1975), and the usual home fish preparation temperatures are well below that, namely, at 97°C as recorded for all treatments by a potentiometer with thermocouple inserted in the center of the fish portion.

In baking, the oven setting was at 190°C; for frying the electric pan was set at 204°C; and for poaching 500 ml boiling water in a kitchen vegetable blancher was used. The fish pieces were turned once during the treatment which lasted until an internal temperature of 97°C had been reached.

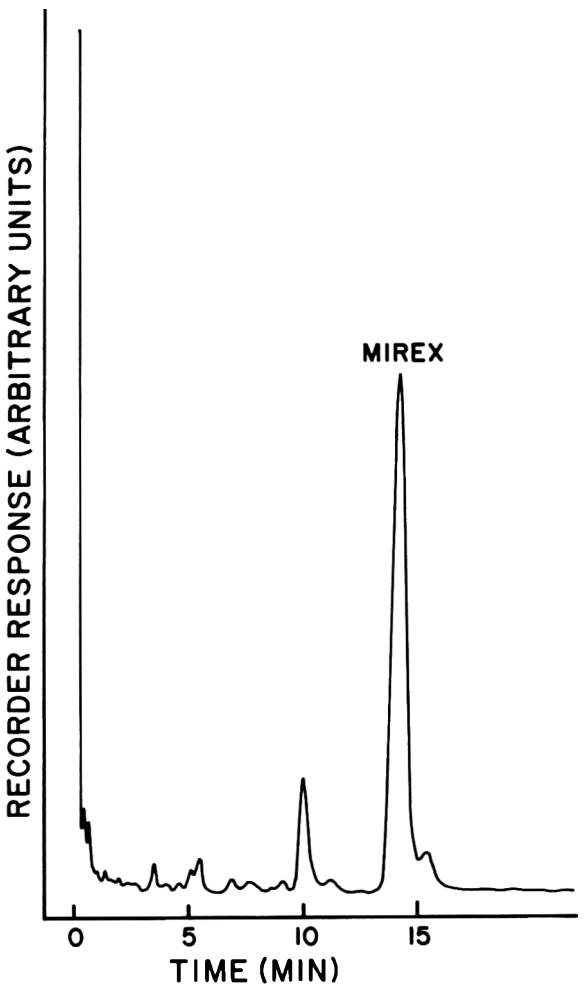


Fig. 1—A representative chromatogram obtained from Spring Creek brown trout showing retention time of mirex. All other peaks were ignored.

Ultraviolet irradiation treatment

A Chromato-Vue Cabinet (Ultra-Violet Products, San Gabriel, CA) was used inside a 5°C cold room. Both the shortwave (15 watts at 2540 Å and longwave (15 watts at 3650 Å) lamps were on during sample irradiation.

Fifteen 25-g samples of pooled, homogenized edible fish tissue were spread thinly and evenly in a Pyrex baking dish. Exposure times were 24, 48 and 72 hr. The samples were approximately 8 inches from the lamps. There was no heat build-up, since there was sufficient cold air circulation. Following exposure, the fish was scraped from the dish. These samples were then stored in a freezer for several days until analyzed for mirex concentration, as well as for percent solids according to standard procedures (Association of Official Analytical Chemists).

Gamma irradiation treatment

The gamma source was ^{60}Co with an activity of 4×10^5 Mrads/hr. Fish samples were irradiated with the assistance of the Nuclear Engineering Department, The Pennsylvania State Univ.

Twenty-five-gram samples of the pooled, homogenized edible fish tissue were placed in glass jars with aluminum foil-lined plastic lids. Irradiation temperature was approximately 20°C. Three jars were irradiated at a time. They were withdrawn from the irradiation chamber at calculated intervals to give dosages of 1, 3, and 5 Mrad. The samples were kept frozen for a maximum of one week prior to mirex analysis. As no change in moisture content was expected, being kept in sealed jars throughout the experiment, no percent solids analysis was done.

RESULTS & DISCUSSION

Effect of heat treatment (baking, frying, poaching, baking after skin removal)

Overall, the heat treatment did not result in statistically significant reductions in mirex concentrations.

Students' t-test was used to seek significant differences in mirex concentrations between raw populations and treated populations. Means of raw samples were not significantly different (p's > 0.05) from the corresponding means of the cooked groups. The means of the cooked groups were compared with each other through analysis of variance. A Duncan's multiple range test showed that the cooked means were not significantly different ($p > 0.05$) from each other.

The two reasons an organochlorine pesticide becomes less concentrated during cooking are (a) chemical degradation and (b) physical removal with the moisture or, primarily, the lipid phase.

Holloman et al. (1975) demonstrated that mirex did not begin to degrade until a temperature of 525°C was reached. Crawfish tissue containing mirex was subjected to a thermal process of 121°C for 13 min by Lane (1973). No dechlorination took place. Crawfish and eggs were autoclaved (1.22 atm) for 6 hr, also without any loss of their mirex burden (Lane, 1973). The endpoint temperature of 97°C and the maximum temperature of 204°C (temperature of the oil used during frying), used in this study, were insufficient to cause any significant degradation of the thermally stable mirex.

Brown trout used in this study had a relatively low fat content in the muscle tissue (2.50%), similar to fat percentages found by Reinert et al. (1972) in perch (0.6%) and by Smith et al. (1973) in chinook salmon (2.65%) and coho salmon (3.59%).

Neither Reinert et al. (1972), Smith et al. (1973) nor the authors of this study observed any significant reductions in fat percentages or insecticide concentration during cooking of the above low-fat fish species.

This is contrasted with the cooking of bloater (27.9% fat in muscle tissue) by Smith et al. (1973) and fat trout (26.8% fat in muscle tissue) by Zabik et al. (1979) who obtained significant reductions in fat percentage and insecticide concentration.

—Continued on next page

That cooking failed to reduce mirex concentrations in low-fat brown trout through lipid loss fits the conclusion of Smith et al. (1973) that such a loss would only occur in fish with a high fat content.

Reinert et al. (1972) also reported that fatty areas under the skin had seven to twelve times more insecticide residue in coho salmon and lake trout.

In an attempt to lower the concentration of insecticides in edible tissue, fish have had their skin and sub-dermal fat removed before cooking. Chinook steaks were cooked with and without skin (Smith et al., 1973) and sections of fat trout were cooked with and without skin (Zabik et al., 1979). No significant differences were found in either case.

In this investigation, to see whether the combination of skin removal and baking would reduce mirex concentration in brown trout, no significant difference between raw halves subjected to this treatment were found ($p > 0.05$).

Effect of ultraviolet irradiation

Pooled trout muscle tissue samples were shown to contain, by multiple analysis, mirex concentrations of 2.54 ppm, 1.96 ppm, and 8.93 ppm. These samples were made up of 6, 1 and 5 fish, respectively.

Changes in mirex concentrations due to ultraviolet light exposure are shown in Table 1. The results are expressed on a ppm solids basis. Average solids contents at 0, 24, 48 and 72 hr of exposure were 23.18%, 88.21%, 86.61% and 87.58%, respectively.

Average values for no exposure (5.23 ppm), 24 hr exposure (3.65 ppm), 48 hr exposure (2.99 ppm) and 72 hr exposure (2.79 ppm) indicate that mirex degradation did occur (Fig. 2).

These means were compared through analysis of variance. Significant differences were found through use of the Waller-Duncan K-Ratio T-test (Table 2).

A significant degradation of mirex occurred during the initial 24 hr of exposure ($p < 0.05$). Following this the rate of degradation became slower. The next significant degradation was evident only after 48 hr of additional exposure. At 24 hr, 70.0% of the original mirex was undegraded. At 48 and 72 hr, 57.2% and 54.4% of the original mirex remained undegraded.

The penetration of ultraviolet radiation through biological tissue is a maximum of 1 mm (Koller, 1965). The lessening of the degradation rate conceivably represents a reduction in the amount of undegraded mirex within the penetration depth of the radiation.

This work is in agreement with other studies showing insecticide burdens can be reduced through ultraviolet irradiation (Li and Bradley, 1967; Archer, 1969; Hallab, 1968; Lane, 1973).

Lane (1973) exposed egg tissue containing 2.5 ppm mirex to ultraviolet irradiation. This is similar to the exposure of muscle tissue containing an average of 1.2 ppm mirex to ultraviolet irradiation done in this work. The major difference appears to be in the source of radiation. Lane (1973) used a lamp with 2.6 watts of output at 2540 Å. The short-wave lamp used to irradiate trout had an output of 15 watts at 2540 Å. In addition, our trout study employed a long-wave lamp with 15 watts of output at 3650 Å.

Although the difference in food systems could have contributed, the difference in degradation achieved between the two studies can be mainly attributed to the difference in the output of ultraviolet energy. Mirex in egg tissue underwent a 20% and a 36% degradation during 24 and 48 hr of exposure, respectively. Mirex in fish muscle tissue decreased 30.0% and 42.8% from the same periods of exposure.

The work by Li and Bradley (1967) on the degradation of organochlorines in milk raises an important point, namely, the concomitant destruction of desirable food components due to ultraviolet irradiation. Such treatment caused destruction of beta-carotene and vitamin A. However, vitamin D was produced.

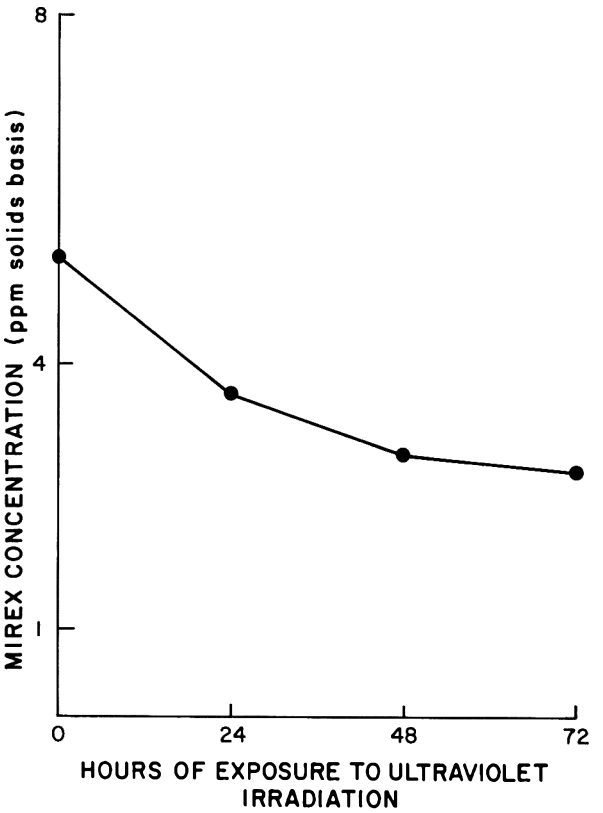


Fig. 2—Plot of mirex concentrations (ppm, solids basis) versus hours of exposure to ultraviolet irradiation. Each point represents the mean of nine determinations.

Table 1—Effect of ultraviolet irradiation upon mirex concentrations (ppm, solids basis) in brown trout edible tissue

Sample no.	Hours of exposure to ultraviolet irradiation			
	0	24	48	72
1-a	2.54	1.59	1.16	1.12
1-b	2.54	1.20	1.34	1.46
1-c	2.54	1.69	2.01	1.77
2-a	1.87	1.22	0.94	1.26
2-b	1.87	1.47	0.95	1.14
3-a	8.93	6.07	4.94	4.98
3-b	8.93	6.54	5.95	4.97
3-c	8.93	6.76	4.67	4.25
3-d	8.93	6.38	4.95	4.18
Means	5.23	3.66	2.99	2.79

Table 2—Results of Waller-Duncan K-ratio T Test for means of fish samples treated with untraviolet irradiation

K-ration = 100 DF = 30

MS = 0.594353 F = 18.4978 LSD — 0.680274

Grouping[a]	Mean	N	Hours
A	5.23	9	0
B	3.66	9	24
C,D	2.99	9	48
C	2.79	9	72

[a] Means with the same letter are not significantly different.

Although sensory aspects were not addressed in this study it should be mentioned that irradiated fish, using the conditions described here, will in all likelihood be rejected by the average consumer.

Effect of gamma irradiation

Two of the pooled samples from the ultraviolet irradiation treatment were used.

Changes in mirex concentrations are shown in Table 3. Average values for no gamma irradiation absorbed (7.51 ppm), 1 Mrad absorbed (6.77 ppm), 3 Mrad absorbed (5.77 ppm) and 5 Mrad absorbed (4.69 ppm) indicate that breakdown of mirex occurred (Fig. 3). Means were compared through analysis of variance. Significant differences were found through use of the Waller-Duncan K-ratio T-Test (Table 4).

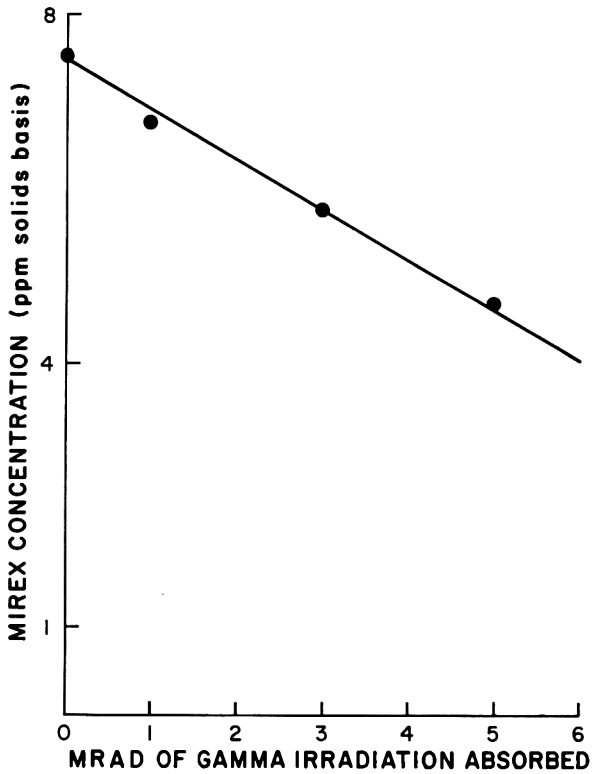


Fig. 3—Plot of mirex concentrations (ppm, solids basis) versus dose of gamma irradiation. Each point represents the mean of nine determinations.

Table 3—Effect of gamma irradiation upon mirex concentrations (ppm, solids basis) in brown trout edible tissue

Sample no.	Mrad of gamma irradiation absorbed			
	0	1	3	5
1-a	2.54	2.37	2.08	2.10
1-b	2.54	2.15	1.78	1.74
3-a	8.93	8.30	8.54	4.52
3-b	8.93	6.06	8.12	6.36
3-c	8.93	6.09	6.95	5.47
3-d	8.93	8.49	5.93	5.10
3-e	8.93	8.83	6.41	5.61
3-f	8.93	9.55	6.71	5.47
3-g	8.93	9.10	5.44	5.87
Means	7.51	6.77	5.77	4.69

Apparently, the first dose of 1 Mrad was not enough to cause a significant degradation of mirex. The means of no irradiation absorbed and 1 Mrad absorbed were not significantly different ($p > 0.05$). All other means were significantly different from each other ($p < 0.05$, Table 4). Percent mirex remaining after 1, 3, and 5 Mrad absorbed were 90.2%, 76.9%, and 62.5%, respectively.

The relationship between mirex concentration and gamma irradiation absorbed is linear (mirex concentration = −0.63050670 × Mrad). Maximum degradation obtained, 37.5% at 5 Mrad, is lower than the degradation of organochlorines found by Solar et al. (1971) and Lane (1973). Solar et al. observed a 42.5% and a 30.1% reduction in heptachlor epoxide and aldrin, respectively, with only 0.87 Mrad of gamma irradiation. This was in a different food system (potatoes) which might explain the discrepancy; or perhaps, it serves as another example of the extreme stability of mirex as compared to the other organochlorines. Carp et al. (1972), however, experienced a lesser degradation of aldrin (20%) in corn oil which had absorbed 6 Mrad. Possibly, the fat afforded a protective effect.

Lane (1973) demonstrated a 76% decrease in mirex with a dose of 4.5 Mrad. The major differences between Lane's (1973) work and this work were the food system and initial concentration of mirex. Brown trout muscle tissue with a maximum burden of 2 ppm was used in this study. Egg tissue with a mirex burden of 233 ppm was used by Lane. It can be assumed that the difference in mirex concentration is a critical factor in the different degradations obtained. The larger concentration of mirex increased the probability of a mirex molecule being affected by a gamma ray or a product of a gamma ray.

Lane (1973) made the point that, when dealing with irradiation-induced changes of compounds which are in small concentrations in an aqueous medium, the indirect effects of irradiation prevail over the direct effects. When diluted aqueous solutions are irradiated, practically all the energy absorbed is deposited in water molecules and the observed chemical changes are brought about indirectly. Direct action due to energy deposited directly in the solute is generally unimportant at solute concentrations below about 0.1M (Spinks and Woods, 1976).

Trout muscle tissue averaged 76% water content. Therefore, the 1.2 ppm mirex in the tissue can be considered a dilute aqueous solution with a concentration of 0.000003M. Thus, one would expect very little direct effect of irradiation upon mirex molecules.

Obviously, further work is required on the problem of removing pesticides from foodstuffs. Attention ought to be devoted to those conditions that would assure palatability of the treated foods. Future applications, particularly of gamma irradiation, will also depend on toxicity tests and evaluations and rulings by food regulatory agencies.

—Continued on next page

Table 4—Results of Waller-Duncan K-ratio T Test for means of fish samples treated with gamma irradiation[a]

K-ratio = 100		DF = 31	
MS = 0.927072	F = 14.5513		LSD = 0.857594
Grouping	Mean	N	Mrad
A	7.51	9	0
A	6.77	9	1
B	5.77	9	3
C	4.69	9	5

[a] Means with the same letter are not significantly different.

REFERENCES

Archer, T.E. 1969. DDT and related chlorinated hydrocarbon residues on alfalfa hay exposed to drying by sunlight, ultraviolet light and air. J. Dairy Sci. 52: 1806.
Benville, P.E. and Tindle, R.D. 1970. Dry ice homogenization procedure of fish samples in pesticide residue analysis. J. Agr. Food Chem. 18: 948.
Carp, A.E., Liska, B,J., and Ziemer, P.L. 1972. Decomposition of aldrin by gamma radiation. 2. In lipid solutions. Bull. Environ. Contam. Toxicol. 7: 321.
Dorough, H.W., Ivie, G.W., Gibson, J.R., Bryant, H.E., Begin, J.J. and Barnett, J.R. 1974. Accumulation, distribution and excretion of mirex —^{14}C in animals exposed for long periods of time to the insecticide in the diet. J. Agr. Food Chem. 22: 646.
Food & Drug Administration. 1978. Action levels for poisonous or deleterious substances in human food and animal feed. Bureau of Foods, FDA, U.S. Dept. of Health & Welfare, 14 p.
Gaines, T. and Kimbrough, R.D. 1970. Oral toxicity of mirex in adult and suckling rats. Arch. Environ. Health. 21: 7.
Hallab, A.H. 1968. Detoxification of pesticide residues in fish and shellfish. Ph.D. dissertation, Louisiana State Univ.
Holloman, M.E., Layton, B.R., Kennedy, M.V., and Swanson, C.R. 1975. Identification of the major thermal degradation products of the insecticide mirex. J. Agr. Food Chem. 23: 1011.
Kefford, F. 1979. Pennsylvania Department of Environmental Resources, Harrisburg, PA. Personal communication.
Koller, L.R. 1965. "Ultraviolet Radiation," 2nd ed. John Wiley and Sons, Inc., New York, NY.
Lane, R.H. 1973. Influence of food processing on mirex. Ph.D. dissertation, Louisiana State Univ.
Li, C.F. and Bradley, R.L. 1967. Degradation of chlorinated hydrocarbons in fluid milk systems by ultraviolet light. J. Dairy Sci. 50: 944.
Reinert, R.E., Stewart, D., and Seagran, H.L. 1972. Effects of dressing and cooking on DDT concentrations in certain fish from Lake Michigan. J. Fish Res. Board Can. 29: 525.
Smith, W.E., Funk, K., and Zabik, M.E. 1973. Effects of cooking on concentrations of PCB and DDT compounds in chinook (Oncorhynchus tschawytscha) and coho (O. kisutch) salmon from Lake Michigan. J. Fish Res. Board Can. 30: 702.
Solar, J.M., Liuzzo, J.A., and Novak, A.F. 1971. Removal of aldrin, heptachlor epoxide and endrin from potatoes during processing. J. Agr. Food Chem. 19: 1008.
Spinks, J.W.T. and Woods, R.J. 1976. "An Introduction to Radiation Chemistry," 2nd ed. John Wiley and Sons, Inc., New York, NY.
Zabik, M.E., Hoojjat, P., and Weaver, C.M. 1979. Polychlorinated biphenyls, dieldrin, and DDT in lake trout cooked by broiling, roasting or microwave. Bull. Environ. Toxicol. 21: 136.
Ms received 6/3/81; revised 9/25/81; accepted 9/28/81.

Paper No. 6069 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
The authors express their gratitude to the Pennsylvania Fish Commission and Dr. Dean Arnold (Biology) for assistance in collecting fish; Mrs. Nancy Warner for technical assistance in the laboratory and computer center; and Mr. Floyd Kefford and other employees of the Pennsylvania Department of Environmental Resources for discussing methodology.

MINCED FISH PRODUCTION FROM CAPELIN . . . From page 349

below 45°C. Thus, the energy requirement is low compared to usual fish oil production where raw material is heated to 85–90°C before the oil is separated by pressing. In addition, it can be concluded that lipids remaining in the mince are mostly structure fat. Consequently, a homogeneous mince can be made from capelin independent of seasonal variation of total fat content in the fish. Secondly, during the washing procedures, both under neutral and acid conditions, skin and black belly lining are broken down, partly dissolved, and the intestines are completely washed off the fish pieces. Depot fat, dark pigments and intestines may thus be separated from the fish muscle in one single step by decanting the process water, resulting in a mince of white appearance after bone separation.

Finally, at low pH the process temperature can be maintained below critical values for the denaturation of the myofibrillar proteins (ca 40°C for myosin, Søbstad, 1977). Hence the low pH process combined with subsequent neutralization yields a capelin mince with good water-holding capacity and a white appearance. This should make it an excellent starting material for further processing into various minced fish products.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.
Bligh, E.G. and Dyer, W.G. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.
Carroll, K.K. 1961. Separation of lipid classes by chromatography on Florisil. J. Lipid Res. 2: 135.
Carroll, K.K. 1963. Acid-treated Florisil as an adsorbent for column chromatography J. Amer. Oil. Chem. Soc. 40: 413.
Carroll, K.K. 1976. Column chromatography of neutral glycerides and fatty acids. In "Lipid Chromatographic Analysis," Vol 1, p. 173. Marcel Dekker Inc., New York.
Galanos, D.S. and Kapoulas, V.M. 1962. Isolation of polar lipids from triglyceride mixtures. J. Lipid Res. 3: 134.
Gildberg, A. and Raa, J. 1979. Solubility and enzymatic solubilization of muscle and skin of capelin (Mallotus villosus) at different pH and temperature. Comp. Biochem. Physiol. 63B: 309.
Glass, R.L. and Christophers, S.W. 1969. A method for the differential analysis of mixture of esterified and free fatty acids. Chem. Phys. Lipids 3: 405.
Hood, R.L., Thompson, E.H., and Allen, C.E. 1972. The role of acetate, propionate, and glucose as substrates for lipogenesis in bovine tissues. Int. J. Biochem 3: 598.
Jangaard, P.M. 1974. The Capelin (Mallotus villosus). Biology, Distribution, Exploitation, Utilization, and Composition. Bull. Fish, Res. Bd. Can. 186: 70.
Lambertsen, G. and Hansen, O. 1978. Fettsyresammensetning i vevslipider fra lodde, makrell og sild, Government Vitamin Institute N 5000 Bergen, Norway.
Mohr, V. 1971. On the constitution and physicalchemical properties of the connective tissue of mammalian and fish skeletal muscle. Ph.D. thesis, Aberdeen.
Mohr, V. 1977. Fettvev i fisk. Rapport 5. Department of Technical Biochemistry, NTH, N-7034 Trondheim, Norway.
Mohr, V., Møller, M., and Flo, A. 1973. Fettvev i fisk. Rapport 1. Dept. of Technical Biochemistry, NTH, N-7034 Trondheim, Norway.
Mohr, V., Møller, M., Ormberg, A., Halvorsen, J., and Padget, E. 1976a. Fettvev i fisk. Rapport nr 2. Department of Technical Biochemistry, NTH, N-7034 Trondheim, Norway.
Mohr, V., Ormberg, A., Halvorsen, J., and Padget, E. 1976b. Fettvev i fisk. Rapport 3. Dept. of Technical Biochemistry, NTH, N-7034 Trondheim, Norway.
Mohr, V. and Ormberg, A. 1977. Fettvev i fisk. Rapport 4. Department of Technical Biochemistry, NTH, N-7034 Trondheim, Norway.
Novak, A.F., Rao, R.M., and Smith, O.A., 1977. Fish proteins. In "Food Colloids," p. 292. Avi Publishing Co., Westport, CT
Søbstad, G. 1977. Protein-Vann-Fettemulsjoner. The Herring Meal & Oil Industry Research Institute, 5000 Bergen, Norway.
Sørensen, N.K. 1978. Kjøttutbytte—lodde. Institute of Fishery Technology Research, N-9000 Tromsø, Norway.
Ms Received 1/21/81; revised 9/28/81; accepted 10/4/81.

This work was supported by The Norwegian Council for Fishery Research under the research projects FTFI 663.1-7 and FTFI 663.1-8. The authors would like to thank Mrs. Grethe Kvåle Dørum, Mrs. Audny Johansen and Mrs. Reidun Dahl for technical assistance. We will further thank Dr. Jan Raa and Mr. Asbjørn Gildberg for helpful suggestions and inspiring discussions.

Effect of Packaging Film and Vacuum Level on Regular and Sorbate-Cured Bacon

C. M. AMUNDSON, J. G. SEBRANEK, A. A. KRAFT, R. E. RUST, M. K. WAGNER and W. H. GEHRKE

ABSTRACT

Bacon was produced with either 40 ppm $NaNO_2$/0.26% potassium sorbate, or 120 ppm $NaNO_2$. Slices were packaged in either nylon/Surlyn or nylon/Saran/Surlyn pouches and sealed at either a high or intermediate vacuum level. At 0, 10, 21 and 28 days after packaging, residual nitrite, rancidity, pH, residual salt and pigment conversion were measured. Residual nitrite and pH were lower while nitrosopigment conversion was higher in bacon packaged with the high-barrier film. Vacuum level, however, had no effect on any measured characteristic. The sorbate/reduced nitrite cure resulted in lower pigment conversion, lower residual nitrite, and a higher TBA number.

INTRODUCTION

SINCE THE DISCOVERY of measurable amounts of carcinogenic nitrosamines in bacon, there has been a trend to decrease the amount of ingoing nitrite allowed in bacon processing. To date, reductions from 200 ppm residual allowed in bacon to 120 ppm ingoing nitrite have occurred. In 1978, the USDA proposed a new cure for bacon incorporating 40 ppm nitrite and 0.26% potassium sorbate in the initial product. The justification for the proposal derived from work by Ivey et al. (1978), Ivey and Robach (1978) and Sofos et al. (1979) who found the sorbate-nitrite cure to be at least as effective as a regular (120 ppm nitrite) cure for inhibition of *Clostridium botulinum* growth and toxin formation. It had previously been determined that sorbic acid was effective in inhibiting other microbial organisms, including Salmonella, Staphylococcus and *Clostridium perfringens* (Tompkin et al., 1974; Robach and Ivey, 1978; Park and Marth, 1972) as well as in preventing the growth of various molds and yeasts (Baldock et al., 1979; Melnick et al., 1954; Gooding et al., 1955). In addition, it was expected that the 1978 proposal would, by decreasing the ingoing nitrite level, result in a decrease in residual nitrite and, consequently, decreased nitrosamine formation (Sen et al., 1979; Pensabene et al., 1974, 1979; Kushnir et al., 1975).

Several factors remained unclear, however. Since nitrite is well known to affect the color, flavor and oxidative properties of cured meat (Mac Dougall et al., 1975; Sebranek et al., 1977), some concern was raised for the effect of a 2/3 reduction in nitrite on these characteristics. Work on the flavor of sorbate-cured meat has produced variable results. Berry and Blumer (1981) found a less acceptable product when using the sorbate cure, and Paquette et al. (1980) found no differences in bacon cured with sorbate.

Inasmuch as many methods of bacon production are employed in the United States, several processing variables were considered for a comparison of the two cure systems and have been previously reported (Amundson et al., 1982a, b). Two of the most important variables affecting shelf life and overall bacon quality are the degree of vacuum under which the bacon is packaged and the oxygen permeability of the package film. According to Gray (1978) and Watts (1954), oxidative rancidity is an important, if not the major cause, of food deterioration. In addition to oxidative rancidity, the presence of oxygen in a package may aid in the oxidation of myoglobin to form an off color in the product (George and Strautmann, 1952). Although cured meat color probably is formed by involvement of a metmyoglobin intermediate; once the nitrosopigment is formed, further exposure to light and air may result in fading of the cured color (Draudt and Deatherage, 1956; Walsh and Rose, 1956).

The purpose of the experiment reported here was to determine the effect of two initial vacuum levels and two types of film pouch combinations for packaging and storage of regular and sorbate-cured bacon.

Author Amundson is with the Dept. of Animal Science, Authors Sebranek and Rust are affiliated with the Dept. of Animal Science and Dept. of Food Technology, and Authors Kraft and Wagner are with the Dept. of Food Technology, Iowa State Univ., Ames, IA 50011. Author Gehrke is with Curwood, Inc., 718 High St., New London, WI 54961.

EXPERIMENTAL

PAIRED BELLIES from 64 hogs (128 bellies) were selected at a commercial packing plant. The hogs were of average fatness as determined by the packer's backfat probe measurement. The bellies were transported to the Iowa State University Meat Laboratory where they were skinned, separated between pairs and chilled for 24 hr. One belly of each pair was pumped to 110% of green weight by using a brine composed of 17% sodium chloride (NaCl), 2.6% sucrose, 2.0% sodium tripolyphosphate (TPP), 1200 ppm sodium nitrite ($NaNO_2$) and 5500 ppm sodium erythorbate (Ery). This resulted in ingoing levels of 120 ppm $NaNO_2$. The second belly of each pair was pumped to 110% of green weight with a brine composed of identical levels of NaCl, sucrose, TPP and Ery, but lowered $NaNO_2$ concentration to 400 ppm and including 2.6% potassium sorbate. This resulted in an ingoing nitrite level of 40 ppm and 0.26% potassium sorbate.

After pumping, the bellies were smoked and thermal processed to an internal temperature of 52°C, followed by chilling at 5°C for 48 hours. After chilling, the bacon was sliced (approximately 3 mm thick) and vacuum packaged in a shingled form with 14 slices per package. Two types of film pouches were used, a high-barrier pouch composed of a nylon/Saran/Surlyn lamination and an intermediate-barrier pouch composed of nylon and Surlyn without Saran. The permeability characteristics of the films are presented in Table 1. Each pouch type was sealed at one of two vacuum levels; high vacuum (590–635 mm Hg) or intermediate vacuum (400–480 mm Hg).

Determination of vacuum level was made by measuring the packages using a bell jar apparatus. The package to be measured was inserted in an empty bell jar together with a Marshalltown vacuum gauge. At the first sign of loosening or separation of the film from the bacon surface, the gauge was read, and air was readmitted to the jar. A Multivac vacuum packaging machine was adjusted to seal at the desired vacuum level, and numerous packages were removed and tested in the bell jar to insure maintenance of the proper vacuum level.

The vacuum levels and pouch types examined were designed to represent a typical industry representative and an ideal package type. In testing several commercial bacon packages obtained in local supermarkets with the bell jar apparatus, it was found that the average vacuum level was approximately 17–20 inches (430–508 mm) Hg.

After processing and packaging, all bacon was boxed and stored at 2–3°C for 14 days. Selected packages then were removed and placed in an open-top display case at 5 ± 3°C under 200 ft-c of cool white fluorescent light for an additional 14 days. Treatment

groups were sampled and data collected at 0, 10, 21 and 28 days after packaging.

Package numbers 5 and 12 (slices 70–84 and 168–182) from each belly were obtained. This was done to lessen variability between samples (Nusbaum et al., 1976). At each sample day, packages from each of 16 bellies were selected. This allowed duplicates to be taken from each cure, film and vacuum combination treatment. Cure differences were always analyzed between paired bellies. Each bacon strip was divided into fat and lean portions by dissecting out the muscle fraction. Fat within the lean portions was not removed. One-fourth of the dissected fat and lean portions were recombined to form a fraction representing the total or average composition. The fat, lean and total portions were frozen in liquid nitrogen and pulverized using a Waring Blendor. Duplicate analyses on each fraction were performed.

Residual nitrite was measured by using the AOAC method (1970) (with the proven carcinogenic activity of naphthylamine, an alternate method using N-(1-naphthyl) ethylenediamine dihydrochloride is suggested for future use). Rancidity was measured by using the 2-thiobarbituric acid technique for malonaldehyde determination of Tarladgis et al. (1960). Nitrosopigment formation was determined by using the acetone-water extraction procedure of Hornsey (1956), and residual salt was found by using the Technicon autoanalyzer technique (1965) for chloride ion. The samples were measured for moisture, fat and ash content (AOAC, 1970), and protein content was determined from nitrogen content found by using the macrokjeldahl procedure. pH was measured by blending 10g of the frozen material in 75 ml of distilled water and reading with a Corning Model 125 pH meter.

Statistical analysis was performed by using the Statistical Analysis System (SAS) program. The standard F-ratio analysis of variance was implemented with Duncan's New Multiple Range Test (1955) used between means where significance was indicated. Variation between pigs was used as the main effect error term and was in every instance larger than the corresponding residual error term.

RESULTS & DISCUSSION

PROXIMATE ANALYSIS of bacon packaged according to the treatments of barrier type and vacuum level was presented in Table 2. As expected, no difference was seen between treatments with the exception of ash values. This similarity was desirable since previous work (Amundson et al., 1982a) indicated that belly compositional differences could have a major effect on residual $NaNO_2$, pH, NaCl content and TBA number. Ash content in this study was statistically but not practically different. There were no significant effects of date (time after packaging) or cure on proximate analysis.

Residual $NaNO_2$ was affected by date and cure and was greater in bacon cured with 120 ppm $NaNO_2$ in every instance. As seen in Table 3, a significant ($P < 0.05$) date by cure interaction occurred resulting in less difference in residual $NaNO_2$ between cures at 28 days after packaging. The vacuum levels in this experiment did not result in a significant difference in residual $NaNO_2$, however, the film barrier did have a significant effect. High-barrier film resulted in a lower residual $NaNO_2$ level at 10 and 21 days after packaging. By 28 days after packaging, however, there was little difference. The decrease in nitrite is in general agreement with Lechowich et al. (1978), who found nitrite levels decreasing to approximately 10–20 ppm and then remaining stable. A possible explanation of the decrease in nitrite under vacuum was found by Walters and Taylor (1964) who determined that, under anaerobic conditions, muscle mitochondria will utilize nitrite as an electron acceptor.

Fig. 1 presents the results of vacuum level and film type on TBA number. The results are presented as averages combining both cures to simplify the effects of the film type or vacuum level. No significant differences ($P \leq 0.05$) were seen with either film or vacuum. There are two general sources for the potential development of rancidity. These are either autoxidation of the fat or the effect of lipolytic bacteria. Monitoring of our bacon for several groups of bacteria including lipolytic bacteria indicated little, if any, growth in our bacon product (Wagner et al., 1982). As a result, autoxidation is probably the primary cause of any rancidity development. Higher oxygen permeability and a lower level of vacuum would be expected to enhance the possibiliteis of rancidity development, however, as was seen in Table 1 the film was sufficiently impermeable and the vacuum adequate to maintain product quality.

Table 4 presents effect of the two cures on TBA number, pH, nitrosopigment conversion, and salt concentration over all packaging conditions. The sorbate-cured bacon resulted in significantly ($P < 0.05$) higher TBA number than regular cured bacon. Neither cure resulted in levels commonly associated with detectable levels of rancidity. Luck (1976) indicated that, under acid conditions with high salt content, sorbate will react with TBA reagent. Nitrosopigment conversion was greater for the regular cure overall but pH and salt content did not differ.

Table 1—Packaging films (materials and data provided by Curwood, Inc.).

Film	Contents	Permeability characteristics °F	R.H.	ml O_2/100 in^2/24 hr
High barrier	0.6 mil nylon Saran	75	35%	0.7
	2.0 mil Surlyn	38	90%	0.05
Intermediate barrier	0.6 mil nylon	75	35%	5.6
	2.0 mil Surlyn	38	90%	0.6

Table 2—Proximate analysis of bacon according to vacuum level and film type

	% Moisture	% Fat	% Protein	% Ash
High barrier High vacuum	33.0[a]	54.3[a]	9.1[a]	2.2[bc]
High barrier Intermediate vacuum	33.3[a]	53.7[a]	9.8[a]	2.4[a]
Intermediate barrier High vacuum	32.3[a]	55.0[a]	9.9[a]	2.1[c]
Intermediate barrier Intermediate vacuum	32.9[a]	54.0[a]	10.3[a]	2.3[ab]

[a] Averages in each column with a common letter are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.

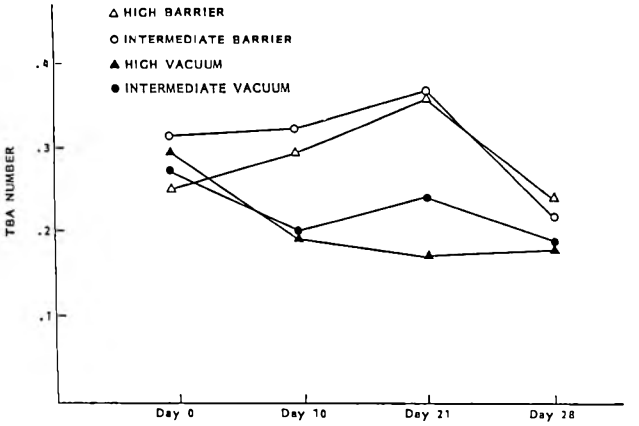


Fig. 1—Effect of film type and vacuum level on TBA number.

Table 3—Effect of days after packaging and cure on residual nitrite (ppm)

	Days after packaging			
	0	10	21	28
Regular				
High barrier High vacuum	48.3 ± 5.0	19.5 ± 3.1	15.9 ± 2.4	19.5 ± 2.6
High barrier Intermediate vacuum	53.2 ± 4.0	20.2 ± 1.6	17.8 ± 1.7	11.7 ± 0.7
Intermediate barrier High vacuum	51.7 ± 4.7	37.4 ± 4.5	28.3 ± 3.5	20.1 ± 2.7
Intermediate barrier Intermediate vacuum	52.3 ± 4.3	37.0 ± 3.8	37.2 ± 4.1	22.9 ± 3.0
Overall	51.4[a]	28.5[a]	24.8[a]	18.6[a]
Sorbate				
High barrier High vacuum	22.8 ± 2.5	13.5 ± 1.2	10.8 ± 0.8	10.9 ± 0.9
High barrier Intermediate vacuum	21.2 ± 1.4	14.6 ± 1.0	14.4 ± 1.4	12.2 ± 1.2
Intermediate barrier High vacuum	21.6 ± 2.0	18.0 ± 1.9	11.9 ± 1.4	8.9 ± 1.3
Intermediate barrier Intermediate vacuum	21.8 ± 1.6	17.9 ± 1.9	15.8 ± 1.3	10.3 ± 0.9
Overall	21.9[b]	16.0[b]	13.2[b]	10.6[b]

[a] Averages in each column with a common letter are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.

pH differences were significant ($P < 0.05$) between film types (Table 5), with high-barrier film resulting in a lower pH than intermediate barrier film. The lower pH in the high-barrier film is likely to contribute to the drop in nitrite found in those packages (Sofos et al., 1979). The higher pH values in intermediate-barrier film could be important if bacon is to be cured using sorbate. The efficacy of sorbate for inhibiting bacterial growth is related to pH, with little effect at pH 6.5 and increasing inhibition as pH drops (Luck, 1976; Monsanto, 1978). Addition of potassium sorbate to bacon was observed by Pierson et al. (1979) to result in a decreased pH. In our experiment, no pH differences were seen between the cures. The buffering action of tripolyphosphate may explain part of this.

Nitrosopigment conversion as a function of cure and day after packaging is shown in Table 6 and the effect of package conditions is shown in Table 7.

A significant difference ($P < 0.05$) between cures was found with regular cure having significantly higher nitrosopigment conversion (0.53 overall in regular cure versus 0.48 overall in sorbate cure). Several authors have indicated that 40 ppm is sufficient for adequate color formation (Mac Dougall et al.,1975; Kerr et al., 1926; Herring, 1973).

In Table 7, it is thought that the high values for intermediate barrier, high vacuum treatments at ten days after packaging are probably unrealistic, however, even including those values for the intermeidate barrier film, the high-barrier film resulted in significantly higher overall pigment conversion ($P < 0.05$) in the analysis of variance. The greatest difference in nitrosopigment conversion as a result of film was seen at 28 days after packaging which may indicate potential long-term storage problems using the intermediate barrier film. A significant ($P < 0.05$) film by vacuum interaction occurred with the intermediate barrier film-intermediate vacuum resulting in a significantly ($P < 0.05$) lower overall average to nitrosopigment conversion. A low nitrite concentration coupled with the intermediate barrier, intermediate vacuum packaging as seen in industry may create a potential color problem.

Table 4—Effect of sorbate and nitrite cure on TBA number, pH, nitrosopigment conversion and salt concentration, averaged over all packaging conditions

	TBA no.	pH	Nitrosopigment/ total pigment	% Salt conc
$NaNO_2$ cure	0.20[a]	6.36[a]	0.53[a]	1.7[a]
Sorbate cure	0.40[b]	6.36[a]	0.48[b]	1.6[a]

[a] Averages in each column with a common letter are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 5—Effect of film type, vacuum level and date on pH[a]

	Days after packaging			
	0	10	21	28
High barrier High vacuum	6.27[c]	6.27[b]	6.20[c]	6.24[b]
High barrier Intermediate vacuum	6.32[c]	6.27[b]	6.11[d]	6.28[b]
Intermediate barrier High vacuum	6.61[a]	6.31[b]	6.38[b]	6.39[a]
Intermediate barrier Intermediate vacuum	6.52[b]	6.38[a]	6.57[a]	6.36[a]

[a] Averages in each column with a common letter are not significantly different at the 5% level fo significance based on Duncan's Multiple Range Test.

The change ($P < 0.05$) in nitrosopigment as a result of date after packaging showed a peak at 10 days. Although as mentioned earlier, the figures at day 10 may be high, this trend was also observed in earlier experiments (Amundson, 1982a, b).

Salt content of the bacon may be a factor in formation of nitrosamines from nitrite (Hildrum et al., 1975; Theiler et al., 1981). As a result, NaCl content was monitored. No

significant differences were seen as a result of cures, films or vacuum levels.

The lean, fat and total fractions were chemically analyzed to determine where the $NaNO_2$ and NaCl was located and what factors may contribute to the pH and rancidity of the bacon. As seen in Table 8, the lean portion contained 53% H_2O and 25% fat while the fat fraction had 16% H_2O and 79% fat. The water-soluble salts distributed much as might be expected from the proximate analysis with nearly three times the overall residual nitrite (34 ppm) found in the lean compared with the fat fraction (14 ppm), and 2.6% NaCl in the lean compared to 0.78% in the fat. Rancidity was also higher in the lean than in fat portions (0.42 to 0.20 TBA numbers, respectively). To a certain extent, this could be due to the increased activity of metabolic enzymes in the lean fraction even though there are much lower fat levels involved. An interesting observation was the relatively high rancidity levels found in the fat fraction of the sorbate-cured becon compared with the regular cure (0.24 vs 0.15 TBA numbers, respectively). pH, as has been seen in a previous study on fat versus lean type bacon, is consistently higher with greater fat content.

Table 6—Nitrosopigment/total pigment conversion as a function of date and cure averaged for all package conditions

	Days after packaging			
	0	10	21	28
Regular cure	0.54[a]	0.62[a]	0.49[a]	0.48[a]
Sorbate cure	0.45[b]	0.62[a]	0.44[b]	0.41[b]

[a] Averages In each column with a common letter are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 7—Nitrosopigment/total pigment conversion as a function of date and package

	Days after packaging				
	0	10	21	28	Overall
High barrier High vacuum	0.56[a]	0.57[b]	0.35[c]	0.49[b]	0.49[b]
High barrier Intermediate vacuum	0.56[a]	0.59[b]	0.65[a]	0.56[a]	0.59[a]
Intermediate barrier High vacuum	0.48[b]	0.80[a]	0.52[b]	0.30[c]	0.53[b]
Intermediate barrier Intermediate vacuum	0.37[c]	0.50[c]	0.35[c]	0.42[b]	0.41[c]

[a] Averages In each column with a common letter are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.

Table 8—Composition of lean, fat and total fractions of cured bacon

	% Moisture	% Fat	% Protein	% Ash
Lean	52.9[a]	24.7[a]	17.6[a]	3.6[a]
Fat	16.5[b]	79.0[b]	3.4[b]	1.1[b]
Total	29.2[c]	59.1[c]	8.7[c]	2.1[c]

	pH	TBA	Nitrite (ppm)	% Salt
Lean	6.23[a]	0.42[c]	33.9[a]	2.6[a]
Fat	6.48[b]	0.20[b]	13.9[b]	0.8[b]
Total	6.34[c]	0.27[c]	21.4[c]	1.5[c]

[a] Averages in each column with a common letter are not significantly different at the 5% level of significance based on Duncan's Multiple Range Test.

CONCLUSIONS

IN THE STUDY PRESENTED, vacuum level had little effect on the chemical characteristics measured except for an interaction with film type for nitrosopigment conversion. The high barrier film resulted in lower residual nitrite, a lower product pH, and greater nitrosopigment converison when compared with the low barrier film. However, none of the barriers or vacuum levels used represented poor-quality packages, nor did the experiment take into account possible package abuse such as elevated storage temperatures.

The use of a sorbate-reduced nitrite cure did decrease residual nitrite as well as decreasing nitrosopigment conversion. The use of sorbate should include consideration for attaining a pH low enough for antimicrobial efficacy. TBA number was higher in sorbate cured bacon; however, an actual increase in detectable rancidity would probably require TBA numbers higher than any of those observed.

In terms of chemical analysis, there was no advantage to sorbate-cured bacon with the exception of decreasing residual nitrite which was shown earlier (Amundson, 1982a, b) to reduce potential nitrosamine formation. Potential disadvantages might develop, however, due to a high product pH or the lower percent of color conversion found in sorbate-cured bacon. The high barrier packaging film appeared to partially compensate for the changes induced by the sorbate cure.

REFERENCES

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

Amundson, C.M., Sebranek, J.G., Kraft, A.A., Rust, R.E., Wagner, M.K., and Robach, M.C. 1982a. The effect of belly composition on sorbate-cured bacon. J. Food Sci. 47(1): 218.

Amundson, C.M., Sebranek, J.G., Kraft, A.A., Rust, R.E., Wagner, M.K., and Robach, M.C. 1982b. Effect of belly handling (chilled, frozen, prerigor) and smoking delay on sorbate-cured bacon. J. Food Sci. 47(1): 222.

Baldock, J.D., Frank, P.R., Graham, P.O., and Ivey, F.J. 1979. Potassium sorbate as a fungistatis agent in country ham processing. J. Food Prot. 42(10): 780.

Berry, B.W. and Blumer, T.N. 1981. Sensory, physical and cooking characteristics of bacon processed with varying levels of sodium nitrite and potassium sorbate. J. Food Sci. 46: 321.

Draudt, H.N. and Deatherage, F.E. 1956. Studies on the chemistry of cured meat pigment fading. Food Res. 21: 122.

Duncan, D.B. 1955. Multiple range and multiple F-tests. Biometrics 11: 1.

George, P. and Strautmann, C.J. 1952. The oxidation of myoglobin to metmyoglobin by oxygen. 2. The relation between the first order rate constand and the partial pressure of oxygen. Biochem. J. 51: 418.

Gooding, C.M., Melnick, D., Lawrence, R.L., and Luckmann, F.H. 1955. Sorbic acid as a fungistatic agent for foods. 9. Physicochemical considerations in using sorbic acid to protect foods. Food Res. 20(6): 639.

Gray, J.I. 1978. Measurement of lipid oxidation: A review. J. Am. Oil Chem. Soc. 55: 539.

Herring, H.K. 1973. Effect of nitrite and other factors on the physico-chemical characteristics and nitrosamine formation in bacon. Proc. Meat Ind. Res. Conf., AMI Foundation, Chicago, IL.

Hildrum, K.I., Williams, J.L., and Scanlan, R.A. 1975. Effect of sodium chloride on the nitrosation of proline at different pH levels. J. Agric. Food Chem. 23: 439.

Hornsey, H.C. 1956. The color of cured pork. J. Sci. Food Agric. 7: 534.

Ivey, F.J. and Robach, M.C. 1978. Effect of potassium sorbate and sodium nitrite on Clostridium botulinum growth and toxin production in canned comminuted pork. J. Food Sci. 43: 1782.

Ivey, F.J., Shaver, K.J., Christiansen, L.N., and Tompkin, R.B. 1978. Effect of potassium sorbate on toxinogenesis of Clostridium botulinum in bacon. J. Food Prot. 41: 621.

Kerr, R.H., Marsh, T.N., Schroeder, W.F., and Boyer, E.A. 1926. The use of sodium nitrite in the curing of meat. J. Agric. Res. 33: 541.

Kushnir, I., Feinberg, J.I., Pensabene, J.W., Piotrowski, E.G., Fiddler, W., and Wasserman, A.E. 1975. Isolation and identification of nitrosoproline in uncooked bacon. J. Food Sci. 40: 427.

Lechowich, R.V., Brown, W.L., Deibel, R.H.,and Somers, I.I., 1978. The role of nitrite in the production of canned cured meat products. Food Technol. 32: 45.

Luck, E. 1976. Sorbic acid as a food preservative. Int. Flavors & Food Additiv. 7: 122.

MacDougall, D.B., Mottram, D.S., and Rhodes, D.N. 1975. Contribution of nitrite and nitrate to the colour and flavour of cured meats. J. Sci. Food Agric. 26: 1743.

Melnick, D., Luckmann, F.H., and Gooding, G.M. 1954. Sorbic acid

—Continued on page 362

Influence of Tumbling and Phosphate on the Yield, Sensory and Chemical Characteristics of Pork Liver Loaf

P. J. PERCEL, N. A. PARRETT, R. F. PLIMPTON, H. W. OCKERMAN, B. KROL and P.S. VAN ROON

ABSTRACT

Yield, sensory and chemical properties of pork liver loaves manufactured using varying processing treatments (tumbling vs immersion) and phosphate levels (0 vs 6.4%) were studied. Tumbling significantly improved liver cure uptake, total cure and loaf cooked yield when compared to immersion as a processing treatment. Tumbling the livers improved uniformity of internal slice color, external loaf color and external loaf appearance. Loaves made from immersed liver however yielded a more intense liver flavor. Increasing moisture levels were likewise noted for tumbled livers. Addition of phosphate resulted in an increase in liver cure uptake, total cure and loaf cooked yield for the tumbled livers. Phosphate however had no effect on these parameters for livers which were immersed. Addition of phosphate improved slice cohesiveness and internal slice consistency. An increase in the percent ash was the only chemical parameter altered significantly through the addition of phosphate.

INTRODUCTION

MANY PROCESSES and techniques have been developed to improve the desirable characteristics of cured meat products. Two of these processes, massaging and tumbling, each with their origins in Europe, are presently being evaluated for use with products other than the traditional ham. Massaging and tumbling, which mechanically agitate the meat tissue, aid in the extraction of the salt soluble proteins, thus enhancing product cohesiveness, increasing yields and bringing forth a more uniform color development (Krause et al., 1978). Viskase Ltd (1971) reported that the primary function of tumbling is to enable several pieces of meat to be bonded together giving a large piece which has a homogeneous appearance.

Vartorella (1975), Krause et al., (1978) and Siegel et al., (1978) indicated that the combination of massaging and/or tumbling with the addition of phosphate to the curing mixture significantly increase the cohesiveness of cured ham. Alkaline phosphates are known to enhance and stabilize cured meat color, increase the retention of soluble proteins and reduce the shrinkage during the curing cycle. As new products are developed, massaging and/or tumbling with the addition of phosphate may very well be a common procedure used during the production process.

One product, common in Europe, but manufactured to a very limited degree in the U.S. is pork liver loaf. Percel (1979) has indicated that the curing of liver, which is to be used in a pork liver loaf, is more effective when using a multiple needle injection system in comparison to either bile duct pumping or immersion. In addition the use of alkaline phosphates was found to exert positive effects regardless of the curing method.

Authors Percel, Parrett, Plimpton, and Ockerman are affiliated with the Dept. of Animal Science, The Ohio State Univ., Columbus, OH 43210 and The Ohio Agricultural Research & Development Center, Wooster, OH 44691. Author Krol is affiliated with the Central Institute for Nutrition & Food Research, TNO, Zeist, The Netherlands and Faculty Veterinary Medicine, State Univ. of Utrecht, Utrecht, The Netherhland. Author van Roon is affiliated with the Faculty Veterinary Medicine, State Univ. of Utrecht, Utrecht, The Netherlands.

The development of techniques used during the formation of a new product is always foremost in the meat processors mind. To date, the use of tumbling when making a liver loaf has not been a part of the manufacturing cycle. The use of phosphates have not been evaluated as a part of pork liver loaf production. This current study was completed to evaluate the effects which phosphate addition and processing treatment (tumbling, immersion) may have upon the yield, sensory and chemical characteristics of a pork liver loaf.

EXPERIMENTAL

Selection and curing

Two hundred forty fresh pork livers were selected directly from the slaughter line of a commercial U.S. pork processing plant. The livers were randomly allotted to eight treatment groups which were replicated five times (Table 1). All livers were immediately trimmed of any connective tissue and chilled to 3°C.

Pork livers allotted to treatment groups A, B, C, and D were pumped using a multiple needle injection system to 5% of their green weight using a brine solution containing 88.90% water, 10.54% salt, 0.50% sodium erythorbate and 0.06% sodium nitrite. Treatment groups E, F, G and H were pumped, using the same system, with a brine solution containing 82.50% water, 10.54% salt, 0.50% sodium erythorbate, 0.06% sodium nitrite and 6.40% sodium tripolyphosphate. Following multiple injection those livers in treatment groups C, D, G and H were immediately placed in an immersion brine containing 91.05% water, 8.90% salt, and 0.05% sodium nitrite. Treatments C and G were immersed for 20 hr while treatments D and H were immersed for 40 hr prior to further processing. Livers in treatment groups A, B, E and F were immediately placed in a 58 cm diameter, three baffle tumbler with 20% by weight additional brine added. All treatments were completed in a 3°C chilled room for the appropriate time period. Tumbling was carried out at the rate of 12 rpm continuously for 2 hr (B and F) or intermittently (6 min/hr) for 20 hr (A and E).

Cure uptake was obtained by dividing the brine uptake resulting from tumbling or immersion by the liver weight after pumping and multiplying by 100. Total cure represented the brine uptake plus the brine pumped divided by the green liver weight times 100. Cooked yield was measured as the weight of the cooked liver loaf divided by the liver loaf weight before cooking times 100.

Table 1—Treatment groups for pork liver

Treatment group	Phosphate %	Multiple needle injected: Tumbled[a] Continuous	Tumbled[a] Intermittent	Immersed[b] 20 hr	Immersed[b] 40 hr
A[c]	0	–	+	–	–
B[c]	0	+	–	–	–
C	0	–	–	+	–
D	0	–	–	–	+
E[c]	6.4	–	+	–	–
F[c]	6.4	+	–	–	–
G	6.4	–	–	+	–
H	6.4	–	–	–	+

[a] Livers were tumbled for 2 hr continuously or intermittently 6 min/hr for 20 hr.
[b] Livers were immersed for either 20 or 40 hr.
[c] Livers which were tumbled received 20% by weight additional brine added to the tumbler.

Processing

Following the curing treatments, livers within a treatment were divided into two equal weight units and layered with porkrindfolio (1 kg liver: 30 g porkrindfolio) into two plastic lined metal loaf molds (28 cm x 16 cm x 14 cm).

Porkrindfolio, a combination of pork rind and liver was made by mixing nine parts of cooked ground (2 mm) pork rind with one part of fresh ground (2 mm) pork liver. After this mixture was allowed to set overnight in a 1°C cooler, it was sliced into 2 mm slices for subsequent layering.

Spring load closure of the filled molds (liver plus porkrindfolio) allowed for the removal of trapped air prior to immersion cooking at 75°C for 3 hr 15 min. Cooling during a 3-hr time period was carried out with cold running tap water. The cooked, cooled loaves were then removed from the molds, rinsed with cold water to remove adhering gelatin or loose particles, and weighed for eventual yield computation. All loaves were individually vacuum packaged prior to placing and holding in a 3°C cooler until subsequent chemical and sensory analysis.

Chemical analysis

One liver loaf from each treatment was ground through a 0.3 cm grinding plate, mixed, and reground through a 0.2 cm grinding plate. Moisture, fat and ash content of the respective liver loaves were determined according to AOAC (1975) procedures. Protein content was established as the difference between the total of the three aforementioned determinations and 100. Salt content and residual nitrite were analyzed using procedures outlined by Ockerman (1976).

Sensory evaluation

The second liver loaf from each treatment group was subjectively evaluated by a six member descriptive attribute panel. The panel was selected and trained using the procedures of Cross et al. (1978). The following attributes were rated on an 8-point structured scale: (a) uniformity of external loaf an internal slice color (1 = very irregular; 8 = very uniform) (b) external loaf appearance (1 = very irregular; 8 = very uniform) (c) slice cohesiveness (1 = noncohesive; 8 = very cohesive) (d) internal slice consistency (1 = soft and wet; 8 = dry and firm) and (e) flavor intensity (1 = extremely bland; 8 = extremely intense).

Statistical anlaysis

All data were analyzed using the least squares analysis of data with unequal subclass numbers procedure as described by Harvey (1975). When significant differences were apparent, the multiple range test of Duncan, (1955) was used to determine where the differences occurred.

RESULTS & DISCUSSION

THE RESULTS will be discussed separately for the effect of phosphate addition and processing treatment.

Phosphate effects

A significant ($P < 0.05$) interaction between phosphate addition and processing treatment (tumbling, immersion) was found. The tumbled livers with phosphate showed a significant ($P < 0.05$) improvement in cure uptake, total cure, and cooked yield when compared to the livers that were immersed or tumbled which had received no phosphate in the pumping brine (Table 2). These results agree with reported results of Wierbicki et al. (1976) and Krause et al. (1978) working with hams.

No significant ($P < 0.05$) interaction between tumbling method (continuous vs intermittent) and the effect of phosphate on either cure uptake, total cure or liver loaf yield was found. The lack of a significant interaction may be due to the liver not needing the rest period between tumbling intervals, because of its cellular structure. The liver lobule, the basic functional unit of the liver, is constructed around a central vein that empties into hepatic veins and ultimately into the vena cava. The lobule itself is composed principally of many hepatic cellular plates. Venous sinosoids which are lined with endothelial cells, lie between the hepatic plates. The endothelial lining of the venous sinusoids has extremely large pores which allow very free exchange of substances in the plasma with the fluids surrounding the hepatic cells. Even the plasma proteins produced by the liver flow freely into these fluids (Guyton, 1961). In hams, the rest periods, characteristic of intermittent tumbling, are often used to permit cure migration (Krause et al., 1978).

There was no significant ($P < 0.05$) interaction between the effects of phosphate and those of tumbling or immersion on the uniformity of liver loaf color or external loaf appearance (Table 3). The addition of phosphate, however, did bring forth a significant increase in slice cohesiveness as well as internal slice consistency. In addition, a significant ($P < 0.05$) decrease in flavor intensity due to phosphate inclusion was noted. The improvement in slice cohesiveness and internal consistency is similar to the improved sliceability and cohesiveness in hams attributed to the addition of phosphate (Krause et al., 1978) and is a function of the protein binding characteristics attributed to phosphates. The decrease in flavor intensity may in part be

Table 3–Least square means (LSM) and standard errors (SE) for parameters used to identify the effects[a] of sodium tripolyphosphate on sensory characteristics of pork liver loaf

	PO_4		No PO_4	
Parameter	LSM	SE	LSM	SE
Internal slice color[b]	4.29[f]	0.14	4.26[f]	0.14
External loaf color[b]	4.35[f]	0.18	4.12[f]	0.18
Slice cohesiveness[c]	4.17[f]	0.20	3.57[g]	0.20
Internal slice consistency[d]	4.82[f]	0.19	4.19[g]	0.19
Flavor intensity[e]	4.80[f]	0.13	5.26[g]	0.13
External loaf appearance[b]	4.56[f]	0.17	4.16[f]	0.17

[a] Tumbling method and immersion time effects absorbed.
[bcde] Values based on an eight point structured scale; [b](8 = very uniform, 1 = very irregular); [c](8 = very cohesive, 1 = noncohesive); [d](8 = dry and firm, 1 = soft and wet); [e](8 = extremely intense, 1 = extremely bland).
[fg] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 2–Effect of sodium tripolyphosphate on cure uptake and product yield for tumbled or immersed liver: LSM = Least Square Means SE = Standard Errors

	PO_4				No PO_4			
	Tumbled[a]		Immersed[a]		Tumbled[a]		Immersed[a]	
Parameter	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Liver cure uptake, %*	13.25[b]	0.50	4.45[c]	0.48	11.28[d]	0.46	4.19[c]	0.46
Liver total cure, %*	18.79[b]	0.54	9.48[c]	0.51	16.67[d]	0.49	9.22[c]	0.49
Loaf cooked yield, %*	93.32[b]	0.74	87.89[c]	0.69	88.96[c]	0.68	87.26[c]	0.67

[a] Tumbling method and immersion time effects absorbed.
[b,c,d] Means in the same row with different superscripts are significantly different ($P < 0.05$).
*Significant interaction ($P < 0.05$).

accounted for by the increased percent of cure uptake in the tumbled livers. The increased cure uptake may have had the effect of diluting flavor intensity.

The only significant ($P < 0.05$) interaction between phosphate and processing treatment for the chemical parameters of the pork liver loaves occurred with percent ash (Table 4). The tumbled livers treated with phosphate were significantly ($P < 0.05$) higher in percent ash while no such phosphate effect was noted in the loaves made from the immersed livers. This increase may be a result of the processing treatment. Those livers which were tumbled received phosphate both from the initial injection as well as the brine added during the tumbling process whereas those livers which were immersed only received phosphate during the initial injection phase. An evaluation of the percent cure uptake indicates greater uptake and ultimate retention of cure for those livers which were tumbled when compared to those only immersed. This could lead to a greater retention of ash. Analysis of the data with processing treatment effects removed yielded a similar pattern with respect to the chemical parameters.

Processing treatment effects

Since there was a significant sodium tripolyphosphate treatment interaction with processing treatment (tumbling vs immersion), as previously discussed for percent cure uptake, percent total cure and cooked loaf yield, these phosphate effects were statistically absorbed during the analysis of processing treatment effects.

Tumbling significantly ($P < 0.05$) improved the uniformity of internal slice color, external loaf color, and external loaf appearance (Table 5). Viskase Ltd. (1971) reported improved color uniformity in tumbled hams and hypothesized that the improved uniformity was due to greater cure distribution throughout the ham. Those liver loaves made from immersed livers possessed a more intense flavor ($P < 0.05$) thereby indicating that the tumbled livers had a greater uptake of the curing solution which would lead to a more bland flavor. Other sensory parameters were not significantly ($P < 0.05$) affected by tumbling procedure or immersion time.

The effects of tumbling and immersion on the chemical characteristics of pork liver loaf may be noted in Table 6.

—Continued on next page

Table 4—Effect of sodium tripolyphosphate on cure uptake and product yield for tumbled liver: LSM = Least Square Means SE = Standard Errors

	PO_4				No PO_4			
	Continuous[a]		Intermittent[a]		Continuous[a]		Intermittent[a]	
Parameter	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Liver cure uptake, [b]%	13.53[e]	0.68	13.03[e]	0.68	11.30[f]	0.68	11.24[f]	0.68
Liver total cure, [c]%	19.10[e]	0.73	18.56[e]	0.73	16.69[f]	0.74	16.63[f]	0.73
Loaf cooked yield, [d]%	93.38[e]	0.73	93.31[e]	0.73	87.83[f]	0.75	90.08[f]	0.74

[a] Tumbled 2 hr continuously or intermittently 6 min/hr for 20 hr.
[b] Cure uptake = brine uptake resulting from tubling divided by liver weight after pumping X 100.
[c] Total cure = brine uptake plus brine pumped divided by green liver weight X 100.
[d] Cooked yield = loaf weight after cooking divided by loaf weight before cooking X 100.
[ef] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 5—Effects of tumbling and immersion on sensory panel evaluations of pork liver loaf as shown by least square means (LSM) and standard errors (SE)

	Tumbled[a]				Immersed[b]			
	Continuous		Intermittent		20		40	
Parameter	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Internal slice color[c]	5.27[g]	0.19	5.42[g]	0.19	3.26[h]	0.19	3.14[h]	0.19
External loaf color[c]	5.26[g]	0.24	5.06[g]	0.24	3.10[h]	0.24	3.52[h]	0.24
Slice cohesiveness[d]	4.41[g]	0.29	4.23[g]	0.29	3.15[g]	0.29	3.67[g]	0.29
Internal slice consistency[e]	4.30[g]	0.27	4.17[g]	0.27	4.47[g]	0.27	5.07[g]	0.27
Flavor intensity[f]	4.79[g]	0.15	4.82[g]	0.15	5.39[h]	0.15	5.12[h]	0.15
External loaf appearance[c]	5.03[g]	0.17	4.60[g]	0.17	3.74[h]	0.17	4.05[h]	0.17

[a] Tumbled 2 hr continuously or 6 min/hr for 20 hr.
[b] Immersed for either 20 or 40 hr.
[cdef] Values based on an eight-point structured scale: [c](8 = very uniform, 1 = very irregular); [d](8 = very cohesive, 1 = noncohesive); [e](8 = dry and firm, 1 = soft and wet); [f](8 = extremely intense, 1 = extremely bland).
[gh] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 6—Effects of tumbling and immersion on chemical parameters of pork liver loaf as shown by least square means (LSM) and standard errors (SE)

	Tumbled[a]				Immersed[b]			
	Continuous		Intermittent		20 hr		40 hr	
Parameter	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Salt, %	2.66[c]	0.12	2.76[c]	0.10	2.39[d]	0.10	2.91[c]	0.11
Moisture, %	70.39[c]	0.43	71.08[c]	0.35	63.94[d]	0.35	69.01[d]	0.39
Ash, %	3.60[c]	0.11	3.69[c]	0.09	2.88[c]	0.09	3.27[c]	0.10
Ether extract, %	4.84[c]	0.31	5.07[cd]	0.25	5.53[cd]	0.25	5.88[d]	0.28
Protein, %	21.19[c]	0.37	20.14[d]	0.31	22.64[e]	0.31	21.86[ce]	0.34
Nitrite, ppm	8.60[c]	1.59	10.46[c]	1.32	7.56[c]	1.31	15.41[d]	1.45

[a] Tumbled 2 hr continuously or 6 min/hr for 20 hr.
[b] Immersed for either 20 or 40 hr.
[cde] Means in the same row with different superscripts are significantly different ($P < 0.05$).

Livers which were tumbled contained a higher concentration of salt ($P < 0.05$) in comparison to those which were immersed for only 20 hr. Those livers which were immersed for 40 hr, however, were no different than those which were tumbled. This signifies that the equalization of salt was not complete at the end of 20 hr. In addition, the significant increase in salt concentration between the tumbled livers and those immersed for 20 hr may be a result of the increased uptake of the curing solution as shown by the increased percent total cure. Tumbling also significnatly ($P < 0.05$) increased the percent moisture, which would lend credence to the fact that tumbled livers have a greater ability to bind moisture and therefore to result in increased liver loaf yields. Liver loaves processed from immersed livers were slightly higher in percent ether extract in comparison to loaves processed from tumbled livers. A significant ($P < 0.05$) increase was found between those livers immersed for 40 hr in relation to those tumbled for 2 hr continuously. These increases may be a direct function of the lower moisture levels associated with the immersed livers. The protein values for the liver loaves varied erratically. In general, the values were slightly higher for the immersed livers than for those which were tumbled. This follows closely the pattern established for ether extract. The fact that moisture levels were higher and ether extraction levels were lower in tumbled livers would lead one to theorize that the pattern established for protein was a direct function of both moisture and ether extract. In this study, increased support of this theory would be valid since percent protein was determined by difference. The nitrite level associated with those livers immersed for 40 hr was significantly ($P < 0.05$) higher than the levels for those livers tumbled or for those livers immersed for 20 hr. This difference however is very small and therefore would be of limited practical importance.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.
Cross, H.R., Moen, R., and Stanfield, R. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 32(7): 48.
Duncan, P.B. 1955. New multiple range and multiple F tests. Biometrics 11:1.
Guyton, A.C. 1961. "Textbook of Medical Physiology." W.B. Sanders Co. Philadelphia, PA.
Harvey, W.R. 1975. Least squares analysis of data with unequal subclass numbers. ARSH-r, USDA. Beltsville, MD.
Krause, R.J., Ockerman, H.W., Krol, B., Moerman, P.C., and Plimpton, R.F. 1978. Influence of tumbling, tumbling time, trim and sodium tripolyphosphate on quality and yield of cured hams. J. Food Sci. 43: 853.
Ockerman, H.W. 1976. "Quality Control of Post-Mortem Muscle Tissue." Animal Science Dept., The Ohio State Univ., Columbus, OH.
Percel, P.J. 1979. Influence of curing method, massaging and phosphate on quality, yield, and chemical composition of pork liver loaf. Master's thesis, Ohio State Univ., Columbus, OH.
Siegel, D.G., Theno, D.M., Schmidt, G.P., and Norton, K.W. 1978. Meat massaing: The effects of salt, PO4 and massaging on cooking loss, bind strength and exudate composition in sectioned and formed ham. J. Food Sci. 43: 331.
Vartorella, T.R. 1975. The effect of tumbling, salt, and tripolyphosphate on selected quality characteristics of cured, canned pork. Master's thesis, The Ohio State Univ., Columbus, OH.
Viskase Limited. 1971. Growing interest in meat tumbling. Food Mfg. Oct.
Wierbicki, E., Hawker, J.J., and Shults, G.W. 1976. Effect of salt, phosphates and other curing ingredients on shrinkage of lean pork meat and the quality of smoked processed ham. J. Food Sci. 41: 1116.
Ms received 5/19/81; revised 9/21/81; accepted 9/23/81.

Journal Article No. 83-81 of the Ohio Agricultural Research & Development Center, Wooster, OH 44691.

PACKAGING FILMS AND BACON CURED WITH SORBATE . . . From page 358

as a fungistatis agent for foods. 5. Resistance of sorbic acid in cheese to oxidative deterioration. Food Res. 19: 33.
Monsanto. 1978. Sorbic acid and potassium sorbate: For preserving food freshness and market quality, p. 3. Monsanto, St. Louis, MO.
Nusbaum, R.P., Rust, R.E. and Topel, D.G. 1976. Predicting chemical composition of pork bellies. J. Anim. Sci. 42: 1348.
Paquette, M.W., Robach, M.C., Sofos, J.N., and Busta, F.F. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on color and sensory qualities of commercially prepared bacon. J. Food Sci. 45: 1293.
Park, H.S. and Marth, E.H. 1972. Inactivation of Salmonella typhimurium by sorbic acid. J. Milk Food Technol. 35(9): 532.
Pensabene, J.W., Fiddler, W., Gates, R.A., Fagan, J.C., and Wasserman, A.E. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. J. Food Sci. 39: 314.
Pensabene, J.W., Feinberg, J.I., Dooley, C.J., Phillips, J.G., and Fiddler, W. 1979. Effect of pork belly composition and nitrite level on nitrosamine formation in fried bacon. J. Agric. Food Chem. 27: 842.
Pierson, M.D., Smoot, L.A., and Stern, N.J. 1979. Effect of potassium sorbate on growth of Staphylococcus aureas in bacon. J. Food Prot. 42: 302.
Robach, M.C. and Ivey, F.J. 1978. Antimicrobial efficacy of potassium sorbate dip on freshly processed poultry. J. Food Prot. 41: 284.
Robach, M.C., Owens, J.L., Paquette, M.W., Sofos, J.N., and Busta, F.F. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on nitrosamine formation in commercially prepared bacon. J. Food Sci. 45: 1280.
Sebranek, J.G., Schroder, B.G., Rust, R.E., and Topel, D.G. 1977. Influence of sodium erythorbate on color development, flavor and overall acceptability of frankfurters cured and reduced levels of sodium nitrite. J. Food Sci. 42: 1120.
Sen, N.P., Seaman, S., and Miles, W.F. 1979. Volatile nitrosamines in various cured meat products: Effect of cooking and recent trends. J. Agric. Food Chem. 27: 1354.
Sofos, J.N., Busta, F.F., and Allen. C.E. 1979. Botulism control by nitrite and sorbate in cured meats: A review. J. Food Prot. 42: 739.
Tarladgis, B.G., Watts, B.G., Younathan, M.T., and Dugan, L. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.
Technicon Corporation. 1965. Technicon Auto Analyzer Methodology, N-206. Technicon Corp., Chauncey, NY.
Theiler, R.F., Sato, K., Aspelund, T.G., and Miller, A.F. 1981. Model system studies on N-nitrosamine formation in cured meats: Effect of curing solution ingredients. J. Food Sci. 45: 996.
Tompkin, R.B., Christiansen, L.N., Shaparis, A.B., and Bolin, H. 1974. Effects of potassium sorbate on salmonellae, staphylococcus, Clostridium perfringens and Clostridium botulinum in cooked, uncured sausage. Appl. Microbiol. 28: 262.
Wagner, M.K., Kraft, A.A., Sebranek, J.G., Rust, R.E., and Amundson, C.M. 1982. Effect of pork belly type on the microbiology of bacon cured with or without potassium sorbate. J. Food Protect. (In press)
Walsh, K.A. and Rose, D. 1956. Factors affecting the oxidation of nitric oxide myoglobin. Agric. Food Chem. 4: 353.
Walters, C.L. and Taylor, A.McM. 1964. Nitrite metabolism by muscle in vitro. Biochim. Biophys. Acta 86: 448.
Watts, B.M. 1954. Oxidative rancidity and discoloration in meat. Adv. Food Res. 5: 1.
Ms received 6/22/81; revised 10/14/81; accepted 10/16/81.

Journal Paper No. J-10293 of the Iowa Agriculture & Home Economics Experiment Station, Ames, IA 50011. Project No. 2175. The authors gratefully acknowledge the support and technical assistance provided by Monsanto, St. Louis, Mo. and Curwood, Inc., New London, WI. The authors acknowledge Sarah Brown, Carol Wysocki, Dave Fish and Dave Kline for their assistance with sample preparation and analysis.

Computer-Assisted Identification of Bacteria on Hot-Boned and Conventionally Processed Beef

C. Y. LEE, D. Y. C. FUNG, and C. L. KASTNER

ABSTRACT

Mesophilic and psychotrophic bacteria (706 isolates) obtained from hot-boned and conventionally processed beef at the time of fabrication and after 14-day vacuum storage at 2°C were analyzed by computer-assisted numerical taxonomy (108 attributes per isolate). There was no significant difference in terms of the number of phenons between hot-boned and conventionally processed beef both at the time of fabrication and after storage. Before storage, 24 mesophilic phenons and 11 psychrotrophic phenons were characterized. After storage in vacuum bags, there were 13 mesophilic phenons and 6 psychrotrophic phenons. The after storage flora consisted mainly of *Streptococcus* and *Lactobacillus* from both hot-boned and conventionally processed beef.

INTRODUCTION

IN CONVENTIONAL meat processing, the carcasses are chilled after slaughter and then fabricated. An alternative procedure, termed hot boning or hot processing, involves fabrication of meat soon after slaughter and before conventional chilling. Hot processing has many potential advantages when compared to conventional processing (Kastner, 1977; Kansas State University, 1980). Since this is a relatively new processing technique, the qualities of hot-boned meat must be ascertained to ensure the acceptability of the product in terms of shelf life and safety.

Due to different processing procedures, the microbiological quality of hot-boned meat may be different from conventionally processed meat. In general, investigators using different hot-boning procedures and experimental animals have reported that hot-boned meat occasionally had higher total bacterial counts and harbored more potential pathogens than conventionally processed meat (Schmidt and Gilbert, 1970, McLeod et al., 1973; Kastner et al., 1976; Fung et al., 1980). Those research studies dealt only with bacterial counts and potential pathogens of hot-boned meat. Little is known about the bacterial profile associated with hot-boned meat. The purpose of this investigation was to characterize the bacteria on hot-boned and conventionally processed beef to elucidate the bacterial profile of these products.

To obtain a reasonable representative profile, we studied a large number of isolates by using miniaturized microbiological techniques and numerical taxonomy to characterize the cultures.

MATERIALS & METHODS

Meat processing

Meat was processed at meat slaughtering facility at the Department of Animal Sciences and Industry at Kansas State University. After five steers were slaughtered, half of each carcass was hot-boned within 2 hr postmortem. The samples were packaged in vacuum bags which were maintained within the range 23.0–26.8 in. Hg and stored at 2°C for 14 days. The initial chilling rate was monitored so that 6 hr was required for chilling the meat surface to 21°C which represents good quality hot-boned beef (Fung et al., 1981). The other half of the carcass was chilled conventionally at 2°C and the temperature decline at the surface of the meat was recorded by a digital thermometer with surface measuring probe. Samples of conventionally processed sides were prepared at 48 hr postmortem and then treated in the same manner as the hot-boned counterparts. Both hot-boned beef and conventionally processed beef were stored at 2°C for 14 days.

Authors Lee, Fung and Kastner are affiliated with the Dept. of Animal Sciences & Industry, Kansas State Univ., Manhattan, KS 66506.

Enumeration and isolation of microorganisms

Mesophilic (32°C for 2 days) and psychrotrophic (7°C for 10 days) standard plate counts were performed at the time of fabrication ("0" time) and after 14 days storage for hot-boned and conventionally processed beef. Then a total of 706 colonies obtained from standard plate count agar plates were isolated randomly. The original plan was to isolate 100 colonies from each of the eight categories listed in Table 1. Due to death of some isolates (9.1%), low number of organisms on meat at "0" time, and the larger number of organisms on meat after storage, and actual numbers of isolates obtained and analyzed are listed in Table 1.

The colonies were purified on Brain Heart Infusion (BHI) agar plates and the purified strains were stored at −20°C in BHI broth containing 50% glycerine as stock cultures. Working cultures were transferred at 6–8 wk intervals from the stock cultures.

Characterizing strains

Each strain was scored in 108 attributes among the following categories: cell character (12 attributes), cell stain (8 attributes), colony character (11 attributes), growth condition (16 attributes), carbohydrate fermentation (22 attributes), biochemical test (18 attributes), special differential agar test (9 attributes), and antibiotics sensitivity test (12 attributes). Unless otherwise specified, all media were inoculated with log-phase culture in BHI broth and were incubated at 32°C for 2 days for studying metabolic activities of mesophile isolates and 21°C for 3 days for studying metabolic activities os psychrotroph isolates. Miniaturized microbiological techniques (Fung and Hartman, 1975) were used for all tests except tests of characterization of cell type, colony morphology, and staining properties.

Procedures and reagents of the biochemical tests were listed in Kovac (1956), Skerman (1969), and Valland (1969). Tests for catalase, oxidase, and growth at various temperatures, pH values, and %NaCl were accomplished using BHI agar as the basal medium. Flagella was stained by Leifson's method (Leifson, 1965). The Methyl Red tests were examined after 5 days incubation. Antibiotic-sensitivity test was scored as sensitive, resistant, or intermediately resistant, according to no growth, growth, and less growth, respectively, when compared to the control.

Table 1—Category of isolated organisms.

Sampling time	Bacteria	Treatment	No. of isolate
"0"-time	Mesophile	HB[a]	63
		CP[b]	64
	Psychrotroph	HB	33
		CP	20
14-day	Mesophile	HB	107
		CP	129
	Psychrotroph	HB	144
		CP	146

[a] Sampling from hot-boned beef
[b] Sampling from conventionally processed beef

Computer analysis

The data comprising 108 attributes scored for 706 strains were divided into four groups: 127 mesophiles and 53 psychrotrophs for the "0" sampling time and 236 mesophiles and 290 psychrotrophs for the 14-day sampling time. The Dice coefficient (Dice, 1945) was used to calculate similarities of organisms. Clustering was by unweighted average linkage (Sneath and Sokal, 1973), from which sorted similarity matrics and dendrograms were constructed by computer program package – DENDROGRAM (Iowa State University, courtesy of P.A. Hartman, Dept. of Microbiology). Twenty-three named, control cultures were included for comparative purposes.

RESULTS & DISCUSSION

DATA OBTAINED in the study were from a university facility which may be somewhat different from that of a commercial processing plant, but will serve as a model for analysis. At the time of fabrication ("0" sampling time), the mesophilic and psychrotrophic counts for hot-boned (HB) beef were log 1.96 Colony Forming Unit (CFU)/cm^2 and 0.8 CFU/cm^2, respectively, and for conventionally processed (CP) beef, log 1.2 CFU/cm^2 and 0.72 CFU/cm^2, respectively. After 14 days of vacuum storage at 2°C

PHENON	IDENTITY	No. of Strains HB	CP
1	Lactobacillus	3	12
2	Leuconostoc	5	0
3	Neisseria	2	1
4	Acinetobacter	3	1
5	Gram + Rod	1	0
6	Moraxella	4	1
7	Micrococcus	5	12
8	Arthrobacter	1	2
9	Pediococcus	0	3
9	Pediococcus	2	0
10	Staphylococcus	2	4
11	Aeromonas	0	1
12	Planococcus	0	1
13	Flavobacterium	0	1
14	Streptococcus	4	8
15	Gram + Coccus	0	6
16	Serratia	0	1
17	Bacillus	11	3
18	Gram - Rod	0	1
4	Acinetobacter	1	0
19	Kurthia	1	0
20	Microbacterium	1	0
21	Pseudomonas	4	0
10	Staphylococcus	14	5

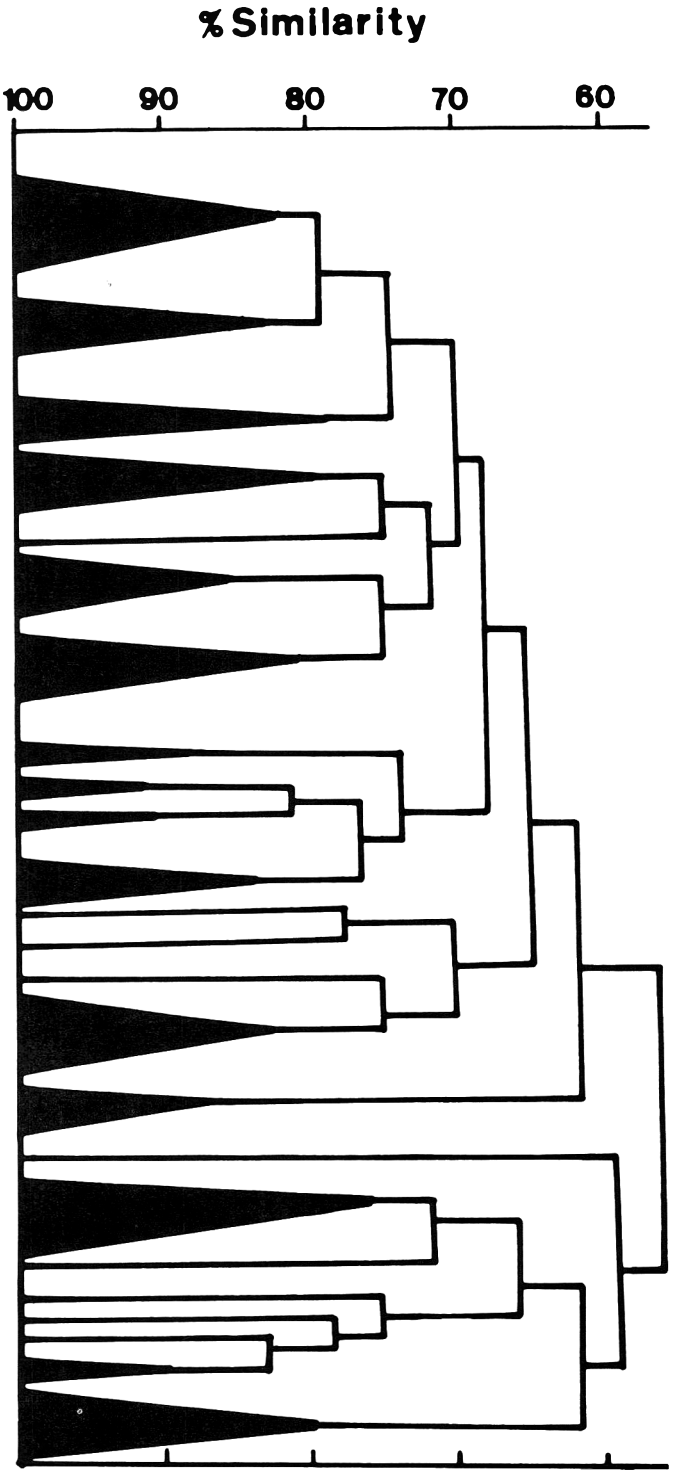


Fig. 1—Simplified dendrogram prepared from mesophilic strains isolated at "0" time.

the mesophilic and psychrotrophic counts for hot-boned beef were log 3.75 CFU/cm^2 and 3.59 CFU/cm^2, respectively and for conventionally processed beef, log 3.13 CFU/cm^2 and 2.92 CFU/cm^2, respectively. There is no significant difference between HB beef and CP beef. These data represented typical counts for properly chilled HB and CP beef (Fung et al., 1981).

The results of numerical taxonomy analysis for four sets of strains (for "0" time mesophiles and psychrotrophs and for 14-day mesophiles and psychrotrophs) are shown in the simplified dendrograms in Fig. 1 to Fig. 4. Each dendrogram shows the numbers and kinds of bacterial genera isolated from HB and CP beef. The phenons were tentatively identified to genus level by matching attributes with known cultures as well as by analyzing the representative strains conventionally according to the diagnostic keys provided by the 8th Edition of *Bergey's manual of Determinative Microbiology* (1974) and special keys including those of

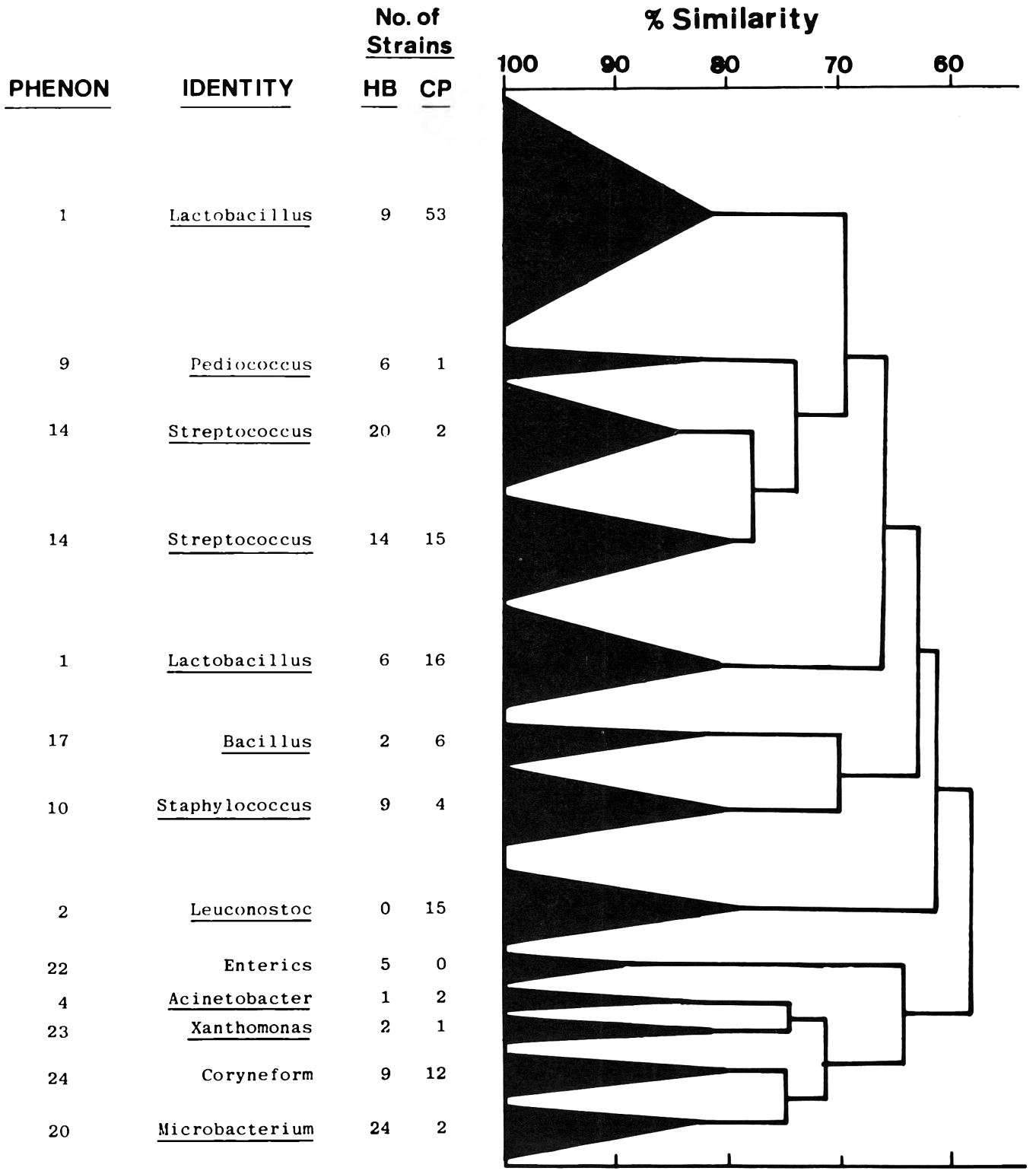
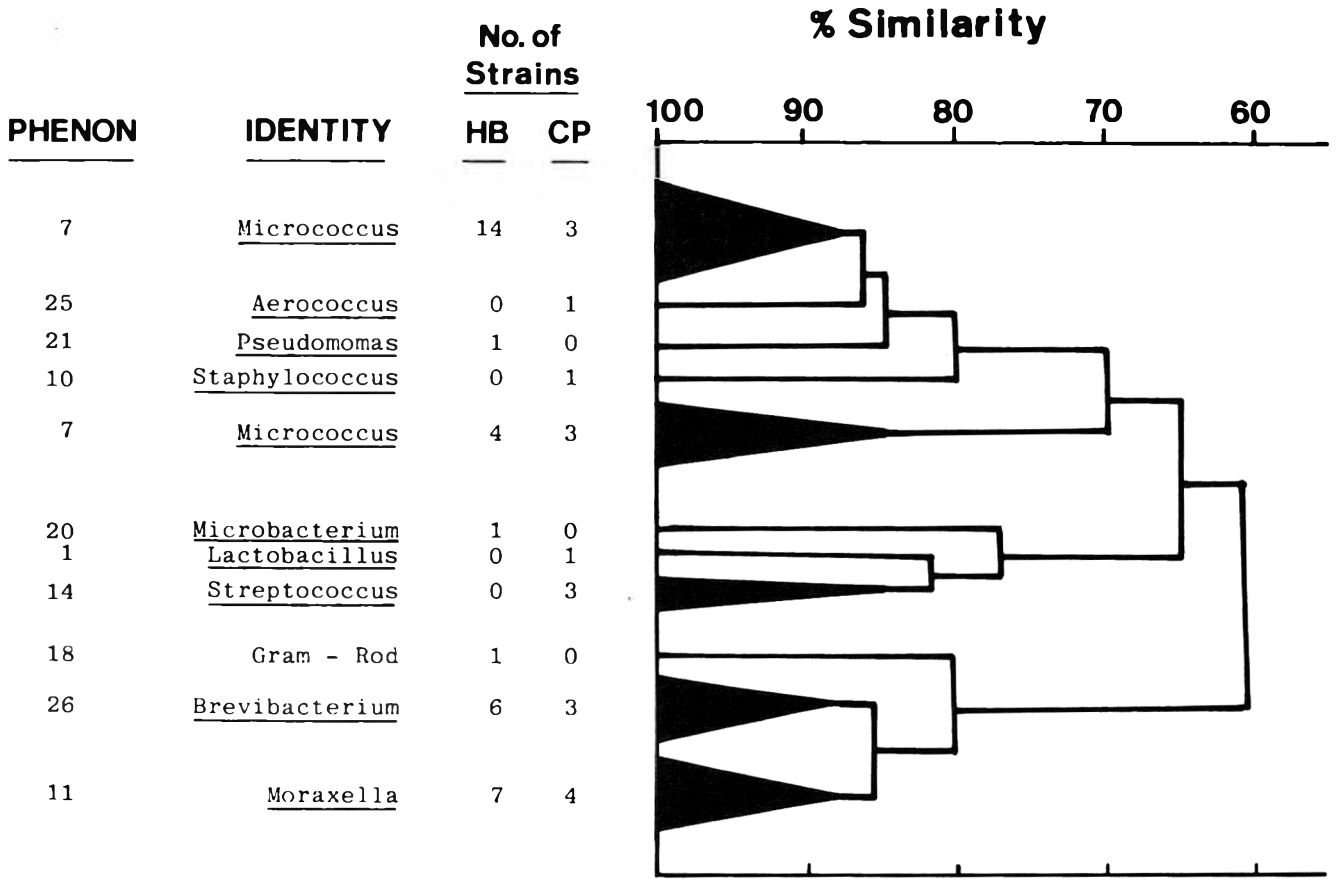
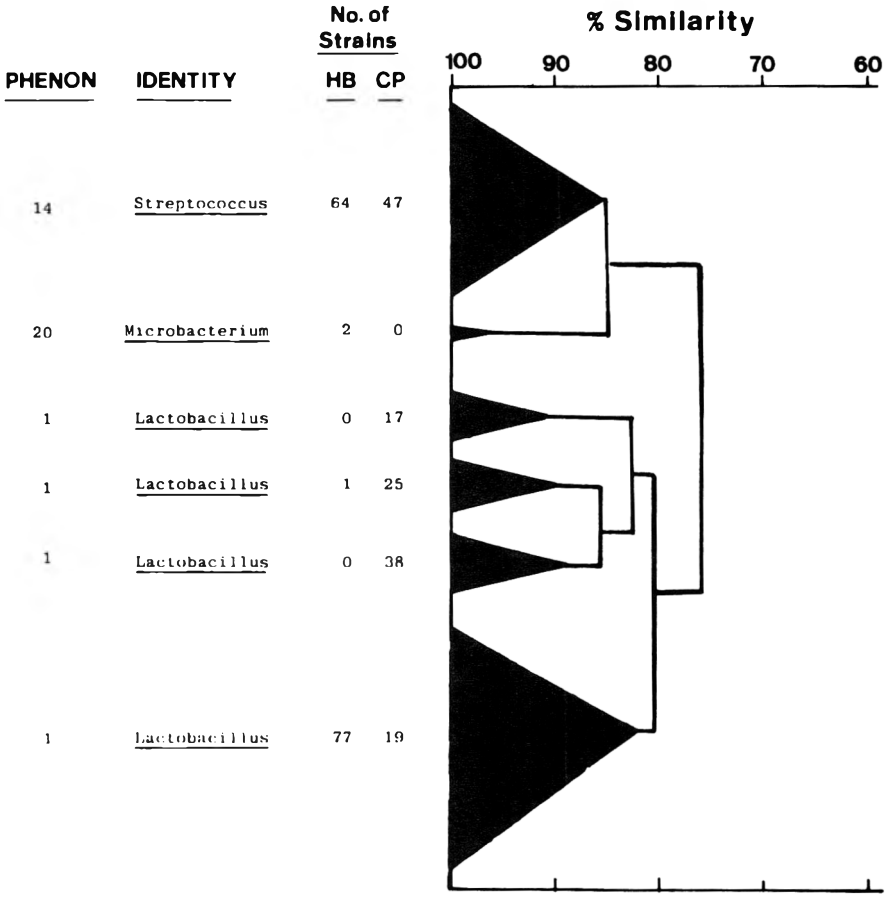


Fig. 2—Simplified dendrogram prepared from mesophilic strains isolated at 14 days storage.



PHENON	IDENTITY	No. of Strains HB	CP
7	Micrococcus	14	3
25	Aerococcus	0	1
21	Pseudomomas	1	0
10	Staphylococcus	0	1
7	Micrococcus	4	3
20	Microbacterium	1	0
1	Lactobacillus	0	1
14	Streptococcus	0	3
18	Gram - Rod	1	0
26	Brevibacterium	6	3
11	Moraxella	7	4

Fig. 3—Simplified dendrogram prepared from psychrotrophic strains isolated at "0" time.



PHENON	IDENTITY	No. of Strains HB	CP
14	Streptococcus	64	47
20	Microbacterium	2	0
1	Lactobacillus	0	17
1	Lactobacillus	1	25
1	Lactobacillus	0	38
1	Lactobacillus	77	19

Fig. 4—Simplified dendrogram prepared from psychrotrophic strains isolated at 14 days storage.

Jones (1975), Shewan et al. (1960), Gibbs and Shapton (1968), and Skerman (1967). In order to differentiate between the genera *Staphylococcus* and *Micrococcus*, and between *Streptococcus, Leuconostoc* and *Pediococcus*, some of the additional tests such as glucose fermentation (hetero- or homo-) and oxidation tests, and hemolysis of blood were performed by conventional methods.

The distribution and change of characterized phenons in each variable are shown in Table 2. There were 17 mesophilic phenons found on both the HB and CP beef at the time of fabrication ("0" sampling time). After 14 days of vacuum storage, the mesophilic phenons for both HB and CP beef decreased to 12 phenons. The psychrotrophic phenons were 7 and 8 before storage for HB and CP beef, respectively. After storage, the phenons decreased to 4 and 5, respectively. The mesophilic flora at the time of fabrication were heterogeneous. *Bacillus* and *Staphylococcus* were more predominant in HB beef, where *Micrococcus* and *Lactobacillus* were predominant in CP beef. The difference in flora at "0" time of HB and CP beef was probably due to the fact that CP beef was chilled aerobically at 2°C for 48 hr, thus allowing growth of psychrotrophic flora and retarding of mesophiles. The low temperature and low a_w of CP beef due to evaporation at meat surface probably favored the growth of *Lactobacillus* and *Micrococcus*. The heterogeneous mesophilic bacteria population at fabrication likely resulted from the contaminated animal hide and gastrointestinal tract, and the environment during slaughter. After storage, *Microbacterium* and *Streptococcus* were the major strains on HB beef, whereas in CP beef *Lactobacillus* dominated. The different microbial flora at the time of vacuum packaging subsequently affected the flora after storage.

Table 2—Percentage of bacteria at "0"-time and after 14-day storage hot-boned beef and conventionally processed beef

	Mesophile				Psychrotroph			
	0-time		14-day		0-time		14-day	
Phenon[a]	HB[b]	CP[c]	HB	CP	HB	CP	HB	CP
Acinetobacter (4)	6	2	1	2	0	0	0	0
Aerococcus (25)	0	0	0	0	0	5	0	0
Aeromonas (11)	0	2	0	0	0	0	0	0
Arthrobacter (8)	2	3	0	0	0	0	0	0
Bacillus (17)	17	5	2	5	0	0	0	0
Brevibacterium (26)	0	0	0	0	18	16	0	0
Coryneform (24)	0	0	8	9	0	0	0	0
Enterics (22)	0	0	5	0	0	0	0	0
Flavobacterium (13)	0	2	0	0	0	0	0	0
Kurthia (19)	2	0	0	0	0	0	0	0
Lactobacillus (1)	5	19	14	54	0	5	54	68
Leuconostoc (2)	8	0	0	12	0	0	0	0
Microbacterium (20)	2	0	22	2	3	0	1	0
Micrococcus (7)	8	19	0	0	53	32	0	0
Moraxella (6)	6	2	0	0	21	21	0	0
Neisseria (3)	3	2	0	0	0	0	0	0
Pediococcus (9)	3	5	6	1	0	0	0	0
Planococcus (12)	0	2	0	0	0	0	0	0
Pseudomonas (21)	6	0	0	0	3	0	0	0
Serratia (16)	0	2	0	0	0	0	0	0
Staphylococcus (10)	25	13	8	3	0	5	0	0
Streptococcus (14)	6	12	32	13	0	16	44	32
Xanthomonas (23)	0	0	2	1	0	0	0	0
Gram + Rod (5)	2	0	0	0	0	0	0	0
Gram − Rod (18)	0	2	0	0	3	0	0	0
Gram + Coccus (15)	0	10	0	0	0	0	0	0
Total percentage[d]	100	100	100	100	100	100	100	100

[a] The number in parenthesis corresponds to the phenon number appearing in Fig. 1—4; these numbers are arbitrarily assigned for ease of identification.
[b,c] The same legend as in Table 1.
[d] Due to rounding off, the total percentage does not add up to be 100%.

In psychrotrophs, *Micrococcus* that could survive at cold temperatures under aerobic conditions was the main organism found at the time of fabrication for both HB and CP beef. After storage, bacterial isolates from both CP and HB beef were exclusively *Lactobacillus* and *Streptococcus*. *Lactobacillus* apparently produced an antimicrobial agent and inhibited other species during storage (Gilliland and Speck, 1972, 1975; Roth and Clark, 1975).

Pseudomonas, the major aerobic spoilage microorganism of fresh meat, comprised only a small portion of the population at the time of fabrication. None was found after storage because they cannot grow anaerobically. Enterics and Gram negative rods were found in small numbers or were not detected at the time of fabrication and after storage. The fast initial chilling rate may have killed 90% of these bacteria by the effect of cold shock (Davis, 1980).

Fung et al. (1981) reported that proper initial chilling rate, namely meat chilled to 21°C within 9 hr was required for producing good quality hot-boned beef. In this study, we chilled HB beef rapidly (to 21°C within 6 hr), therefore, the psychrotrophic microbial flora did not differ much from those of CP beef. Although we found some difference in mesophilic flora before and after storage, this should not significantly affect the quality of cold stored HB beef since psychrotrophs play a more important role in this environment than mesophiles.

In conclusion, we have obtained the mesophilic and psychrotrophic bacterial profiles of HB beef and CP beef chilled at reasonably fast rates. Both HB and CP beef consisted of heterogenous bacterial flora at the time of fabrication, but consisted mainly of *Streptococcus* and *Lactobacillus* after 14-day storage. The fast chilling rate of HB beef used in these experiments probably accounted for low bacterial counts of HB beef and similar bacterial profiles of HB beef and CP beef after storage. The results of this study shows the HB beef is as safe as CP beef under our experimental condition. We are investigating similar profiles from HB meat chilled at slow and intermediate rates and will compare these to CP beef.

REFERENCES

Buchanan, R.E. and Gibbons, N.E. (Ed.) 1974. "Bergey's Manual of Determinative Bacteriology," 8th ed. The Williams and Wilkins Co., Baltimore, MD.

Davis, B.D. 1980. Bacterial physiology. In "Microbiology," 3rd ed. Ed. B.D. Davis, R. Dulbecco, H.N. Eisen, and H.G. Ginsberg, p. 62. Harper and Row, New York.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26: 297.

Fung, D.Y.C. and Hartman, P.A. 1975. Miniaturized microbiological techniques for rapid characterization of bacteria, Chap. 21. In "New Approaches to the Identification of Microorganisms," Ed. C.-G. Heden and T. Illeni. John Wiley and Son, New York.

Fung, D.Y.C., Kastner, C.L., Hunt, M.C., Dikeman, M.E., and Kropf, D.H. 1980. Mesophilic and psychrotrophic populations on hot-boned and conventionally processed beef. J. Fd. Protect. 43: 547.

Fung, D.Y.C., Kastner, C.L., Lee, C.Y., Hunt, M.C., Dikeman, M.E. and Kropf, D.H. 1981. Initial chilling rate effects on bacterial growth of hot-boned beef. J. Fd. Protect. 44: 539.

Gibbs, B.M. and Shapton, D.A. 1968. "Identification Methods for Microbiologists," Part A and B. Academic Press, London.

Gilliland, S.E. and Speck, M.L. 1972. Interactions of food starter cultures and food borne pathogens: Lactic streptococci versus staphylococci and salmonellae. J. Milk Fd. Technol. 35: 307.

Gilliland, S.E. and Speck, M.L. 1975. Inhibition of psychrotrophic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. J. Food Sci. 40: 903.

Jones, D. 1975. A numerical taxonomic study of coryneforms and related organisms. J. Gen. Microbiol. 87: 52.

Kansas State University. 1980. Economic feasibility of hot processing beef carcasses. Kansas Agric. Exp. Sta. Bull. No. 639. December, 1980.

Kastner, C.L. 1977. Hot processing-update on potential energy and related economics. Proceeding Meat Industry Conference, Chicago. March 22—24, 1977. Univ. of Chicago.

Kastner, C.L., Luedecke, L.D., and Russell, T.S. 1976. A comparison of microbial counts on conventionally and hot-boned bovine carcasses. J. Milk Fd. Technol. 39: 684.

Kovac, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature 74: 703.

—Continued on page 373

Effect of Time-on-Feed on the Palatability of Rib Steaks from Steers and Heifers

H. G. DOLEZAL, G. C. SMITH, J. W. SAVELL, and Z. L. CARPENTER

ABSTRACT

Rib steaks from steers (n=326) and heifers (n=68) that were either grass-fed or fed high-concentrate diets for 30–230 days were used in palatability studies. Extending time-on-feed beyond 100 days (steers) or 90 days (heifers) provided little additional palatability assurance. Within time-on-feed strata from 100 through 230 days, few differences in palatability were found between rib steaks from carcasses of different USDA quality grades. The minimum marbling requirement for the U.S. Choice grade could be lowered with no appreciable loss in palatability if the stipulation was made that cattle had been fed a high-concentrate diet for at least 90 days.

INTRODUCTION

McBee and Wiles (1967) reported strong relationships between USDA quality grades and tenderness, juiciness and flavor of cooked beef. Conversely, Tuma et al. (1962), Romans et al. (1965), Breidenstein et al. (1968) and Parrish et al. (1973) concluded that USDA quality grades were not significantly related to cooked beef tenderness. Additional investigations have found that USDA quality grades provide little assurance that beef will be palatable (Campion et al., 1975; Berry et al., 1974; Garcia-de-Siles et al., 1977; Tatum et al., 1980). There is increasing concern within the industry that present USDA quality grades unnecessarily emphasize marbling and–as a result–encourage over-fattening of cattle and carcasses (NCA, 1981).

The length of time that cattle have been fed a high-energy diet ("time-on-feed") has been proposed as an adjunct to or substitute for use of marbling (intramuscular fatness) for predicting cooked beef palatability. Adams et al. (1977) and Harrison et al. (1978) suggested that beef from cattle that have been fed a high-energy diet for a specified period of time will be acceptable in palatability regardless of marbling amounts or quality grades. Other researchers have reported that once cattle have been fed for a certain period of time on a high-concentrate diet, little additional benefit in ultimate cooked beef palatability is attained by extending the feeding period (Epley et al., 1968; Zinn et al., 1970b; Campion et al., 1975; Tatum et al., 1980).

The purpose of the present study was to further investigate the effectiveness of using time-on-feed to predict the ultimate eating satisfaction of cooked beef from steers and heifers and to identify a point in high-concentrate feeding time beyond which additional time-on-feed does little to further enhance cooked beef palatability.

EXPERIMENTAL PROCEDURE

STEERS (n=326) and heifers (n=68) that had never been fed grain or that had been fed grain for 30–230 days were obtained from a number of sources. The experimental design (Table 1) describes the class designations and diet energy levels of each time-on-feed group.

All authors were affiliated with the Meats & Muscle Biology Section, Dept. of Animal Science, Texas Agricultural Experiment Station, Texas A&M Univ., College Station, TX 77843 at the time this study was conducted. Author Dolezal is presently employed by the Dept. of Animal Sciences, Colorado State Univ., Fort Collins, CO 80523.

Included in the sample population were cattle of Brahman, British (Shorthorn, Angus, Hereford), Continental European (Charolais, Maine-Anjou, Simmental, Limousin), dairy (Holstein, Jersey, Brown Swiss) and Longhorn breeding and crosses of most of these breeds; cattle of multiple breed origin were included in every group in Table 1. The grass-fed cattle (group 1 and group 10) were from two sources (La. Agr. Expt. Sta., Homer, LA; Texas Agr. Expt. Sta., Overton, TX) and were maintained for 12–18 months post-weaning on millet-bermuda grass or coastal bermuda grass pastures until the time of slaughter. The grain-fed cattle (groups 2, 3, 4, 5, 6, 7, 8, 9, 11 and 12) were from five sources (La. Agr. Expt. Sta., Homer, LA; Texas Agr. Expt. Sta., Overton, TX; Miller Feedyard, LaSalle, CO; Monfort of Colorado, Gilchrest, CO; Harrell Cattle Company, Gonzales, TX) and were fed high-concentrate finishing diets of generally similar energy-density for the designated periods of time (Table 1).

Upon the termination of each feeding period the cattle were slaughtered in the conventional manner. Approximately 24 hr postmortem, yield and quality grade (USDA, 1975) data were obtained.

Following a 14- to 16-day postmortem aging period (2±1°C), two steaks (3.2 cm thick) were obtained from the 9th through 11th rib section of each of the 394 wholesale ribs. Steaks were wrapped in polyethylene-coated freezer paper, frozen at −34°C, stored at −20°C, thawed (2°C) and broiled on Farberware Open-Hearth broilers to an internal temperature of 70°C. Samples from one cooked steak (9th rib) from each animal were served, while warm, to an experienced 8-member sensory panel for evaluations of palatability using 8-point descriptive scales (8=extremely juicy, extremely tender, no detectable connective tissue, extremely desirable flavor and extremely desirable overall palatability; 1=extremely dry, extremely tough, abundant connective tissue, extremely undesirable flavor and extremely undesirable overall palatability). The second steak (10th rib) was cooked to 70°C, cooled to room temperature (23°C) and then cored (1.27 cm) for Warner-Bratzler shear force determinations.

Data were analyzed using analysis of variance (Snedecor and Cochran, 1967) and mean separation (Duncan, 1955; Kramer, 1956) with the use of the Statistical Analysis System package (Barr et al., 1979).

Table 1–Class designations and diet energy levels characteristic of each time-on-feed group.

Group	Sex class	Age class[a]	N	Time-on -feed (days)	Diet energy level[b] NEm, Mcal/kg	NEg Mcal/kg
1	Steer	Yearling	39	0	–	–
2	Steer	Yearling	29	30	1.54	.98
3	Steer	Yearling	20	60	1.58	.85
4	Steer	Yearling	38	90	1.57	.89
5	Steer	Yearling	40	100	1.52	.95
6	Steer	Yearling	43	130	1.52	.96
7	Steer	Yearling	45	160	1.52	.96
8	Steer	Calf	37	200	1.47	.96
9	Steer	Calf	35	230	1.47	.96
10	Heifer	Yearling	13	0	–	–
11	Heifer	Yearling	15	90	1.56	.93
12	Heifer	Calf	39	200	1.47	.96

[a] Age class at the start of the feeding treatment.
[b] Expressed on a 100% dry matter basis; values for diet energy levels were calculated from actual diets fed to each group of animals using values from NRC (1976).

RESULTS & DISCUSSION

MEAN, minimum and maximum values for certain live and carcass characteristics and palatability attributes for steers and heifers are presented in Table 2. Carcasses ranged in USDA quality grade from Standard to Prime for both sex-classes and in USDA yield grade from 0.0–5.3 (steers) and 1.2–3.8 (heifers). Among palatability attributes, values for shear force, tenderness ratings (myofibrillar and overall) and overall palatability rating were the most variable for steaks for both sex classes.

As time-on-feed is extended, there are increases in marbling score and quality grade (Zinn et al., 1970a; Campion et al., 1975; Harrison et al., 1978; Schroeder et al., 1980; Tatum et al., 1980), fat thickness (Campion et al., 1975; Harrison et al., 1978; Schroeder et al., 1980; Tatum et al., 1980) and numerical yield grade (Harrison et al., 1978; Schroeder et al., 1980; Tatum et al., 1980). In this study (Table 3), marbling score, quality grade and most of the yield grade factors generally increased as time-on-feed increased but these increases were not always directionally consistent or statistically significant. Grass-fed steer carcasses had the lowest numerical values for fat thickness and longissimus muscle area; grass-fed heifer carcasses had lower ($P<0.05$) values for fat thickness, longissimus muscle area, carcass weight and numerical yield grade than did carcasses from heifers fed grain for either 90 or 200 days. Steers fed for 200 days had the highest marbling score and quality grade. For heifers, marbling score and quality grade increased ($P<0.05$) with each successive increase in time-on-feed (0, 90 and 200 days) which agrees with Zinn et al. (1970a).

—Continued on next page

Table 2—Mean, minimum and maximum values for certain live and carcass characteristics and palatability attributes for steers and heifers

Trait	Steers (n=326)			Heifers (n=68)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Live characteristic						
Chronological age, days	521.4	440.0	638.0	596.1	576.0	627.0
Frame size score[a]	5.0	1.0	9.0	4.8	2.0	8.0
Muscling score[b]	4.8	1.0	9.0	4.8	2.0	7.0
Slaughter weight, kg	515.3	319.8	767.5	412.4	315.2	521.2
Carcass characteristic						
USDA maturity score[c]	A^{45}	A^{10}	B^{99}	A^{48}	A^{25}	B^{25}
USDA marbling score[d]	Sl^{68}	PD^{00}	Ab^{10}	Sm^{20}	PD^{50}	Ab^{20}
USDA quality grade[e]	Gd^{51}	St^{00}	Pr^{70}	Gd^{81}	St^{25}	Pr^{73}
Fat thickness, mm	9.44	0.0	30.5	10.8	2.5	22.9
Longissimus muscle area, cm^2	75.5	41.9	110.3	71.7	53.5	92.2
Estimated kidney, pelvic and heart fat, %	1.9	0.5	3.5	2.4	1.0	4.5
Carcass weight, kg	287.6	135.2	477.2	255.7	167.8	316.2
USDA yield grade	2.4	0.0	5.3	2.6	1.2	3.8
Palatability attribute						
Juiciness[f]	5.0	3.1	6.9	5.0	3.4	6.0
Myofibrillar tenderness[g]	5.6	1.9	7.7	6.2	3.6	7.6
Connective tissue amount[h]	6.7	4.0	7.9	7.1	5.6	7.8
Overall tenderness[g]	5.5	1.8	7.7	6.1	3.5	7.4
Flavor desirability[i]	5.5	3.4	7.0	5.7	3.5	7.0
Overall palatability[i]	5.2	1.9	7.3	5.6	3.8	6.9
Shear force, kg	5.0	2.3	16.0	3.7	2.4	7.4

[a] 9=small-minus; 5=medium-typical; 1=large-plus.
[b] 9=thin-minus; 5=medium-typical; 1=thick-plus.
[c] A^{00} to A^{100} in maturity score represents a range in chronological age from about 9 months to about 30 months; B^{00} to B^{100}=about 30 months to about 42 months.
[d] Marbling score: Ab=abundant: Sm=small; Sl=slight; PD=practically devoid.
[e] Quality grade: Pr=Prime; Ch=Choice; Gd=Good; St=Standard (USDA, 1975).
[f] 8=extremely juicy; 1=extremely dry.
[g] 8=extremely tender; 1 = extremely tough.
[h] 8=none; 1=abundant.
[i] 8=extremely desirable; 1=extremely undesirable.

Table 3—Mean values for steer and heifer carcass characteristics stratified according to time-on-feed

Item	Steers									Heifers		
	Time-on-feed (days)									Time-on-feed (days)		
	0	30	60	90	100	130	160	200	230	0	90	200
Number of observations	39	29	20	38	40	43	45	37	35	13	15	39
Carcass characteristic												
USDA maturity score[a]	A^{37de}	A^{44ef}	A^{37de}	A^{41def}	A^{50fg}	A^{54gh}	A^{59h}	A^{33d}	A^{43ef}	A^{48d}	A^{51d}	A^{57d}
USDA marbling score[b]	Tr^{19g}	Sl^{06f}	PD^{72g}	Sl^{09f}	Sl^{95e}	Sm^{40de}	Sm^{42de}	Sm^{85d}	Sm^{22e}	Tr^{59f}	Sl^{57e}	Sm^{02d}
USDA quality grade[c]	St^{62g}	Gd^{13f}	St^{36g}	Gd^{20f}	Gd^{72e}	Gd^{89de}	Gd^{92de}	Ch^{16d}	Gd^{85de}	St^{84f}	Gd^{56e}	Ch^{25d}
Fat thickness, mm	2.12^{d}	7.31^{e}	2.22^{d}	6.88^{e}	10.64^{f}	11.34^{fg}	13.01^{gh}	12.97^{gh}	14.19^{h}	5.17^{d}	12.19^{e}	12.21^{e}
Longissimus muscle area, cm^2	64.70^{f}	70.10^{ef}	64.76^{ef}	70.22^{e}	81.32^{d}	81.00^{d}	79.78^{d}	81.95^{d}	78.41^{d}	63.39^{e}	72.93^{d}	74.29^{d}
Estimated kidney, pelvic and heart fat, %	1.21^{d}	2.40^{g}	1.00^{d}	1.74^{e}	2.00^{f}	2.07^{f}	2.37^{g}	2.07^{f}	2.17^{fg}	1.71^{d}	3.17^{f}	2.44^{e}
Carcass weight, kg	198.16^{f}	260.43^{e}	197.13^{f}	249.91^{e}	321.40^{d}	322.70^{d}	330.00^{d}	325.41^{d}	326.46^{d}	203.11^{e}	256.90^{d}	270.70^{d}
USDA yield grade	1.36^{d}	2.36^{ef}	1.32^{d}	2.08^{e}	2.58^{fg}	2.61^{fg}	3.03^{h}	2.89^{gh}	3.13^{h}	1.86^{d}	2.89^{e}	2.81^{e}

[a] A^{00} to A^{100} in maturity score represents a range in chronological age from about 9 months to about 30 months.
[b] Marbling score: Sm=small; Sl=slight; Tr=traces; PD=practically devoid.
[c] Quality grade: Ch=Choice; Gd=Good; St=Standard (USDA, 1975).
[d,e,f,g,h] Means in the same row and for the same sex class bearing a common superscript letter are not significantly ($P>0.05$) different.

Cattle fed grain have advantages over those fed grass in tenderness (Bowling et al., 1977; Harrison et al., 1978; Leander et al., 1978), flavor desirability (Bowling et al., 1977; Harrison et al., 1978), and overall palatability and shear force (Bowling et al., 1977). As cattle are fed grain for longer periods of time before slaughter, there is a resulting increase in tenderness (up to 139 days, Epley et al., 1968; up to 150 and 180 days, Zinn et al., 1970b). In the present study (Table 4) steers fed grain for 100 days or longer produced steaks with higher ($P<0.05$) ratings for tenderness and overall palatability and lower ($P<0.05$) values for shear force than did steers fed 90 days or less; grain-feeding for 130 days or longer resulted in the highest ($P<0.05$) flavor desirability ratings for steer beef, as was previously reported by Tatum et al. (1980). Steaks from heifers fed grain for 90 days were less ($P<0.05$) desirable in juiciness and shear force than either grass-fed beef or beef from heifers fed grain for 200 days. Steaks from heifers fed grain for 200 days had higher ($P<0.05$) ratings for flavor desirability and overall palatability than steaks from grass-fed heifers. Grass-fed steers and heifers produced steaks with lower ($P<0.05$) flavor ratings and numerically lower overall palatability ratings than did their grain-fed counterparts. Steers fed grain for 100 days produced steaks that were as palatable as those from steers fed 130, 160, 200 or 230 days, heifers fed grain for 90 days produced steaks that were as palatable as those from heifers fed 200 days; thus, extending time-on-feed beyond 100 days for steers or 90 days for heifers provided little additional assurance of eating satisfaction.

For grass-fed cattle, 36% (steer) and 43% (heifer) of steaks were "undesirable" in flavor and 49% (steer) and 43% (heifer) of steaks were "undesirable" in overall palatability (Table 5). More than 90% of the steaks from steers fed grain for 100 days or longer or from heifers fed grain for 90 or 200 days received at least "desirable" ratings for overall tenderness, flavor desirability and overall palatability. Tatum et al. (1980) concluded that increasing time-on-feed beyond 100 days did not substantially reduce the incidence of "undesirable" ratings for flavor, tenderness or overall palatability.

Mean values for palatability attributes of steaks from steer and heifer carcasses stratified by time-on-feed within USDA quality grade are presented in Table 6. Within Prime,

Table 4—Mean values for palatability attributes of steaks from steer carcasses stratified by time-on-feed

Sex class	Time-on-feed (days)	Number of observations	Sensory panel rating						Shear force, kg
			Juiciness[a]	Myofibrillar tenderness[b]	Connective tissue amount[c]	Overall tenderness[b]	Flavor desirability[d]	Overall palatability[d]	
Steer	0	39	5.14^{e}	5.07^{gh}	6.15^{i}	4.77^{gh}	4.73^{h}	4.42^{g}	8.25^{g}
Steer	30	29	4.54^{g}	4.62^{h}	6.26^{hi}	4.56^{h}	5.35^{fg}	4.53^{fg}	6.42^{f}
Steer	60	20	4.61^{fg}	5.07^{gh}	6.35^{hi}	5.06^{gh}	5.11^{g}	4.75^{fg}	5.36^{f}
Steer	90	38	4.96^{ef}	5.29^{g}	6.50^{gh}	5.19^{g}	5.27^{fg}	4.85^{f}	5.64^{f}
Steer	100	40	5.15^{e}	6.04^{ef}	6.93^{ef}	5.97^{ef}	5.51^{f}	5.56^{e}	4.31^{e}
Steer	130	43	4.90^{ef}	5.82^{f}	6.68^{fg}	5.72^{f}	5.87^{e}	5.58^{e}	4.34^{e}
Steer	160	45	5.16^{e}	5.90^{f}	6.77^{f}	5.76^{f}	5.86^{e}	5.56^{e}	4.21^{e}
Steer	200	37	4.97^{ef}	5.90^{f}	6.90^{ef}	5.78^{f}	5.90^{e}	5.61^{e}	3.85^{e}
Steer	230	35	4.92^{ef}	6.40^{e}	7.09^{e}	6.36^{e}	5.93^{e}	5.93^{e}	3.55^{e}
Heifer	0	13	5.06^{e}	6.22^{e}	7.12^{ef}	6.20^{e}	4.68^{f}	5.02^{f}	3.69^{e}
Heifer	90	15	4.65^{f}	6.11^{e}	7.87^{f}	5.96^{e}	5.74^{e}	5.47^{ef}	4.89^{f}
Heifer	200	39	5.15^{e}	6.20^{e}	7.18^{e}	6.19^{e}	5.97^{e}	5.83^{e}	3.30^{e}

[a] 8=extremely juicy; 1=extremely dry.
[b] 8=extremely tender; 1=extremely tough.
[c] 8=none; 1=abundant.
[d] 8=extremely desirable; 1=extremely undesirable.
[e,f,g,h,i] Means in the same column and for the same sex class bearing a common superscript letter are not significantly ($P>0.05$) different.

Table 5—Frequency percentages of steaks from steer and heifer carcasses within each of three levels of overall tenderness, flavor desirability and overall palatability stratified according to time-on-feed

Item	Steers									Heifers		
	Time-on-feed (days)									Time-on-feed (days)		
	0	30	60	90	100	130	160	200	230	0	90	200
Number of observations	39	29	20	38	40	43	45	37	35	13	15	39
Overall tenderness[a]												
"Very desirable"	33.3	13.8	10.0	31.6	55.0	41.8	46.7	40.5	80.0	71.4	60.0	76.9
"Desirable"	25.7	34.5	80.0	36.8	45.0	51.2	53.3	54.1	20.0	21.4	33.3	23.1
"Undesirable"	41.0	51.7	10.0	31.6	–	7.0	–	5.4	–	7.2	6.7	–
Flavor desirability[a]												
"Very desirable"	–	3.4	10.0	15.8	22.5	48.8	57.8	48.6	42.9	–	33.3	53.8
"Desirable"	64.1	89.7	80.0	63.2	72.5	51.2	42.2	51.4	57.1	57.1	66.7	46.2
"Undesirable"	35.9	6.9	10.0	21.0	5.0	–	–	–	–	42.9	–	–
Overall palatability[a]												
"Very desirable"	7.7	–	5.0	15.8	37.5	34.9	33.3	40.5	48.6	14.3	26.7	46.2
"Desirable"	43.6	58.6	65.0	47.4	55.0	55.8	60.0	54.1	48.6	42.9	66.7	48.7
"Undesirable"	48.7	41.4	30.0	36.8	7.5	9.3	6.7	5.4	2.8	42.8	6.6	5.1

[a] "Very desirable"=mean sensory panel ratings of 6.00 or higher; "Desirable"=mean sensory panel ratings of 4.50—5.99; "Undesirable"=mean sensory panel ratings lower than 4.50.

no differences ($P>0.05$) in any of the palatability attributes were attributable to differences in time-on-feed of the cattle that produced Prime beef. Admittedly, the numbers of observations in certain grade X time-on-feed cells in Table 6 are very limited but there is some evidence that beef from steers fed forage only and from those fed grain for 30 days and graded Choice or Good is deficient in tenderness and overall palatability in comparison to beef from steers fed grain for 130 days or longer that graded Choice or Good, respectively. Steaks from heifers fed 200 days that graded Choice received higher ($P<0.05$) ratings for all sensory panel characteristics and had lower ($P<0.05$) shear force values than steaks from carcasses grading Choice from heifers fed grain for 90 days. Within the Good grade, steaks from heifers fed 0 days on grain were scored the lowest ($P<0.05$) in flavor desirability but did not differ in any other sensory attribute.

Mean values for palatability attributes of steaks from steer and heifer carcasses stratified by USDA quality grade within time-on-feed are presented in Table 7. If grade was more important than feeding history in determining palatability of cooked beef in this population of rib steaks, the predominance of comparisons of palatability ratings and shear force values between grades (within time-on-feed strata) should be significantly different; such was not the case. There are 259 possible comparisons of ratings or values (between grades within time-on-feed strata) in Table 7; higher grading beef (e.g., Prime in a Prime vs Choice comparison) was significantly more palatable than lower grading beef (e.g., Good in a Choice vs Good comparison) in only 24 of those 259 comparisons. For overall palatability, Prime had higher ($P<0.05$) ratings than Choice in 0% (0 of 4) time-on-feed strata; comparable percentages were 25% (1 of 4) for Prime vs Good, 50% (1 of 2) for Prime vs. Standard, 20% (2 of 10) for Choice vs Good, 37.5% (3 of 8) for Choice vs Standard and 11.1% (1 of 9) for Good vs Standard. In a composite of all comparisons between grades within time-on-feed strata, higher grading carcasses produced rib steaks with higher ($P<0.05$) palatability ratings or lower ($P<0.05$) shear force values 9.3% (24 of 259 comparisons) of the time and with higher ($P<0.05$) overall palatability ratings 21.6% (8 of 37 comparisons) of the time. Within the 100, 130, 160, 200 and 230 day time-on-feed strata for both steers and heifers (200 days only), few (16 of 168 possible comparisons of ratings or values) significant differences in palatability were found between rib steaks from carcasses of different USDA quality grades. Similar results were reported by Tatum et al. (1980) who concluded

Table 6—Mean values for palatability attributes of steaks from steer and heifer carcasses stratified by time-on-feed within USDA quality grade

Sex class	USDA quality grade	Time-on-feed (days)	N	Sensory panel rating						Shear force, kg
				Juiciness[a]	Myofibrillar tenderness[b]	Connective tissue amount[c]	Overall tenderness[b]	Flavor desirability[d]	Overall palatability[d]	
Steer	Prime	130	2	5.60^{e}	6.75^{e}	7.25^{e}	6.60^{e}	6.40^{e}	6.50^{e}	3.63^{e}
Steer	Prime	160	2	5.60^{e}	5.40^{e}	6.60^{e}	5.40^{e}	6.45^{e}	5.70^{e}	5.22^{e}
Steer	Prime	200	1	5.80^{e}	4.90^{e}	6.10^{e}	4.60^{e}	5.00^{e}	5.00^{e}	4.83^{e}
Steer	Choice	0	1	4.80^{e}	1.90^{h}	4.90^{g}	1.80^{h}	3.55^{g}	2.00^{h}	12.24^{h}
Steer	Choice	30	2	4.40^{e}	4.60^{g}	6.55^{f}	4.70^{g}	5.70^{ef}	4.60^{g}	6.60^{g}
Steer	Choice	90	4	5.05^{e}	5.70^{fg}	6.88^{ef}	5.75^{fg}	5.33^{f}	5.33^{fg}	4.50^{f}
Steer	Choice	100	20	5.32^{e}	6.12^{ef}	6.93^{ef}	6.09^{ef}	5.49^{f}	5.65^{fg}	4.04^{f}
Steer	Choice	130	22	5.11^{e}	6.05^{f}	6.68^{f}	5.98^{f}	5.99^{e}	5.84^{ef}	4.04^{f}
Steer	Choice	160	25	5.24^{e}	5.97^{f}	6.72^{f}	5.78^{fg}	5.90^{e}	5.58^{fg}	3.91^{f}
Steer	Choice	200	26	5.05^{e}	5.95^{f}	6.94^{ef}	5.85^{fg}	5.96^{e}	5.70^{f}	3.87^{f}
Steer	Choice	230	20	5.18^{e}	6.57^{e}	7.18^{e}	6.51^{e}	6.02^{e}	6.11^{e}	3.38^{e}
Steer	Good	0	3	4.70^{e}	6.07^{e}	6.73^{ef}	5.83^{e}	4.70^{f}	4.80^{ef}	10.71^{h}
Steer	Good	30	13	4.84^{e}	4.62^{f}	6.26^{f}	4.52^{f}	5.38^{ef}	4.54^{f}	6.30^{g}
Steer	Good	90	17	5.13^{e}	5.97^{e}	6.82^{e}	5.85^{e}	5.50^{e}	5.44^{e}	4.81^{f}
Steer	Good	100	10	5.00^{e}	5.93^{e}	6.89^{e}	5.78^{e}	5.41^{ef}	5.34^{e}	4.41^{ef}
Steer	Good	130	9	4.49^{e}	5.49^{e}	6.74^{e}	5.41^{e}	5.80^{e}	5.21^{ef}	4.52^{ef}
Steer	Good	160	9	5.21^{e}	5.88^{e}	6.82^{e}	5.86^{e}	5.80^{e}	5.64^{e}	4.64^{ef}
Steer	Good	200	10	4.68^{e}	5.88^{e}	6.89^{e}	5.70^{e}	5.82^{e}	5.44^{e}	3.72^{e}
Steer	Good	230	10	4.86^{e}	6.22^{e}	7.04^{e}	6.26^{e}	5.87^{e}	5.89^{e}	3.75^{e}
Steer	Standard	0	35	5.18^{e}	5.07^{ef}	6.14^{h}	4.77^{f}	4.77^{g}	4.45^{gh}	7.92^{f}
Steer	Standard	30	14	4.29^{gh}	4.64^{f}	6.21^{gh}	4.58^{f}	5.28^{ef}	4.51^{gh}	6.50^{ef}
Steer	Standard	60	20	4.61^{fgh}	5.07^{ef}	6.35^{fgh}	5.06^{ef}	5.11^{fg}	4.75^{fgh}	5.36^{e}
Steer	Standard	90	17	4.77^{fg}	4.51^{f}	6.10^{h}	4.39^{f}	5.02^{fg}	4.16^{h}	6.74^{ef}
Steer	Standard	100	10	4.98^{ef}	5.98^{e}	6.98^{e}	5.92^{e}	5.67^{ef}	5.61^{e}	4.77^{e}
Steer	Standard	130	10	4.68^{efg}	5.42^{ef}	6.49^{efgh}	5.25^{ef}	5.55^{ef}	5.17^{efg}	4.99^{e}
Steer	Standard	160	9	4.80^{efg}	5.87^{e}	6.89^{ef}	5.71^{e}	5.71^{e}	5.38^{ef}	4.41^{e}
Steer	Standard	230	5	3.98^{h}	6.10^{e}	6.88^{efg}	5.96^{e}	5.68^{ef}	5.30^{efg}	3.85^{e}
Heifer	Choice	90	3	4.53^{f}	5.07^{f}	6.13^{f}	4.97^{f}	5.40^{f}	4.73^{f}	5.38^{f}
Heifer	Choice	200	26	5.26^{e}	6.28^{e}	7.20^{e}	6.27^{e}	6.08^{e}	5.97^{e}	3.18^{e}
Heifer	Good	0	4	5.10^{e}	6.20^{e}	7.05^{e}	6.15^{e}	4.75^{f}	5.33^{e}	3.78^{ef}
Heifer	Good	90	10	4.66^{e}	6.52^{e}	7.08^{e}	6.37^{e}	5.89^{e}	5.82^{e}	4.85^{f}
Heifer	Good	200	10	4.74^{e}	5.95^{e}	7.21^{e}	5.98^{e}	5.76^{e}	5.51^{e}	3.72^{e}
Heifer	Standard	0	9	5.04^{e}	6.23^{e}	7.16^{e}	6.22^{e}	4.64^{e}	4.89^{e}	3.65^{e}
Heifer	Standard	90	2	4.75^{e}	5.60^{e}	6.95^{e}	5.40^{e}	5.50^{e}	4.85^{e}	4.38^{e}

[a] 8=extremely juicy; 1=extremely dry.
[b] 8=extremely tender; 1=extremely tough.
[c] 8=none; 1=abundant.
[d] 8=extremely desirable; 1=extremely undesirable.
[e,f,g,h] Means in the same column, for the same sex class and USDA quality grade, bearing a common superscript letter are not significantly ($P>0.05$) different.

that steaks from steers fed 100 days or longer on a high-concentrate diet were similar in palatability, irrespective of quality grade.

In data not presented in tabular form, steaks from all steer and heifer carcasses grading Choice (N=149) were rated higher ($P<0.05$) for juiciness, tenderness, flavor desirability and overall palatability and had lower ($P<0.05$) shear force values than did all steer and heifer carcasses grading Good (n=105). As a test of the hypothesis originally made by Tatum et al. (1980) that some required time-on-feed minimum could be used to make possible a lowering of the minimum marbling requirement for the U.S. Choice grade, data presented in Table 8 were assembled. The postulation to be tested was that carcasses with a minimum-Slight (the present minimum marbling requirement for the U.S. Good grade) amount of marbling would produce steaks which are as palatable as those from U.S. Choice carcasses if the stipulation was that the cattle which produced carcasses with minimum-Slight marbling had been fed a high-concentrate ration for 90 days or more or for 100 days or more.

Data in Table 8 support such a hypothesis—low-Good carcasses from cattle fed 0–230 days produced steaks which differed ($P<0.05$) from those of Choice carcasses in flavor, tenderness, shear force and overall palatability; however, low-Good carcasses from cattle fed 90 days or more produced steaks that were statistically equivalent in palatability to those steaks from Choice carcasses. Identical results were obtained when steaks from low-Good carcasses from cattle fed 100 days or more were compared to steaks from Choice carcasses—steaks from low-Good carcasses were statistically equivalent in palatability to those steaks from Choice carcasses. Applying similar logic in an attempt to support inclusion of carcasses that presently grade high Standard in a "new" Choice grade was only partially successful—rib steaks from high-Standard carcasses were not interchangeable with those from Choice carcasses if the time-on-feed constraint was 90 days but they were interchangeable with those from Choice carcasses if the time-on-feed constraint was 100 days. Data suggest that the minimum marbling requirement for the U.S. Choice grade could be lowered from minimum-Small to minimum-Slight with

Table 7—Mean values for palatability attributes of steaks from steer and heifer carcasses stratified by USDA quality grade within time-on-feed

Sex class	Time-on-feed (days)	USDA quality grade	N	Sensory panel rating						Shear force, kg
				Juiciness[a]	Myofibrillar tenderness[b]	Connective tissue amount[c]	Overall tenderness[b]	Flavor desirability[d]	Overall palatability[d]	
Steer	0	Choice	1	4.80[e]	1.90[f]	4.90[e]	1.80[f]	3.50[e]	2.00[e]	12.24[e]
Steer	0	Good	3	4.70[e]	6.07[e]	6.73[e]	5.83[e]	4.70[e]	4.80[e]	10.71[e]
Steer	0	Standard	35	5.18[e]	5.07[e]	6.14[e]	4.77[e]	4.77[e]	4.45[e]	7.92[e]
Steer	30	Choice	2	4.40[e]	4.60[e]	6.55[e]	4.70[e]	5.70[e]	4.60[e]	6.60[e]
Steer	30	Good	13	4.84[e]	4.62[e]	6.26[e]	4.52[e]	5.38[e]	4.54[e]	6.30[e]
Steer	30	Standard	14	4.29[e]	4.64[e]	6.21[e]	4.58[e]	5.28[e]	4.51[e]	6.50[e]
Steer	60	Standard	20	4.61	5.07	6.35	5.06	5.11	4.75	5.36
Steer	90	Choice	4	5.05[e]	5.70[ef]	6.88[e]	5.75[e]	5.33[ef]	5.33[e]	4.50[e]
Steer	90	Good	17	5.13[e]	5.97[e]	6.82[e]	5.85[e]	5.50[e]	5.44[e]	4.81[e]
Steer	90	Standard	17	4.77[e]	4.51[f]	6.10[e]	4.39[f]	5.02[f]	4.16[f]	6.74[f]
Steer	100	Choice	20	5.32[e]	6.12[e]	6.93[e]	6.09[e]	5.49[e]	5.65[e]	4.04[e]
Steer	100	Good	10	5.00[e]	5.93[e]	6.89[e]	5.78[e]	5.41[e]	5.34[e]	4.41[ef]
Steer	100	Standard	10	4.98[e]	5.98[e]	6.98[e]	5.92[e]	5.67[e]	5.61[e]	4.77[f]
Steer	130	Prime	2	5.60[e]	6.75[e]	7.25[e]	6.60[e]	6.40[e]	6.50[e]	3.63[e]
Steer	130	Choice	22	5.11[e]	6.05[e]	6.68[e]	5.98[e]	5.99[e]	5.84[e]	4.04[e]
Steer	130	Good	9	4.49[e]	5.49[e]	6.74[e]	5.41[e]	5.80[e]	5.21[f]	4.52[ef]
Steer	130	Standard	10	4.68[e]	5.42[e]	6.49[e]	5.25[e]	5.55[e]	5.17[f]	4.99[f]
Steer	160	Prime	2	5.60[e]	5.40[e]	6.60[e]	5.40[e]	6.45[e]	5.70[e]	5.22[f]
Steer	160	Choice	25	5.24[e]	5.97[e]	6.72[e]	5.78[e]	5.90[e]	5.58[e]	3.91[e]
Steer	160	Good	9	5.21[e]	4.88[e]	6.82[e]	5.86[e]	5.80[e]	5.64[e]	4.64[f]
Steer	160	Standard	9	4.80[e]	5.37[e]	6.89[e]	5.71[e]	5.71[e]	5.38[e]	4.41[ef]
Steer	200	Prime	1	5.80[e]	4.90[e]	6.10[e]	4.60[e]	5.00[f]	5.00[e]	4.83[e]
Steer	200	Choice	26	5.05[e]	5.95[e]	6.94[e]	5.85[e]	5.96[e]	5.70[e]	3.87[e]
Steer	200	Good	10	4.68[e]	5.88[e]	6.89[e]	5.70[e]	5.82[e]	5.44[e]	3.72[e]
Steer	230	Choice	20	5.18[e]	6.57[e]	7.18[e]	6.51[e]	6.02[e]	6.11[e]	3.38[e]
Steer	230	Good	10	4.86[e]	6.22[e]	7.04[e]	6.26[e]	5.87[e]	5.89[ef]	3.75[e]
Steer	230	Standard	5	3.98[f]	6.10[e]	6.88[e]	5.96[e]	5.68[e]	5.30[f]	3.85[e]
Heifer	0	Good	4	5.10[e]	6.20[e]	7.05[e]	6.15[e]	4.75[e]	5.33[e]	3.78[e]
Heifer	0	Standard	9	5.04[e]	6.23[e]	7.16[e]	6.22[e]	4.64[e]	4.89[e]	3.65[e]
Heifer	90	Choice	3	4.53[e]	5.07[f]	6.13[f]	4.97[f]	5.40[e]	4.73[f]	5.38[e]
Heifer	90	Good	10	4.66[e]	6.52[e]	7.08[e]	6.37[e]	5.89[e]	5.82[e]	4.85[e]
Heifer	90	Standard	2	4.75[e]	5.60[ef]	6.95[e]	5.40[ef]	5.50[e]	4.85[ef]	4.38[e]
Heifer	200	Prime	3	5.53[e]	6.27[e]	6.90[e]	6.17[e]	5.77[e]	5.63[ef]	2.95[e]
Heifer	200	Choice	26	5.26[e]	6.28[e]	7.19[e]	6.27[e]	6.08[e]	5.97[e]	3.18[e]
Heifer	200	Good	10	4.74[f]	5.95[e]	7.21[e]	5.98[e]	5.76[e]	5.51[f]	3.72[f]

[a] 8=extremely juicy; 1=extremely dry.
[b] 8=extremely tender; 1=extremely tough.
[c] 8=none; 1=abundant.
[d] 8=extremely desirable; 1=extremely undesirable.
[e,f] Means in the same column and for the same sex class and time-on-feed group bearing a common superscript letter are not significantly ($P>0.05$) different.

Table 8—Mean values for palatability attributes of steaks from steer and heifer carcasses (combined) for specified groupings, by USDA quality grade, of rib steaks

USDA quality grade[a]	Choice (0 to 230 days); Good and/or Standard (0 to 230 days)[b] N	Flavor rating[e]	Overall tenderness rating[f]	Overall palatability rating[e]	Shear force kg	Choice (0 to 230 days); Good and/or Standard (90 to 230 days)[c] N	Flavor rating[e]	Overall tenderness rating[f]	Overall palatability rating[e]	Shear force, kg	Choice (0 to 230 days); Good and/or Standard (100 to 230 days)[d] N	Flavor rating[e]	Overall tenderness rating[f]	Overall palatability rating[e]	Shear force kg
Choice	149	5.87[g]	5.99[g]	5.73[g]	3.88[g]	149	5.87[g]	5.99[g]	5.73[g]	3.88[g]	149	5.87[g]	5.99[g]	5.73[g]	3.88[g]
High Good	35	5.62[gh]	5.74[gh]	5.42[gh]	4.82[h]	31	5.68[g]	5.86[g]	5.52[g]	4.38[g]	21	5.62[g]	5.69[g]	5.35[g]	4.19[g]
Avg Good	27	5.66[gh]	6.03[g]	5.61[gh]	4.83[h]	21	5.75[g]	5.99[g]	5.63[g]	4.51[g]	13	5.84[g]	6.00[g]	5.68[g]	4.09[g]
Low Good	43	5.55[h]	5.55[h]	5.20[h]	4.62[h]	33	5.71[g]	5.88[g]	5.48[g]	4.19[g]	24	5.79[g]	5.88[g]	5.56[g]	4.05[g]
Choice	149	5.87[g]	5.99[g]	5.73[g]	3.88[g]	149	5.87[g]	5.99[g]	5.73[g]	3.88[g]	149	5.87[g]	5.99[g]	5.73[g]	3.88[g]
High Good	35	5.62[gh]	5.74[gh]	5.42[gh]	4.82[h]	31	5.68[gh]	5.86[gh]	5.52[g]	4.38[g]	21	5.62[g]	5.69[g]	5.35[g]	4.19[g]
Avg Good	27	5.66[gh]	6.03[g]	5.61[gh]	4.83[h]	21	5.75[g]	5.99[g]	5.63[g]	4.51[g]	13	5.84[g]	6.00[g]	5.68[g]	4.09[g]
Low Good	43	5.55[h]	5.55[hi]	5.20[hi]	4.62[h]	33	5.71[gh]	5.88[gh]	5.48[gh]	4.19[g]	24	5.79[g]	5.88[g]	5.56[g]	4.05[g]
High Std	74	5.24[i]	5.29[i]	4.86[i]	5.71[i]	40	5.55[h]	5.40[h]	5.07[h]	5.26[h]	27	5.72[g]	5.75[g]	5.43[g]	4.61[h]

[a] USDA (1975).
[b] Choice carcasses were from steers fed 0—230 days and from heifers fed 0—200 days; Good and/or Standard carcasses were from steers fed 0—230 days and from heifers fed 0—200 days.
[c] Choice carcasses were from steers fed 0—230 days and from heifers fed 0—200 days; Good and/or Standard carcasses were from steers fed 90—230 days and from heifers fed 90 or 200 days.
[d] Choice carcasses were from steers fed 0—230 days and from heifers fed 0—200 days; Good and/or Standard carcasses were from steers fed 100—230 days and from heifers fed 200 days.
[e] 8=extremely desirable; 1=extremely undesirable.
[f] 8=extremely tender; 1=extremely tough.
[g,h,i] Means in the same column and for the same grade comparison bearing a common superscript letter are not significantly (P>0.05) different.

no appreciable loss of cooked rib steak palatability if the stipulation was made (and adherence to the proviso could be "certified") that cattle with lower than presently required marbling amounts had been fed a high-concentrate ration for at least 90 days.

REFERENCES

Adams, N.J., Smith, G.C., and Carpenter, Z.L. 1977. Carcass and palatability characteristics of Hereford and crossbred steers. J. Anim. Sci. 46: 438.

Barr, A.J., Goodnight, J.H., Sall, J.P., Blair, H., and Chilko, D.M. 1979. "SAS User's Guide." SAS Institute, Raleigh NC.

Berry, B.W., Smith, G.C., and Carpenter, Z.L. 1974. Beef carcass maturity indicators and palatability attributes. J. Anim. Sci. 38: 507.

Bowling, R.A., Smith, G.C., Carpenter, Z.L., Dutson, T.R., and Oliver, W.M. 1977. Comparison of forage-finished and grain-finished beef carcasses. J. Anim. Sci. 45: 209.

Breidenstein, B.B., Cooper, C.C., Cassens, R.G., Evans, G. and Bray, R.W. 1968. Influence of marbling and maturity on the palatability of beef muscle. 1. Chemical and organoleptic considerations. J. Anim. Sci. 27: 1532.

Campion, D.R., Crouse, J.D., and Dikeman, M.E. 1975. Predictive value of USDA beef quality grade factors for cooked meat palatability. J. Food Sci. 40: 1225.

Duncan, D.B. 1955. New multiple range and multiple F tests. Biometrics 11: 1.

Epley, R.J., Stringer, W.C., Hedrick, H.B., Schupp, A.R., Cramer, C.L., and White, R.H. 1968. Influence of sire and length of feeding on palatability of beef steaks. J. Anim. Sci. 27: 1277.

Garcia-de-Siles, J.L., Ziegler, J.H., and Wilson, L.L. 1977. Effects of marbling and conformation scores on quality and quantity characteristics of steer and heifer carcasses. J. Anim. Sci. 44: 36.

Harrison, A.R., Smith, M.E., Allen, D.M., Hunt, M.C., Kastner, C.L. and Kropf, D.H. 1978. Nutritional regime effects on quality and yield characteristics of beef. J. Anim. Sci. 47: 383.

Kramer, C.Y. 1956. Extension of multiple range test to group means with unequal numbers of replications. Biometrics 12: 307.

Leander, R.C., Hedrick, H.B., Stringer, W.C., Clark, J.C., Thompson, G.B. and Matches, A.G. 1978. Characteristics of bovine longissimus and semitendinous muscles from grass- and grain-fed animals. J. Anim. Sci. 46: 965.

McBee, J.L. and Wiles, J.A. 1967. Influence of marbling and carcass grade on the physical and chemical characteristics of beef. J. Anim. Sci. 26: 701.

NCA. 1981. A proposal to modernize the USDA beef grading system. Presented to USDA by the National Cattlemen's Association, Denver, CO.

NRC. 1976. "Nutrient Requirements of Beef Cattle," No. 4., 5th Rev. ed. National Research Council-National Academy of Sciences, Washington, DC.

Parrish, F.C. Jr., Olson, D.G., Miner, B.E., and Rust, R.E. 1973. Effect of degree of marbling and internal temperature of doneness on beef rib steaks. J. Anim. Sci. 37: 430.

Romans, J.R., Tuma, H.J., and Tucker, W.L. 1965. Influence of carcass maturity and marbling on the physical and chemical characteristics of beef. 1. Palatability, fiber diameter and proximate analysis. J. Anim. Sci. 24: 681.

Schroeder, J.W., Cramer, D.A., Bowling, R.A., and Cook, C.W. 1980. Palatability, shelflife and chemical differences between forage- and grain-fed beef. J. Anim. Sci. 50: 852.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. Iowa State Univ. Press, Ames, IA.

Tatum, J.D., Smith, G.C., Berry, B.W., Murphey, C.E., Williams, F.L., and Carpenter, Z.L. 1980. Carcass characteristics, time on feed and cooked beef palatability attributes. J. Anim. Sci. 50: 833.

Tuma, H.J., Henrickson, R.L., Stephens, D.F., and Moore, R. 1962. Influence of marbling and animal age on factors associated with beef quality. J. Anim. Sci. 21: 848.

USDA. 1975. "Official United States Standards for Grades of Carcass Beef." AMS-USDA, Washington, DC.

Zinn, D.W., Durham, R.M., and Hedrick, H.B. 1970a. Feedlot and carcass grade characteristics of steers and heifers as influenced by days on feed. J. Anim. Sci. 31: 302.

Zinn, D.W., Gaskins, C.T., Gann, G.L., and Hedrick, H.B. 1970b. Beef muscle tenderness as influenced by days on feed, sex, maturity and anatomical location. J. Anim. Sci. 31: 307.

Ms received 7/10/81; revised 9/11/81; accepted 9/15/81.

Technical article 17202 from the Texas Agricultural Experiment Station. This study was partially supported by King Ranch, Inc., Kingsville, TX and by the Texas Cattle Feeders Association, Amarillo, TX.

COMPUTER-ASSISTED IDENTIFICATION OF BACTERIA . . . From page 367

Leifson, E. 1951. Staining, shape and arrangement of bacterial flagella. J. Bacteriol. 62: 377.

McLeod, K., Gilbert, K.V., Wyborn, R., Wenham, L.M., Davey, C.L., and Locker, R.H. 1973. Hot cutting of lamb and mutton. J. Fd. Technol. 8: 71.

Roth, L.A. and Clark, D.S. 1975. Effect of lactobacilli and carbon dioxide on the growth of Microbacterium thermosphactum on fresh beef. Can. J. Microbiol. 21: 629.

Schmidt, G.R. and Gilbert, K.V. 1970. The effect of muscle excision before the onset of rigor mortis on the palatability of beef. J. Fd. Technol. 5: 331.

Shewan, J.M., Hobbs, G. and Hodgkiss, W. 1960. A determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to Pseudomonadaceae. J. Appl. Bacteriol. 23: 379.

Skerman, V.B.D. 1967. "A Guide to the Identification of the Genera of Bacteria," 2nd ed. The Williams and Wilkins Co., Baltimore, MD.

Skerman, V.B.D. (Ed.) 1969. "Abstracts of Microbiological Methods." Wiley-Interscience, Division of John Wiley and Sons, New York.

Sneath, P.H.A. and Sokal, R.R. 1973. "Numerical Taxonomy," p. 211. W.H. Freeman and Co., San Francisco, CA.

Valland, M. 1969. Replica plating and computer analysis of psychrotrophic bacteria on aged beef. Master thesis, Iowa State Univ., Ames.

Ms received 6/25/81; revised 9/9/81; accepted 10/21/81.

Contribution No. 81-467-j, Dept. of Animal Sciences & Industry, Kansas Agricultural Experimental Station, Manhattan, KS 66506.

The authors thank the Cryovac Division of W.R. Grace and Co., South Carolina, for supplying the vacuum bags and the Meat Animal Research Center, Clay Center, Nebraska, for providing the cattle.

Storage, Functional and Processing Characteristics of Pre- and Postrigor Beef Preblends for Wiener Production

A. ABU-BAKAR, J. O. REAGAN, R. L. WYNNE, and J. A. CARPENTER

ABSTRACT

Pre- and postrigor coarse ground beef was preblended with either salt, salt plus antioxidants or salt plus nitrite and stored for an extended period of time at 2°C. Although prerigor preblends had a lower pH and more salt-extractable protein, smokehouse yields for pre- and postrigor wiener batters were similar. Wieners prepared from prerigor raw materials were generally more acceptable in appearance, flavor and juiciness and more desirable than wieners prepared from postrigor raw materials. Desirable sausage making qualities of prerigor beef raw material can be maintained by preblending raw material with 3% salt plus 60 ppm nitrite, packaging preblends to minimize exposure to air and storing at 2°C for up to 28 days. Addition of a mixture of butylated hydroxyanisole, butylated hydroxytoluene and citric acid to the preblend had no effect on functional quality of the raw materials.

INTRODUCTION

INDUSTRY EXPERIENCE has shown that more desirable products are manufactured when prerigor raw materials are used in meat batter formulations. Acton and Saffle (1969) demonstrated that prerigor meat could emulsify 22.4% more fat than postrigor meat. These workers also reported that preblending prerigor meat with ice, sodium chloride, ascorbic acid and sodium nitrite 14–24 hr before processing resulted in a greater emulsifying ability (ca 70%) than frozen postrigor meats similarly preblended. The addition of 2% salt to prerigor beef inhibits total glycogen breakdown in the muscle fibers, thus, the ultimate pH of the meat remains higher than for meat which has gone into rigor without the addition of salt (Hamm, 1977).

At the present time, only a portion of the processors are taking advantage of the superior sausage-making qualities of prerigor raw materials in the preparation of processed meat items. With the increasing cost of energy, the use of prerigor raw materials should receive greater acceptance by the industry, making prerigor preblended raw materials commercially important. Few studies have been conducted to evaluate the changes in the chemical, microbiological or processing properties of prerigor raw materials during extended storage periods at cooler temperatures. In a previous study, Reagan et al. (1981) stored prerigor beef preblends containing either 3 or 5% salt for a period of 21 days without significant increases in microbial numbers or deterioration of processing properties of the preblends. However, wieners prepared from preblends which had been stored for more than 7 days had low flavor ratings due to the development of a rancid type flavor (Reagan et al., 1981).

Branen (1978) stated that the development of undesirable flavors in the refrigerated storage of muscle foods may be due to autoxidative rancidity of fat and microbial spoilage, the former takes place before microbial spoilage if the number of psychrotropic organisms in the meat is less than $\log_{10}$ 8. Lipid oxidation in meat is enhanced by the presence of added salt and the degree of lipid oxidation generally increases as the level of added salt increases (Ellis et al., 1968; Waldman et al., 1974). When nitrite is added to cured meat, the development of lipid oxidation is considerably delayed (Watts, 1954; Cross and Ziegler, 1965; Hadden et al., 1975). In addition, nitrite is also effective in controlling the growth of microorganisms in meat without masking any evidence of spoilage (Anon., 1978). Lew and Tappel (1956), Sweet (1973) and Olson and Rust (1973) indicated that lipid oxidation in meat could be controlled by using antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) alone, or in combination with metal chelators such as citric acid (CA) and ethylenediamine tetra-acetic acid (EDTA). Among phenolic antioxidants, BHA has the highest antimicrobial activity (Branen, 1978).

This study was conducted to evaluate (a) changes in the storage, functional and processing characteristics of pre- and postrigor beef preblends containing either salt, salt plus antioxidants or salt plus sodium nitrite during extended periods of storage at 2°C and (b) the acceptability of wieners prepared from the preblends at the end of five storage periods.

Authors Abu-Bakar, Reagan, and Carpenter are with the Dept. of Food Science, Univ. of Georgia, Athens, GA 30602. Author Wynne, formerly with the Univ. of Georgia, is now with Holland International Foods, P.O. Box 729, Dawson, GA 31742.

MATERIALS & METHODS

Meat sources

Prerigor and postrigor meats were obtained from the forequarters of bull and steer carcasses for experiments I and II, respectively. In experiment I, prerigor meat was obtained from a 425 kg bull carcass by deboning one forequarter within 1 hr postmortem. The meat was coarsely ground through a 1.27 cm plate prior to adjusting the fat content to 10 ± 2%. Postrigor meats were treated in a similar manner with the exception that the forequarters was not deboned until 24 hr postmortem. For experiment II, pre- and postrigor meats were derived from two slaughter steers of similar breeding and management systems. The prerigor and postrigor meats were treated identical to those utilized in experiment I. Trimmed pork fat back (94% fat) was used as the source of fat for wiener production.

Experimental design

Each experiment was established using a completely randomized design with a factorial arrangement of treatments. Sources of variation for each experiment consisted of: (1) meat type (prerigor, postrigor), (2) preblending mixture (salt only, salt plus antioxidants, and salt plus sodium nitrite and (3) length of storage (0, 7, 14, 21 or 28 days) at 2°C.

Samples of each meat type were obtained for pH measurements, salt-soluble protein extraction for emulsifying capacity determination, microbial analysis and for the preparation of 0-day wieners. For the preblending treatments, the pre- and postrigor meats were mixed (5 min) with either (1) 3% salt only, (2) 3% salt plus 0.001% butylated hydroxyanisole (BHA), 0.001% butylated hydroxytoluene (BHT) and 0.0005% citric acid (CA) or (3) 3% salt plus 60 parts per million (ppm) sodium nitrite using a Butcher Boy Meat mixer. The mixer was washed between batches to prevent contamination among preblend treatment batches. Each batch was placed in a polyethylene bag, removing as much air as possible without using vacuum as the bag was twisted, sealed with a twist lock tab and stored in a 2°C cooler.

Samples were taken for chemical and microbial analyses and for wiener preparation upon completion of each storage period. Wieners were prepared using a 2.52 kg meat block/preblending treatment/meat type. Thiobarbituric acid (TBA) values of the preblends were

determined at the end of each storage period following the method described by Tarladgis et al. (1960). Zipser and Watts (1962) recommended a modified method for determining TBA values in cured meats since the presence of nitrite reduced the recovery of malonaldehyde during distillation. The TBA values of meat samples were reduced by 8 and 21% when the nitrite levels in the meat samples were 25 and 50 ppm respectively (Zipser and Watts, 1962). In the present experiment, the level of residual nitrite in the preblends was only 26 ppm or less; thus, the reduction in TBA values for preblends containing nitrite would be less than 10%.

In experiment II, the meat was treated similar to that in experiment I except that prior to storage, the preblended meats were subdivided into four equal portions of 3.6 kg each. The meat batches were then vacuum packaged separately in Barrier Bag® (oxygen transmission rate of 30–40 cc/m^2/24 hr/23°C; moisture vapor transmission rate of 0.5–0.6 g/645 cm^2/24 hr/37.8°C/100% R.H.) using a chamber-type vacuum machine, randomly assigned to one of the four storage periods and stored in a 2°C cooler. As in experiment I, chemical and microbial analyses were conducted and wieners were prepared upon completion of each storage period. Values for nitrite were determined for samples containing sodium nitrite following the method recommended by AOAC (1975).

Wiener production

Wiener batters were prepared in duplicate upon completion of each storage interval within each preblending treatment and meat type. Batters were prepared using a formula which would approximate a commercial all-meat product (fat = 30%; added water = 4 x protein % + 10%; salt = 2.5%). Raw materials for wiener production consisted to either pre- or postrigor beef (10% fat) and trimmed pork fat back (94% fat). Seasonings included salt, sugar, wiener seasoning mix, sodium nitrite and sodium erythorbate. Batters were chopped for 5 min and then passed through a Stephan Microcut (Type MCV 12B with 1.0 mm cutting ring) before stuffing into 22 mm cellulose casings. Stuffed casings were linked with a "Tylinker" to a length of approximately 12.7 cm. Each batch was labeled, placed in wire baskets, covered with a damp cotton shroud and stored overnight at 2°C. Approximately 12 hr after stuffing, the uncooked wieners were weighed and placed in an Alkar-Rasmussen Processing Oven for cooking. The initial temperature of the house was held at 54°C for 30 min, increased to 66°C (relative humidity = 60%) for 30 min, then increased to 77°C (relative humidity = 60%) until the internal temperature of the wieners was 68°C. The approximate cooking time was 75 min. After cooking, the wieners were weighed to determine smokehouse shrinkage and then chilled at 2°C.

The wieners were peeled after 12 hr of chilling. Thirty wieners were randomly selected, vacuum packaged in Barrier Bags® (10 wieners/bag) and frozen for not more than 4 wk at −35°C for subsequent moisture, fat and protein determinations, sensory evaluation and Warner-Bratzler shear force determinations. In experiment II, additional wieners were randomly selected and similarly packaged for the determination of Hunter-Color values.

pH Determination

At the end of each storage period, pH of the raw materials was determined by placing a 10 g meat sample in 50 ml of distilled water and blending for 2 min in a Virtis '23' Homogenizer. The pH of the resulting slurry was determined using a Corning Model 10 pH meter.

Microbial number determinations

Ten gram samples were obtained from the raw materials each day of wiener production and duplicate one gram samples from each treatment were placed in sterile 99 ml dilution blanks containing a 0.85% saline solution. Following appropriate dilutions, duplicate pour plates of Standard Plate agar were inoculated and incubated at 30°C for 72 hr. Total counts were reported as $\log_{10}$ values.

Extraction of Salt-soluble protein

The method described by Acton and Saffle (1969) was used for the extraction of salt-soluble proteins form the preblended batches of both meat types. Samples of the preblended meat (51.5 g) were blended with 200 ml of a 2.23% sodium chloride solution (w/w). This amount of preblended meat and salt solution contained the same weight of meat, water and salt as used in the meat that was not preblended (0 day raw materials). Salt-soluble protein concentration was determined by Biuret method (Gornall et al., 1949) and the results were reported as mg salt-soluble protein/g of raw materials.

Emulsifying capacity determinations

The method of Swift et al. (1961), as modified by Carpenter and Saffle (1964), was used as a basis for the comparison of the emulsifying capacity of the soluble protein extracts. Twenty-five ml aliquots of the protein extract and 50 ml of corn oil were poured into an inverted one-pint Ball glass jar with a 3/8-in hole bored in the bottom and placed on an Osterized mixer base. The contents were blended for 30 sec, then oil was added to the emulsion at a constant rate of 0.6 ml/sec using a Polystaltic pump until a visible collapse of the emulsion occurred. Visibility of emulsion collapse was aided by staining the corn oil with Oil-Red-O biological stain following the method of Marshall et al. (1975). Blending speed was held constant at 10,800 rpm. The protein extracts were at an initial temperature of 2°C and the oil temperature at 22°C. The final temperature of the emulsion at the point of collapse ranged from 25–30°C. Three replications were made for each protein extract.

Moisture, fat and protein determinations

Ten wieners were ground twice through a 0.31 cm plate to facilitate sampling for moisture, fat and protein determinations according to AOAC (1975) procedures.

Shear force determinations

Five wieners per treatment were sheared three times (2.54 cm from each end and in the center of each wiener) using a single blade shearing device attached to an Instron Universal Testing Machine. Data were reported for the average maximum force in kg required to shear through the wiener.

Sensory evaluation

A consumer-type sensory panel of 40–50 members consisting of faculty and students of the Food Science Department evaluated the wieners for appearance, firmness, juiciness and overall desirability using 8-point rating scales (1 = extremely undesirable, 8 = extremely desirable). Samples were also evaluated for degree of saltiness where a rating of 1 was considered extremely bland and a rating of 8 was considered extremely salty. Samples were prepared by placing five wieners (thawed at 2°C for approximately 8 hr) in pans of boiling water. The pans were covered and removed from the heating element. The wieners were held in the covered pans for 4 min prior to slicing into approximately 2.54 cm cross-sections. Five samples from different treatments were presented to the panelists at each sensory session.

Hunter-Color values determinations

Five wieners per treatment were used to determine L, a_L and b_L Hunter-Color values using a Hunter Lab Color/Difference Meter D25-2. The instrument was standardized with reflectance-color standard No. C2-6713 which has the following values; L = 67.0, a_L = 22.6 and b_L = 10.2. The L, a_L and b_L values of each wiener were determined at four different places, equally spaced along the length of the wiener. The reported values are the average of 20 determinations.

Statistical analysis

Data were analyzed using the Statistical Analysis System of Barr and Goodnight (1979) and the mean separation technique of Duncan (1955). Significance was determined by the F-test and significant differences were accepted at the 5% level of probability.

RESULTS & DISCUSSION

Influence of meat type

Mean values for raw material pH, overall preblending treatments and storage times within meat types revealed that meat type significantly influenced the pH of the preblends (Table 1). The higher pH values for the prerigor preblends were probably due to the effect of adding salt to the prerigor muscle, thus inhibiting the complete breakdown of glycogen in the muscle (Hamm, 1977).

–Continued on next page

Although prerigor raw materials had higher pH values and a greater concentration of salt extractable protein which theoretically should bind more moisture than the postrigor raw materials, smokehouse yields were not significantly influenced by meat type. The observed similarity in the smokehouse yields of the wieners could be attributed to the similarity in the water binding capacity. However, the water binding capacity of the raw materials was not determined.

Differences in mean TBA values between meat types in both experiments were not statistically significant which would indicate that the extent of lipid oxidation in the preblends was not affected by meat type. The effect of meat type on microbial total plate count (TPC) of the preblends was not consistent (Table 1). The TPC of pre- and postrigor preblends in experiment I were similar; however, in experiment II prerigor preblends had significantly higher TPC than the postrigor preblends. Emswiler and Kotula (1979) reported that meat type did not influence TPC of beef, but Lin et al. (1979) demonstrated that whole hog sausage prepared from prerigor pork had higher TPC than that for sausage prepared from postrigor pork. The inconsistency of the effect of meat type on TPC in the present study may be due to contamination of the prerigor meat (experiment II) during grinding as indicated by the initial high TPC (Fig. 2).

The level of residual nitrite in the preblends containing nitrite was significantly higher in the prerigor preblends (Table 1). Comparatively low residual nitrite levels in postrigor preblends may be attributed to the lower pH of postrigor raw materials since low muscle tissue pH promotes the depletion of nitrite in meat (Nordin, 1969 and Zaika et al., 1976).

Differences in the residual nitrite level between prerigor and postrigor preblends significantly influenced the yellowness (b_L) values of the wieners. However, the practical significance of the observed difference is questionable since they differed by only 0.4 units. The redness (a_L) and lightness (L) values were not significantly affected by meat type.

Effect of preblending treatment and raw material storage time

In both experiments the addition of antixoidants or nitrite to the preblends in addition to 3% salt had no effect on the functional quality indicators (pH, salt-soluble protein concentration, EC of the protein extracts and smokehouse yield) of the preblends or on the Hunter-Color Difference Meter Values of the wieners (data not presented in tabular form). Mean values for chemical and microbial characteristics of the preblends, smokehouse yields and Hunter-Color Difference Meter Values of the wieners stratified by raw material storage time are presented in Table 2. Raw material pH generally remained the same throughout the 28-day storage period.

During the first 7 days of storage, the solubility of the salt extractable protein decreased ($P < 0.05$) (Table 2). After day 7, the differences in salt-extractable protein were not significantly different. Goll et al. (1964) reported that

Table 1—Overall mean values for chemical and microbial characteristics of p•blends, emulsifying capacity of protein extracts, smokehouse yields and Hunter Color Difference Meter Values of wieners stratified by meat type

Exp.	Meat type	Traits									
		pH	TBA values	TPC (log_{10})	Salt-extractable protein (mg/g)	EC (ml oil/100 mg) Protein	Smokehouse yield (%)	Residual nitrite (ppm)[c]	L[d]	a_L[e]	b_L[f]
I	Prerigor	6.1[a]	0.55	4.6	46.6[a]	43.4[a]	93.2				
	Postrigor	5.8[b]	0.64	4.8	38.8[b]	35.8[b]	93.9				
II	Prerigor	6.1[a]	0.32	6.7[a]	40.0[a]	39.3	92.8	21.7[a]	54.6	10.9	11.5[a]
	Postrigor	5.8[b]	0.26	4.8[b]	33.2[b]	38.5	92.4	11.6[b]	54.0	11.0	11.9[b]

[ab] Means in the same column within each study bearing different superscripts are significantly different ($p < 0.05$).
[cdef] Not determined in Study I.

Table 2—Mean values for chemical and microbial characteristics of preblends, smokehouse yields and Hunter Color Difference Values of wieners stratified by raw material storage time.

Exp.	Traits	Raw Material Storage Times (days)				
		0	7	14	21	28
I	pH	6.0	5.9	5.9	6.0	5.9
	TPC (log_{10})	3.7[a]	4.4[a]	4.8[b]	5.0[b]	5.8[c]
	TBA values	0.05[a]	0.33[b]	0.46[b]	0.45[b]	0.64[b]
	Salt-extractable protein (mg/ml)	12.51[a]	10.42[b]	10.56[b]	10.12[b]	10.18[b]
	EC (ml oil/100 mg protein)	—[d]	32.9[a]	33.3[a]	36.3[a]	49.2[b]
	Smokehouse yield (%)	93.9	*	93.0	93.9	93.6
II	pH	5.9	6.0	6.0	6.0	6.0
	TPC (log_{10})	4.6[ac]	4.3[a]	5.8[bc]	6.2[b]	7.1[d]
	TBA values	0.08[a]	0.28[b]	0.27[b]	0.37[b]	0.32[b]
	Salt-extractable protein (mg/ml)	10.37[a]	9.12[b]	9.01[b]	8.95[b]	9.01[b]
	EC (ml oil/100 mg protein)	34.4[a]	38.8[b]	40.6[c]	38.6[b]	39.1[bc]
	Smokehouse yield (%)	92.1	91.8	92.8	93.0	93.0
	Residual nitrite (ppm)		26.05[a]	17.18[b]	13.06[c]	10.28[c]
	L	52.95[a]	53.98[a]	54.22[ab]	54.23[ab]	55.12[b]
	a_L	11.80	10.93	11.05	10.08	10.55
	b_L	11.65	11.67	11.57	11.9	11.73

[abc] Means in the same row within each study bearing different superscripts are significantly different ($p < 0.05$).
[d] The values were not determined
*Due to mechanical problems with the processing oven, data for day 7 samples were not obtained.

sarcoplasmic, myofibrillar and salt-soluble proteins decreased in solubility as time postmortem increased.

Smokehouse yields generally remained the same as raw material storage time increased. Reagan et al. (1981) showed that smokehouse loss of wiener batters prepared from raw materials held for 14 or 21 days were lower than those prepared from raw materials stored for 7 days. The smokehouse loss on day 7 was 12.7% compared to only 8.1 and 7.9% on day 14 and day 21 respectively (Reagan et al., 1981). Converting the smokehouse loss to smokehouse yield, the smokehouse yields would be 87.3, 91.9 and 92.1% for day 7, 14 and 21, respectively. These data suggest that storing the preblended raw materials up to 28 days may not significantly increase smokehouse yield of wiener batters if the smokehouse yield is already above 90%.

Residual nitrite in preblends prepared with nitrite decreased significantly with storage time (Table 2). The residual nitrite level of the postrigor preblends were lower than those of the prerigor preblends.

The preblending treatment and raw material storage time significantly influenced the TPC and TBA values of the preblends (Table 2 and Fig. 1 and 2). The TBA values of the preblends increased significantly after 7 days of storage (Table 2); however, holding the preblends beyond 7 days did not result in any significant increase in the TBA values. Fig. 1 and 2 show that the rate of microbial growth in the preblends containing nitrite was slower than preblends containing no nitrite. In experiment 1, the initial TPC for the preblends were similar; however, after 28 days in storage the preblends containing nitrite had a TPC of log 4.9, whereas the average TPC for preblends that contained salt only and salt plus antioxidants were log 6.1 and log 6.0, respectively. The TPC for prerigor preblends in experiment II (Fig. 2) did not increase significantly on day 7. However, on day 14, the TPC of the preblends without nitrite increased sharply to log 8.0. During the same period, the TPC of the preblends with nitrite increased to only log 5.2. On day 14 and subsequent storage periods, upon opening the packages, a yeasty off-odor was detected from the preblends prepared without nitrite, while no off-odors were detected for preblends prepared with nitrite. In constant to the TPC of hot-boned preblends without nitrite, the TPC of the postrigor counterparts did not show any sharp increase on day 14. None of the postrigor preblends developed off-odors during storage. The off-odors associated with the high TPC in the prerigor preblends could be due to the growth of bacteria, however, the amount of acid present was probably not sufficient to lower the pH of the preblends.

These results indicate that at the level used in the present experiment, nitrite retarded microbial growth, whereas antioxidants did not (Fig. 1 and 2). Chang and Branen (1975) reported that 150–200 ppm of BHA inactivated an initial log 6 innoculum of *Staphylococcus aureus*.

In the present study, BHA was added at the rate of 0.01% of the fat content. Since the preblends contained 10% fat, the concentration of BHA in the preblends was 0.001% (10 ppm), less than 10% of the concentration reported by Chang and Branen (1975). Branen (1978) reported that the antimicrobial effect of antioxidants was reduced by the presence of fat.

TBA values for the preblends in experiment I indicated that the lipid oxidation in the preblends prepared with salt only, generally increased during storage; however, for preblends prepared with nitrite and antioxidants, lipid oxidation remained the same after a slight increase on day 7 (Fig. 1). The TBA values for the preblends with salt increased to above 1.0 after 21 days, but preblends with nitrite and antioxidants had TBA values below 0.5 throughout the 28-day storage period. Preblends in experiment II showed similar trends (data not presented in tabular form). These data showed that lipid oxidation in coarse ground beef containing 3% salt during extended times of storage at 2°C was depressed by the presence of 60 ppm nitrite or antioxidants added at the rate of 0.025% (0.01% BHA, 0.01% BHT and 0.005% CA) of the fat content.

Proximate analysis and panel evaluation of wieners

Mean values for proximate composition of wieners in experiment I stratified by meat type, preblending treatment

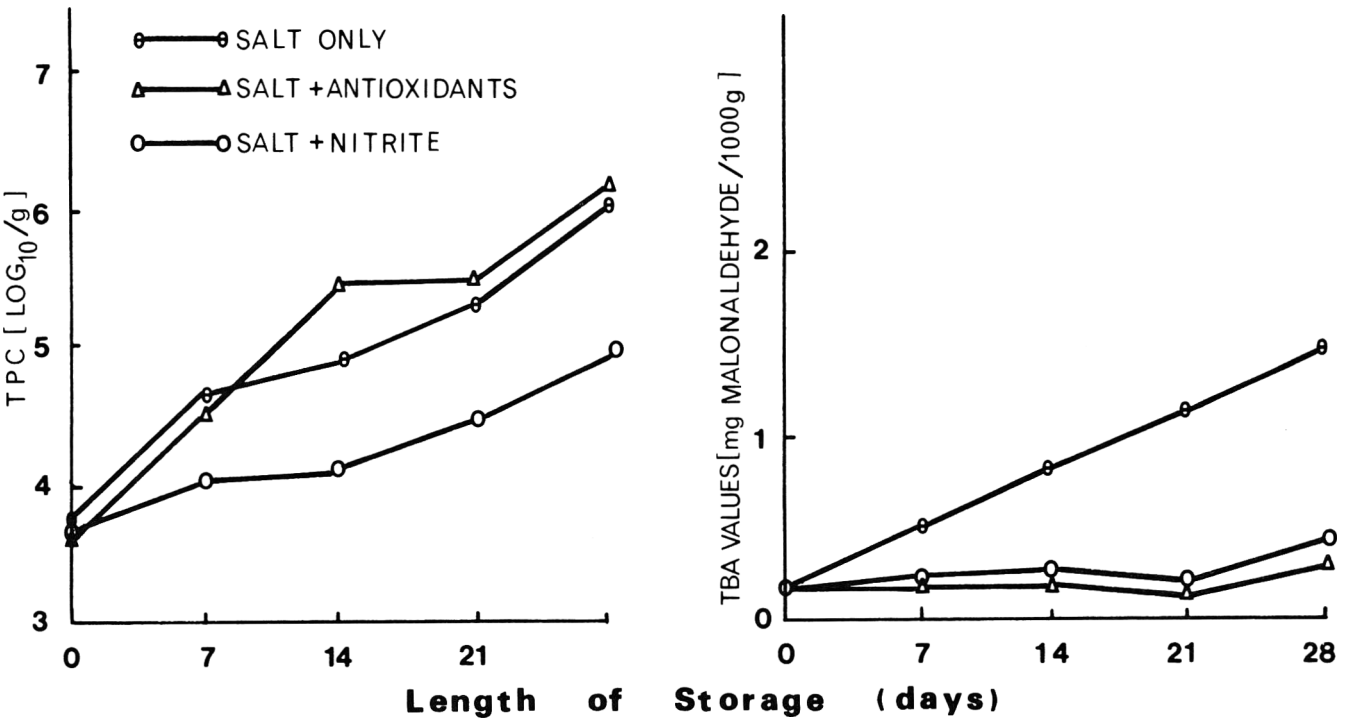


Fig. 1—Mean TPC and TBA number of preblends in experiment I during storage.

and raw material storage time are presented in Table 3. Values for percentage fat, moisture and protein were significantly affected by meat type. The wieners prepared from prerigor preblends exhibited significantly higher percentage values for moisture, fat and protein than the wieners prepared from the postrigor raw materials. Values for percentage fat increased with storage time and values for the percentage moisture decreased as raw material storage time increased up to day 21.

In experiment II (data not presented in tabular form), values for percentage fat and moisture of the wieners were significantly affected by raw material storage time. In contrast to experiment I, the percentage values for fat and moisture decreased as raw material storage time increased.

Various sensory traits of wieners in experiment I were significantly affected by meat type, preblending treatment and raw material storage time (Table 4). Meat type significantly influenced the firmness, flavor and overall desirability ratings of cooked wieners. The panel ratings indicated that wieners prepared from prerigor preblends were firmer, more acceptable in flavor and more desirable than wieners prepared from postrigor raw materials. The significantly higher firmness ratings for prerigor wieners were also reflected in the shear values of the wieners; a greater amount of force was required to shear the prerigor wieners. None of the sensory traits of wieners in experiment II were significantly affected by meat type (data not presented in tabular form). However, for all traits, except for saltiness, prerigor wieners received higher panel ratings than postrigor wieners. The average ratings were 5.5 which would indicate an acceptable product.

When panel ratings were stratified by preblending treatment, only differences in average appearance ratings of wieners in experiment I were statistically significant. Wieners prepared from preblend with nitrite had significantly lower appearance ratings than those prepared from preblends with salt only and salt plus antioxidants. Examination of the appearance ratings of wieners from each preblend indicated that wieners from preblends with nitrite generally decreased in appearance whereas appearance ratings of wieners prepared from preblends without nitrite increased as raw material storage time increased. Also,

Table 3—Mean values for percentage moisture, fat and protein of wieners in experiment I

Treatment factors	Moisture	Fat	Protein
Meat type			
Prerigor	55.3[a]	30.7[a]	12.0[a]
Postrigor	53.4[b]	28.9[b]	11.4[b]
Preblending treatment			
Salt only	55.0	30.4	11.7
Salt + antioxidants	54.2	29.6	11.7
Salt + nitrite	54.1	29.5	11.8
Raw material Storage time (days)*			
0	54.4[ab]	27.2[a]	11.7
14	54.7[a]	30.2[bc]	11.5
21	53.0[b]	30.9[b]	12.0
28	55.4[a]	29.3[c]	11.7

[abc] Means for a treatment factor within a column bearing different superscrips are significantly different ($p < .05$).
*Due to mechanical problems with the processing oven, data for day 7 samples were not obtained.

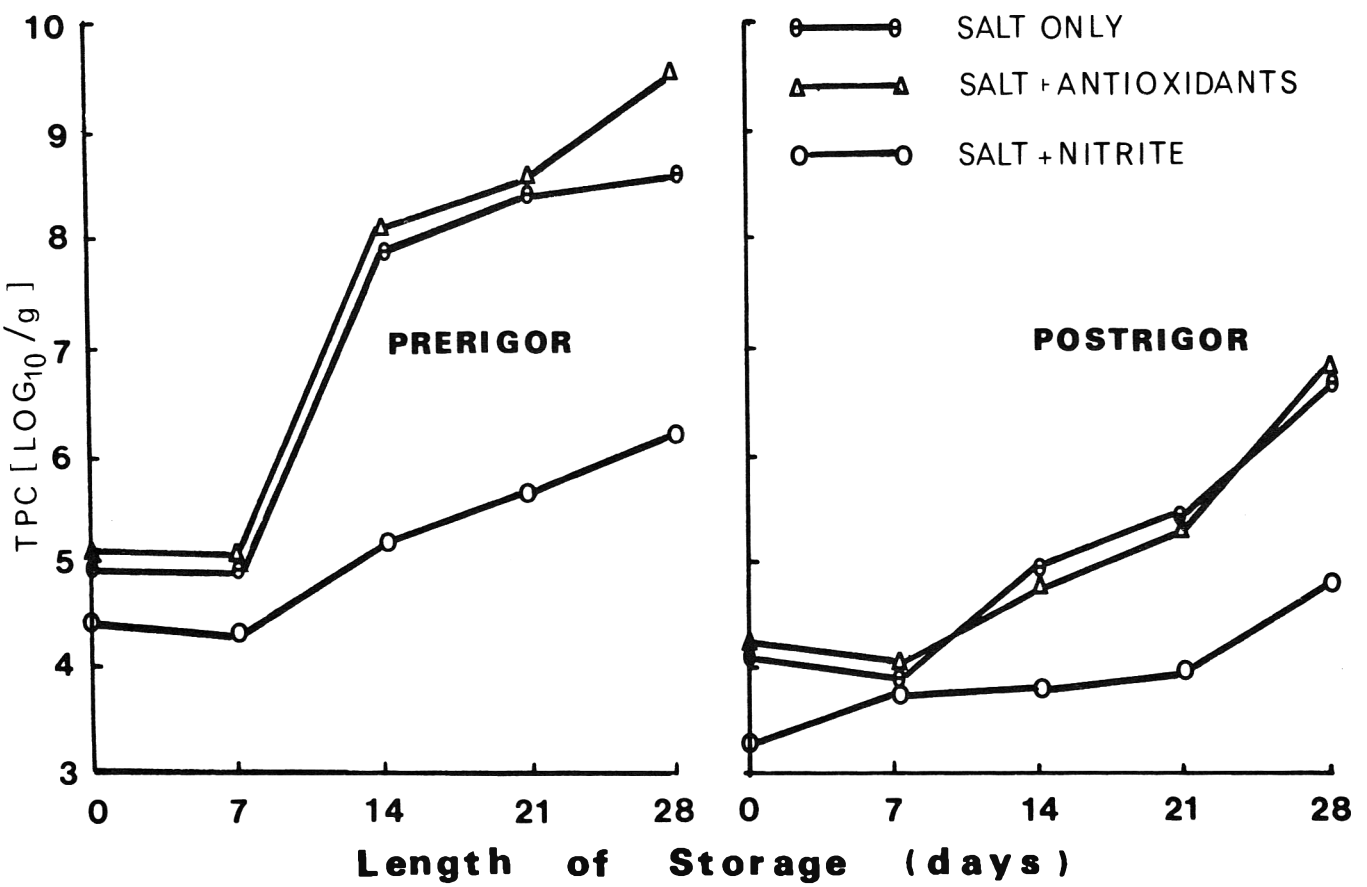


Fig. 2—Mean TPC of pre- and postrigor preblends in experiment II during storage.

Table 4—Sensory evaluation and WBS force of wieners in experiment I

Treatment factors	Sensory traits						
	Appearance	Firmness	Flavor	Juiciness	Saltiness	Overall desirability	WBS (kg)
Meat type							
Prerigor	5.9	6.2[a]	5.7[a]	5.8	5.4	5.8[a]	2.7[a]
Postrigor	5.6	5.5[b]	5.4[b]	5.7	5.3	5.3[b]	2.2[b]
Preblending treatment							
Salt only	6.0[a]	5.8	5.4	5.6	5.3	5.5	2.4
Salt + antioxidants	6.4[a]	5.9	5.9	5.9	5.4	6.0	2.3
Salt + nitrite	4.7[b]	5.9	5.4	5.8	5.5	6.3	2.6
Raw material Storage time (days)*							
0	5.5	6.2[a]	6.0	5.3[a]	5.3	5.1	2.7
14	5.7	5.6[b]	5.3	6.0[b]	5.4	5.6	2.1
21	5.6	6.1[a]	5.7	5.6[ac]	5.4	5.6	2.6
28	5.9	5.8[ab]	5.8	5.8[bc]	5.4	5.8	2.5

[abc] Means for a treatment factor within a column with different superscripts are significantly different ($p < .05$).
*Due to mechanical problems with the processing oven, data for day 7 samples were not obtained.

panelists gave higher appearance ratings to wieners prepared from prerigor preblends with nitrite than to wieners prepared from the postrigor counterparts. The average appearance ratings of prerigor wieners compared to postrigor wieners were 6.0 vs 4.1, 5.0 vs 3.4 and 5.4 vs 4.3 for day 14, 21, and 28, respectively (data not presented in tabular form).

The color of wieners prepared from preblends without nitrite was observed to be pink, typical of non-smoked all meat wieners, whereas wieners prepared from preblends with nitrite were light pink. The color acceptability of wieners has been shown to decrease with decreasing nitrite concentration (Sebranek et al., 1977; Sales et al., 1980). Sales et al. (1980) indicated that turkey frankfurters prepared with lower nitrite levels were lighter in color compared to wieners prepared with higher nitrite levels. The observed didference inthe color of the wieners in this experiment could be due to the depletion of added nitrite during storage of raw material. As a result, when another 60 ppm of nitrite was added during batter preparation, the final concentration of nitrite in the batter would be lower than that for batters prepared from preblends without nitrite in which 120 ppm of nitrite was added during batter preparation. The difference in appearance ratings between wieners prepared from pre- and postrigor preblends with nitrite was probably due to the pH difference between the two preblends. In contrast to experiment I, the color ratings of wieners in experiment II were not significantly affected by preblending treatment (data not presented in tabular form). The only difference in treatment between experiment I and experiment II was in the method of packaging the preblends during storage. It is possible that vacuum packaging of preblends in experiment II reduced nitrite depletion during storage. However, residual nitrite was not determined in experiment I to compare with that in experiment II.

Raw materials storage time significantly influenced firmness and juiciness ratings of wieners in both studies. In experiment I, firmness ratings increased significantly on day 21 after a significant decrease on day 14. However, the ratings on day 21 and 28 were not significantly different from the ratings on day 0. Warner-Bratzler shear force values for wieners in both studies were not significantly affected by raw material storage time. Average juiciness ratings in experiment I increased significantly on day 14 and decreased slightly after day 21. In general, average appearance, flavor and overall desirability ratings increased as raw material storage time increased. Flavor ratings of wieners in experiment II, however, increased up to day 21 and decreased on day 28. Examination of the data indicated that on day 0 flavor ratings of prerigor wieners were lower than that for postrigor wieners. The panelists commented that prerigor wieners prepared on day 0 had a strong beef flavor, atypical of all meat type wieners. Only a few of the panelists detected rancid flavor in wieners prepared from prerigor preblends without nitrite; most of the panelists rated the wieners as very acceptable.

This study showed that wieners prepared from prerigor preblends were as acceptable as wieners prepared from postrigor preblends. The study also suggests that desirable characteristics of beef raw materials for wiener production can be maintained for as long as 28 days by preblending the coarsely ground material with 3% salt and 60 ppm nitrite, packaging the preblends to minimize exposure to air and storing them at 2°C. Additional research is needed to determine a system in which superior EC of prerigor preblends can be utilized more efficiently.

REFERENCES

Acton, J.C. and Saffle, R.L. 1969. Preblended and prerigor meat in sausage emulsions. Food Technol. 23: 367.

Anonymous. 1978. Nitrite in meat curing: risks and benefits. Report No. 74. Council for Agricultural Science & Technology.

AOAC. 1975. "Official Methods of Analysis," 12th ed., 2nd Suppl. Sec. 24.B 04. Association of Official Chemists, Washington, D.C.

Barr, A.J. and Goodnight, J.H. 1979. "User's Guide to the Statistical Analysis System." N.C. State Univ., Raleigh, NC.

Branen, A.L. 1978. Interaction of fat oxidation and microbial spoilage in muscle foods. Proceedings 31st Annual Rec. Meat Conf., p. 156.

Carpenter, J.A. and Saffle, R.L. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29: 774.

Chang, H.C. and Branen, A.L. 1975. Antimicrobial effects of butylated hydroxyanisole (BHA). J. Food Sci. 40: 349.

Cross, C.K. and Ziegler, P. 1965. A comparison of the volatile fractions from cured and uncured meat. J. Food Sci. 30: 610.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1.

Ellis, R., Currie, G.T., Thornton, F.E., Bollinger, N.C., and Gaddis, A.M. 1968. Carbonyls in oxidizing fat. 2. The effect of prooxidant activity of sodium chloride on pork tissue. J. Food Sci. 33: 555.

Emswiler, B.S. and Kotula, A.W. 1979. Bacteriological quality of ground beef prepared from hot and chilled beef carcasses. J. Food Protect. 42: 561.

Goll, D.E., Henderson, D.W. and Kline, E.A. 1964. Post-mortem changes in physical and chemical properties of bovine muscle. J. Food Sci. 29: 590.

—Continued on page 385

Microbiological Quality of Vacuum Packaged Poultry With or Without Chlorine Treatment

A. A. KRAFT, K. V. REDDY, R. J. HASIAK, K. D. LIND, and D. E. GALLOWAY

ABSTRACT

Dry packed broilers were cut up or kept as whole carcasses and vacuum packaged in a high barrier or low barrier film or stretch wrapped in a tray package. In an additional study, chicken was treated with 20 ppm chlorine in a chilled water dip; control chicken had no chlorine added to the water. The poultry was then packaged as previously indicated. The chicken was stored in a display case at about 5°C and examined for various bacteria and odor development at intervals up to 10 days. Vacuum packaging dry packed broilers resulted in significantly lower bacterial counts and longer keeping time compared with stretch wrapped chicken. Longest shelf life was obtained with a high barrier film, and chlorine as a dip did not provide increased storage life compared with vacuum packaged broilers maintained in the dry state.

INTRODUCTION

THE INFLUENCE of packaging methods and materials on keeping quality of fresh or frozen poultry has been well established (Ayres, 1966; Wells et al., 1958). Shelf life is associated with growth of spoilage organisms. Since typical spoilage bacteria, such as *Pseudomonas*, are highly aerobic, the permeability of materials to oxygen and effect of vacuum packaging are factors to be considered in extending shelf life of poultry (Arafa and Chen, 1975; Debevere and Voets, 1973; Shank and Lundquist, 1963). Vacuum packaging of poultry has generally resulted in longer keeping time than observed when poultry was packaged in air. In our laboratory, we found that bacterial fluorescence on chicken was inhibited by packaging the poultry in evacuated, heat shrunk Cryovac bags (Kraft and Ayres, 1961). In our later work (Rey and Kraft, 1971) we observed that vacuum packaging in a highly impermeable film, a 3-ply laminate, resulted in reduction in numbers and activity of spoilage organisms when compared with other methods tested. When chicken was stored in evacuated polyethylene bags at about 4°C, Walker and Ayres, (1956) found that off odor occurred at about 5 or 6 days, followed by slime formation on the chicken. However, differences may exist in time for spoilage of whole vs cut up birds, and one of the purposes of this investigation was to examine the two types of products for microbial flora and keeping time.

Although information described above has shown the worth of vacuum packaging in highly impermeable materials, the combination of vacuum packaging individual carcasses for retail sale after bulk packaging chickens in quantity in carbon dioxide in a sealed plastic bag warranted investigation as a means of further increasing shelf life. The primary objective of this study was to determine effects of these processes on keeping quality and microbial numbers and types associated with dry packed fresh refrigerated broilers after vacuum packaging and holding in a retail type display case. Specific consideration was given to barrier characteristics of packaging films, effect of vacuum packaging compared with stretch wrap packaging in air, whole vs cut up carcasses, and effects of a chlorine dip on microbial populations. Although chlorine treatment of poultry has also been studied extensively, the current work was concerned with the proposal by the U.S. Department of Agriculture to use water containing 20 ppm available chlorine in continuous chillers (Federal Register, 1978).

Author Kraft is with the Dept. of Food Technology and author Hasiak is with the Dept. of Animal Science, Iowa State Univ., Ames, IA 50011. Author Reddy is at the Univ. of Londrina, Londrina, PR 86100, Brazil. Authors Lind and Galloway are with the American Can Co., P.O. Box 702, 1915 Marathon Ave., Neenah, WI 54956.

EXPERIMENTAL

EVISCERATED dry packed whole broilers, approximately 70 lb or about 20–25 birds per box, were obtained from local commercial distributors. The poultry was originally packed in a plastic film bag in the paperboard container with air removed from the bag, carbon dioxide added to the bulk package, and the bags sealed. The boxed chicken was shipped by refrigerated truck from Southern processing plants to the local supplier within 48 hr after processing. One-half of the carcasses (about 10–12 birds) were cut up in the laboratory and the remainder were packaged as whole birds. The broilers were vacuum packaged (about 27–29 in. Hg) in a highly impermeable film and in a film providing a lower barrier to oxygen (about 18 cc/M^2/24 hr compared with 2000 cc/M^2/24 hr at 23°C and 0% R.H., respectively). Oxygen transmission rates were in the order of 100 times difference between films. Broilers were also tray packaged in air in a stretch wrap film which was very permeable to oxygen (6500 cc/M^2/24 hr at 23°C and 0% R.H.). In another series of tests, the chicken was dipped momentarily in a 20 ppm active chlorine solution and compared with similar poultry dipped in chilled water. Such treatment obviously was not comparable with chlorination of a continuous chiller, but it was expected to yield information relative to the value of the use of the specified level of disinfectant. The broilers were drained to remove excess moisture and then vacuum packaged and tray packaged as described previously. Following packaging, the poultry was stored in a display case at about 5°C and examined at intervals of 0, 3, 6, and 10 days of storage. The initial examination was performed on whole and cut-up birds. An area of 2 cm^2 on the surface of each side of the breast was swabbed (total of 4 cm^2 of breast surface) for sampling for enumeration of mesophiles, psychrotrophs, enterococci, coliforms, and lactobacilli. The remainder of the breast was swabbed on both sides for determining incidence of salmonellae, *C. perfringens* and coagulase-positive staphylococci.

Analyses were made at each interval for psychrotrophic and mesophilic aerobic bacteria for typical spoilage types, facultative "souring" types such as lactobacilli, and potential pathogens including salmonellae, staphylococci, and *Clostridium perfringens*. All samples were also evaluated for off odors by a panel of three trained judges. Salmonellae isolates were serotyped at the State Hygienic Laboratory at the University of Iowa. Microbiological test methods are outlined in Table 1. Three replications were performed and the data wereanalyzed statistically.

RESULTS & DISCUSSION

Phase I—Effect of packaging methods and cutting

Fig. 1 and 2 show that tray packed stretch wrapped poultry had the most rapid increase in bacterial counts and to the highest levels, regardless of whether the chicken was whole (Fig. 1) or cut up (Fig. 2). Spoilage occurred fastest with stretch wrapped chicken, and bacterial numbers reached levels of 10 million per cm^2 on whole birds and 100 million per cm^2 on cut-up birds within 6 days at 5°C. In contrast, vacuum packaged chicken showed only gradual increases in bacterial numbers. Bacterial counts reached about 1 million

for the low barrier (LB) whole carcasses and between 100 thousand and 1 million per cm^2 for high barrier (HB) whole birds at 10 days. Cut-up carcasses showed higher bacterial populations by comparison with whole birds.

Longest shelf life and acceptability, as determined by development of off odor, was provided by the HB film with whole carcasses, and only slightly greater counts were observed for HB cut-up poultry. All cut-up poultry spoiled faster than whole birds, but differences for HB vacuum packaged chicken were only slight. With the LB film, bacterial counts increased rather sharply between 3 and 6 days for cut-up birds as compared with whole birds. For HB whole poultry, it is possible that keeping time could be extended beyond the 10 day limit in these trials to about 12 or 13 days. These conditions probably would not apply to LB whole birds, which showed incipient spoilage and had populations of about 1 million per cm^2 by the tenth day of storage. Stretch wrapped chicken demonstrated suffi-

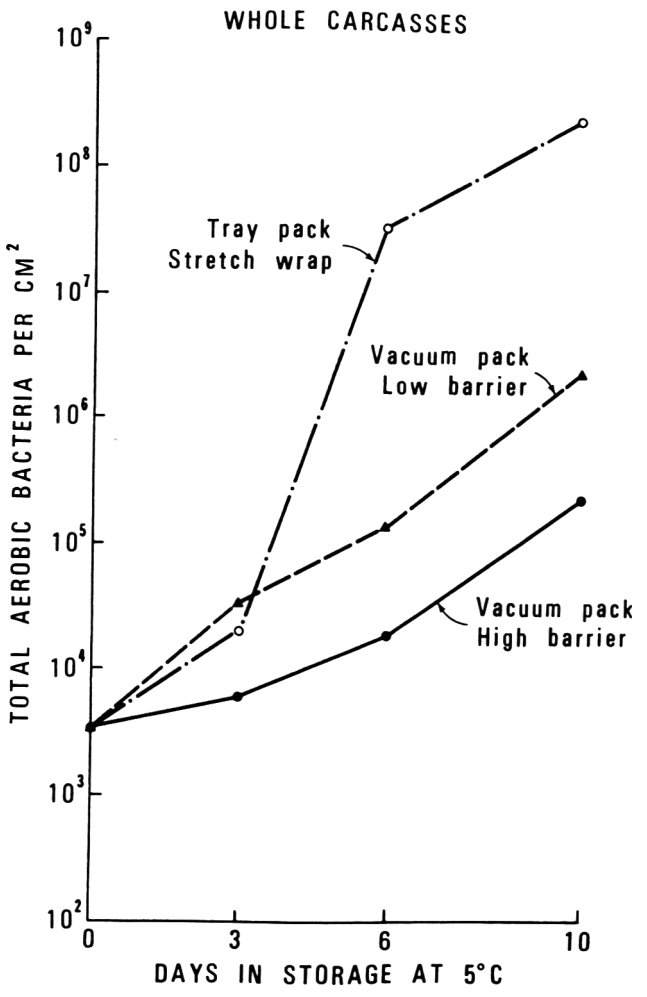


Fig. 1—Counts of total aerobic mesophilic bacteria from packaged whole chickens.

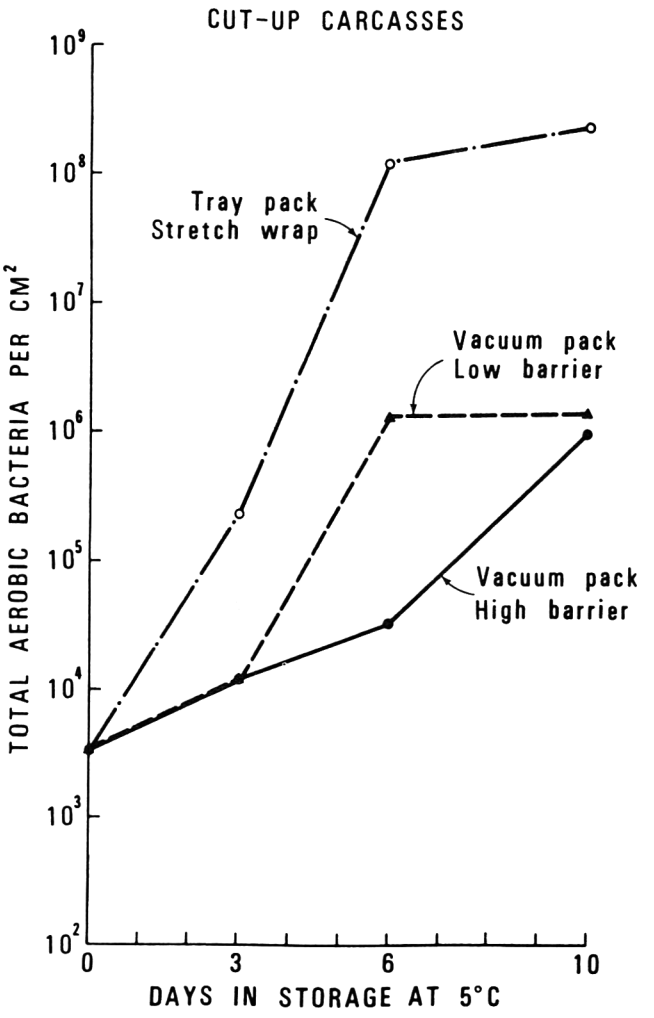


Fig. 2—Counts of total aerobic mesophilic bacteria from packaged cut-up chickens.

Table 1—Bacteriological procedures employed to determine bacterial densities or incidence of organisms

Types of microorganisms	Growth media	Plating technique	Incubation	Confirmatory tests
Mesophiles	Trypiticase soy agar (TSA, BBL)[a]	Pour plates	32°C/24–36 hr	
Psychrotrophs	TSA,BBL[a]	Pour plates	5°C/6 days	
Enterococci	KF Streptococcus medium (Difco)[b]	Pour plates	37°C/24 hr	According to Barnes (1956)
Lactobacilli	LBS agar (BBL)[b]	Pour plates	37°C/48 hr	Gram stain
Coliforms	Violet Red Bile agar (Difco)[b]	Pour plates	37°C/24–36 hr	Levine EMB agar (Difco)[b]
Staphylococcus	Staph 110 medium with egg yolk (Herman and Morelli, 1960)	Surface plating	37°C/48 hr	Tube coagulase test
Salmonella	Procedures for meats (Galton et al. 1968)	Procedures for meats (Galton et al. 1968)	37°C/24 hr	TSI and agglutination
C. perfringens	SPS agar (Angelotti et al. 1962)	Anaerobic pouches (Bladel and Greenberg, 1965)	37°C/24 hr	Motility and H_2S (Angelotti et al., 1962)

[a] BBL division of Bio Quest, Cockeysville, MD
[b] Difco Laboratories, Detroit, MI

cient coalescence of bacterial colonies to produce slime, but no slime formation occurred with either HB or LB vacuum packaged chicken, whole or cut-up.

Psychrotrophic bacteria were favored by the LB film in comparison with the HB material. This may have been due to the greater oxygen permeability of the LB film which resulted in proliferation of typical aerobic spoilage types. However, vacuum packaging retarded development of psychrotrophs and other organisms in comparison with stretch wrapping, regardless of materials used for vacuum packaging.

Lactobacilli grew fairly well on vacuum packaged chicken, reaching levels of 100,000 per cm^2 by 10 days of storage at 5°C. These organisms produced an atypical odor, not characteristic of definite spoilage, nor as pronounced as the typical odors formed by *Pseudomonas* on chicken.

In general, odor determinations correlated subjectively with bacterial counts. All stretch wrapped poultry were judged to show incipient spoilage based on odor at about 3 days. Off-odor was not evident on LB vacuum packaged chicken until about 10 days. Sour odor was associated with lactobacilli and did not become evident on HB chicken until 10 days. At that time, the odor was only moderate. HB whole chicken was still judged to be acceptable at that time. HB vacuum packaged birds probably would have been acceptable beyond 10 days by a day or two. Differences between comparably packaged whole or cut-up chicken were not great enough to be detected, although total numbers of bacteria were greater for cut up birds than for whole birds.

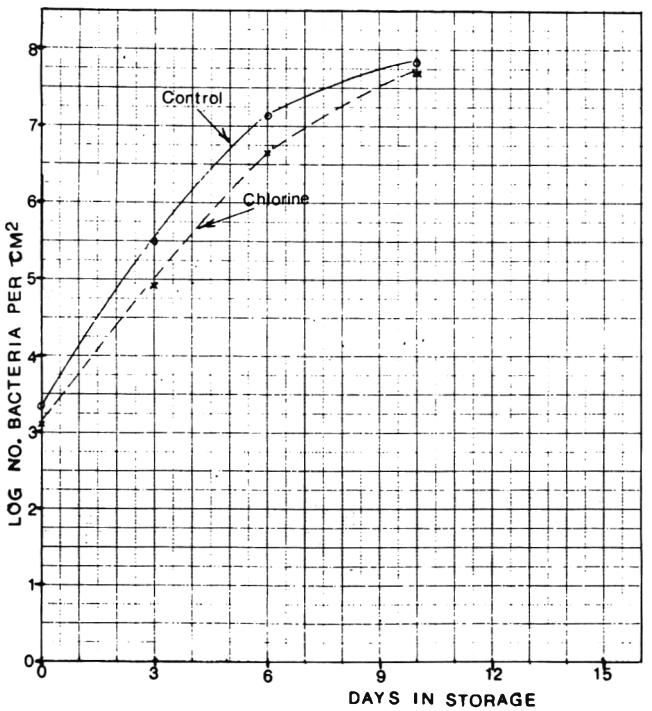


Fig. 3—Effect of chlorine treatment on total aerobic mesophilic bacteria on whole and cut-up chickens.

An analysis of variance was performed for the three replications. In addition, bacterial counts were subjected to a "t" test by pooled variance and least significant differences were calculated (Table 2). All statistical analyses in the Tables refer to overall means. Statistical analysis of counts indicated the following: As may be expected, increase in storage time produced significant increases in levels of all types of microorganisms with the exception of psychrotrophs. Psychrotrophic bacteria reached about as high numbers after 6 days as after 10 days. However, it is also possible that the actual peak in psychrotroph populations occurred between 6 and 10 days, and the 10 day count may have been associated with a downward trend. Nevertheless, growth of spoilage types over time were related to development of off-odors, as mentioned previously. Packaging materials and methods significantly affected bacterial growth, although the HB and LB films were not highly different from each other in effect for mesophilic organisms, lactobacilli, or enterococci. Counts of mesophiles on chicken packaged with HB or LB films differed significantly at the 5% level, as indicated by the shared superscript 'a' in 4.75^{a} and 5.22^{ab}. Similar statistical significance was observed for enterococci in comparing HB and LB films (1.99^{a} vs 2.65^{ab}). The principal significant difference in bacterial growth was between HB or LB vacuum packaged chicken and stretch wrapped birds. With regard to differences between populations on whole or cut up poultry, only mesophilic spoilage organisms differed significantly.

Incidence of salmonellae differed considerably between whole and cut up birds, but not among chickens packaged by vacuum with HB or LB film. Salmonellae were recovered from 11 of 27 cut up birds (40.7%), but not from any whole chickens. Cutting apparently caused contamination by salmonellae, which also was more apparent with stretch wrapped chicken. Of the salmonellae recoveries from cut-up poultry, three were from birds packaged with HB film, two from LB film packaged chicken, and six from stretch

Table 2—Effect of packaging, cutting and storage on bacterial counts of chicken

Treatment		No. of Samples	Log no. bacteria per cm^2			
			Mesophiles	Psychrotrophs	Lactobacilli	Enterococci
Days in storage	3	18	4.23^{a}	3.33^{a}	1.75^{a}	1.64^{a}
	6	18	5.85^{b}	5.33^{b}	2.99^{b}	2.63^{b}
	10	18	6.45^{c}	5.65^{c}	4.66^{c}	3.18^{bc}
Packaging material	HB	18	4.75^{a}	3.12^{a}	3.40^{a}	1.99^{a}
	LB	18	5.22^{ab}	4.80^{b}	3.75^{a}	2.65^{ab}
	SW	18	6.57^{c}	6.40^{c}	2.04^{b}	2.82^{bc}
Type of poultry	Whole	27	5.26^{a}	4.60^{a}	3.12^{a}	2.31^{a}
	Cut up	27	5.76^{b}	4.95^{a}	3.01^{a}	2.66^{a}

^{abc}For each column within a treatment, counts having different single letter superscripts are significantly different from each other at the 1% level. Counts with superscripts "ab" differ from those with "a" at the 5% level, but not at the 1% level. Counts having superscripts "bc" differ from those with "ab" or "b" at the 5% level, but not at the 1% level. Counts having the same single superscripts are not significantly different at either level.

wrapped chicken. Salmonellae isolates were serotyped by the State Hygienic Laboratory at the Univ. of Iowa College of Medicine. Four isolates were *S. heidelberg* and seven were *S. litchfield.* The latter serotype has been relatively uncommon on poultry, but its significance in this work is now known.

Staphylococci followed trends similar to those for salmonellae; 16 of 27 (59.3%) of whole birds demonstrated presence of coagulase-positive staphylococci, and almost all (25 of 27, or 92.6%) of cut-up carcasses were positive for the organisms. A high incidence of staphylococci in cut-up poultry, while not especially desirable, is also not surprising in view of handling procedures.

No *Clostridium perfringens* were recovered from any samples of packaged poultry or fresh chicken.

Phase II—Effect of chlorine dip before packaging

Fig. 3 represents the effect of 20 ppm chlorine in water on growth of mesophilic bacteria on stored packaged chicken. From the figure, it can be noted that chlorine in a water solution as a dip provided lower counts than for control chicken dipped in water with no chlorine added. In preliminary work, the chlorine concentration was found to decrease to 11 ppm after dipping the first two birds and at the end of a second dip of two birds, it further decreased to 6 ppm available chlorine. In the present study, birds were dipped at the rate of two per dip, with a fresh solution used each time. Although the initial 20 ppm availble chlorine in the water was not maintained absolutely at that level during the time of dipping, differences in bacterial counts were highly significant (Table 3) for chlorine treatment and no chlorine treatment for mesophiles and psychrotrophs on chicken. Keeping time was extended about 2 days by the use of chlorine in water (8 days compared with about 6 days for controls). The effect of in-plant chlorination on microbial flora on poultry was reported by Barnes (1972) and Ranken (1973). Further, Ziegler and Stadelman (1955) demonstrated an extended shelf life of chicken by using 10 or 20 ppm chlorinated water as a post chill dip for 5 min. Significant resistance of certain bacterial types, particularly fecal streptococci and staphylococci, was reported by Patterson (1968) when he used 20 ppm chlorine water in continuous immersion chillers in poultry processing plants. The bactericidal effect of chlorine depends upon certain important factors such as concentration, contact time, temperature, etc. Under the experimental conditions presented in this work, chlorine at 20 ppm concentrations did not significantly reduce lactobacilli, enterococci or coliforms. However, decreases were noted for these organisms for chlorine-treated samples compared with controls (Table 3).

Of the three packaging materials, the HB film provided greatest reduction of mesophilic growth, the low barrier film (LB) was next, and the stretch wrap (SW) least effective (Fig. 4), similar to the previous study (Fig. 1 and 2). Also, it may be noted that packaging materials and methods did not have as great an effect on bacterial numbers or shelf life for carcasses treated with chlorine in a dip or for controls dipped in water as they did for dry packed chicken evaluated in the previous study.

For mesophiles, differences were significant at the 5% level between HB and LB films and between LB and SW films, and at the 1% level (highly significant) between HB

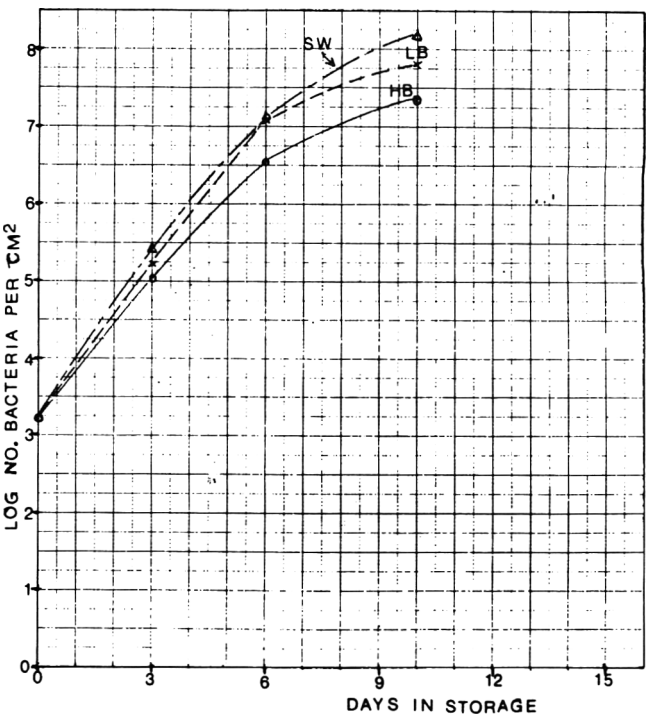


Fig. 4—Relations of packaging materials to total aerobic mesophilic bacteria on chickens.

Table 3—Effect of packaging, cutting, chlorine treatment and storage on bacterial counts of chicken

Treatment		No. of samples	Log no. bacteria per cm^2				
			Mesophiles	Psychrotrophs	Lactobacilli	Enterococci	Coliforms
Days in storage	3	36	5.22^a	4.73^a	2.60^a	1.65^a	1.55^a
	6	36	6.88^b	6.66^b	3.92^b	2.39^b	2.37^b
	10	36	7.76^c	7.60^c	4.42^{bc}	2.73^b	2.74^b
Control	0 ppm	54	6.81^a	6.54^a	3.67^a	2.35^a	2.02^a
Chlorine	20 ppm	54	6.43^b	6.12^b	3.62^a	2.16^a	2.24^a
Packaging material	HB	36	6.30^a	5.68^a	4.59^a	2.47^a	2.10^a
	LB	36	6.68^{ab}	6.45^b	4.67^a	2.56^a	2.62^a
	SW	36	6.89^{bc}	6.86^b	1.68^b	1.73^b	1.93^b
Type of poultry	Whole	54	6.48^a	6.21^a	3.54^a	2.17^a	2.13^a
	Cut-up	54	6.76^{ab}	6.45^b	3.76^a	2.34^a	2.31^a

^{abc}For each column within a treatment, counts having different single letter superscripts are significantly different from each other at the 1% level. Counts with superscripts "ab" differ from those with "a" at the 5% level, but not at the 1% level. Counts having superscripts "bc" differ from those with "ab" or "b" at the 5% level, but not at the 1% level. Counts having the same single superscripts are not significantly different at either level.

and SW films (Table 3). Psychrotrophs were significantly inhibited in growth by the HB film, with no significant difference between LB and SW packaging. For other organisms (lactobacilli, enterococci or coliforms), LB and HB were comparable in effect, but both allowed significantly lower microbial growth than the stretch wrap material. From the previous study, inhibition of aerobic spoilage from growth of psychrotrophs would be expected by the use of HB film.

Similar to earlier results with dry pack chicken (Fig. 1 and 2), cut-up poultry generally spoiled at a faster rate than whole birds (Fig. 5). However, an important observation to be noted here is that cavity odors in whole carcasses became pronounced during storage (from 3 to 6 days). This was not detected on dry packed poultry in the earlier work reported. Use of HB or LB films did not produce consistent differences regarding cavity odors. In later stages of storage, odor was more marked from cavities of whole carcasses than from cut-up chicken. Reasons for these differences are unknown, but odors were likely to be caused by spread of contamination resulting from the wetting procedure. However, in all cases, the SW chicken was least acceptable, as previously observed for dry packed birds maintained in the dry state.

Table 4 shows the occurrence of coagulase positive staphylococci, *Salmonella* spp. on the chicken samples. Incidence of these potential food poisoning types was decreased by the use of chlorine treatment. Nineteen of 54 samples treated with chlorine showed coagulase positive staphylococci compared with 45 of 54 control samples dipped in water with no chlorine. Salmonellae were present on only four of 54 chlorine treated samples compared with eleven of 54 control samples. Contamination by these organisms was favored by cutting up the chicken in comparison with whole chicken, as in the earlier study. Although highly sanitary techniques were practiced, cutting of birds resulted in spread of contamination by the potential pathogens. Chlorine treatment was effective in reducing salmonellae growth on cut-up processed poultry. The bactericidal effect of chlorine on salmonellae was demonstrated by Thomson et al. (1967) and by Dixon and Pooley (1961) using 100–200 ppm chlorine on artificially inoculated poultry carcasses. The results of this study suggest that 20 ppm chlorine is also effective in reducing salmonellae on chicken carcasses. The serotypes isolated were *S. braenderup* (7 of 15) and *S. heidelberg* (8 of 15). These two serotypes may be somewhat more resistant than other types, or they may have had a higher incidence initially on chicken samples in this study.

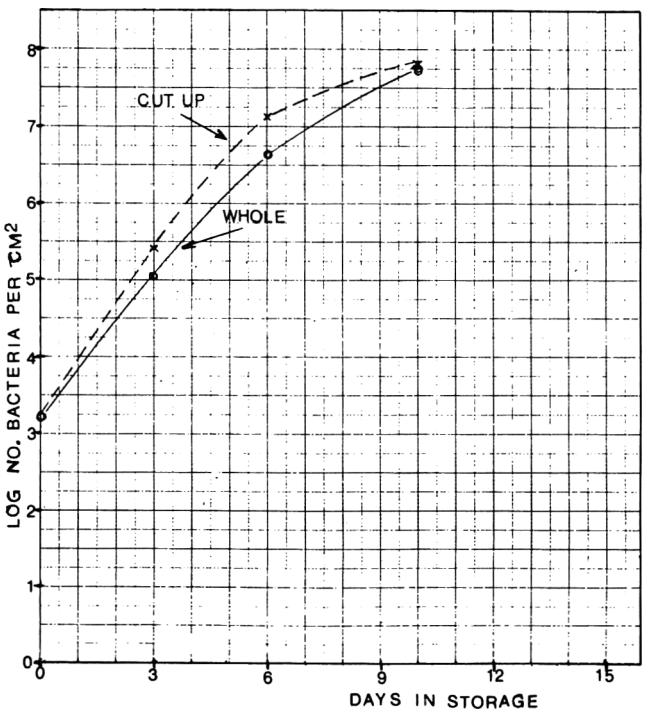


Fig. 5—Comparison of total aerobic mesophilic bacteria counts for whole and cut-up chickens.

Clostridium perfringens was recovered from only two samples before wet dip treatments and none was isolated after either of the treatments. If these organisms are considered as an index of the effect of vacuum packaging on growth of anaerobes on poultry, little or no hazard may be anticipated, assuming no temperature abuse.

From these studies, it may be noted that use of a wet dip, with or without chlorine, did not provide as good microbiological quality or shelf life as the dry pack did. In no instance were dipped birds acceptable after 10 days.

Results of this work may be summarized as follows:

1. Tray packed stretch wrapped poultry had the most rapid increase in bacterial counts, and to the highest level, compared with vacuum packaged chicken in a high or low barrier film, for both whole or cut-up chicken. Consequently, spoilage occurred most rapidly of all packaging methods with stretch wrapped poultry. In contrast, vacuum packaging dry pack broilers resulted in significantly lower bacterial counts and longer keeping time, particularly with a film having providing a high barrier to oxygen transfer.
2. Longest shelf life and best quality as determined by off odor development was provided by the high barrier film with whole carcasses.
3. Although bacterial counts were generally higher on cut-up poultry than on whole birds, these differences were not great for a given packaging material and method.
4. Incidence of salmonellae differed considerably between whole and cut-up birds, but not among chickens packaged by vacuum with HB or LB film. Salmonellae were recovered only from cut-up chickens, and were more apparent with hand wrapped stretch packaged chicken.
5. In the second phase of this work, it was shown that in general, use of a wet dip, with or without chlorine, did not provide as good microbiological quality or shelf life as maintaining the poultry in a dry condition before vacuum packaging.

Table 4—Incidence of staphylococci and salmonellae (54 samples) over the entire storage period

	Control				Total		Chlorine treated				Total	
	Whole (N = 27)		Cut up (N = 27)				Whole (N = 27)		Cut up (N = 27)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Coagulase + Staphylococci	21	77.8	24	88.9	45	83.3	7	25.9	12	44.4	19	35.2
Salmonella +	2	7.4	9	33.3	11	20.3	2	7.4	2	7.4	4	7.4

6. However, with a wet dip, when chlorine was used at a level of 20 ppm in a water solution as a dip, it provided lower total aerobic bacterial counts than observed for control chicken dipped in water with no chlorine added. Keeping time was extended about 2 days by the use of 20 ppm chlorine (to about 7 or 8 days compared with about 6 days for controls). Differences in bacterial counts were highly significant for mesophiles and psychrotrophs, but chlorine treatment did not significantly affect numbers of lactobacilli, enterococci, or coliforms. However, decreases were noted for chlorine treated carcasses.

7. Packaging materials did not have as great an effect on bacterial numbers or shelf life for carcasses treated with chlorine in a dip or for controls dipped in water as they did for dry packed chicken evaluated in earlier work. Of the three films and packaging methods, the high barrier (HB) material provided greatest restriction of bacterial growth, the low barrier film (LB) was next, and the stretch wrap (SW) least effective, similar to earlier work.

8. Similar to studies with dry pack broilers, cut-up poultry generally spoiled at a faster rate than whole birds. However, an important observation to be noted here is that cavity odors became pronounced during storage (from 3 to 6 days) of whole carcasses. This was not detected on dry packed poultry in the earlier work reported. Use of HB or LB films did not produce consistent differences regarding cavity odors. In later stages of storage, odor was more marked from cavities of whole carcasses than from cut-up chicken. In all cases, the SW chicken was least acceptable.

9. In comparing these results with the earlier phase of this work, it may be concluded that dry packed poultry provides the most acceptable product over the longest time period (at least 10 days and possibly about 12 days at 5°C), and that the HB film may be used to advantage for the greatest shelf life. If wet chilled poultry is used, a 20 ppm chlorine dip (or other method of application) is recommended, regardless of packaging materials used.

10. Incidences of salmonellae and coagulase positive staphylococci were decreased by the use of the chlorine dip. Greater incidence of these organisms occurred with cut up chicken than with whole birds. Very few *C. perfringens* were recovered, regardless of treatment.

11. Overall, dry packed chicken vacuum packaged in a high barrier film had the longest shelf life.

REFERENCES

Angelotti, R., Foter, M.J., and Lewis, K.H. 1962. Quantitation of C. perfringens in foods. Appl. Microbiol. 10: 193.

Arafa, A.S. and Chen, T.C. 1975. Effect of vacuum packaging on microorganisms on cut-up chickens and in chicken products. J. Food Sci. 40: 50.

Ayres, J.C. 1966. Microbial implications in the processing of poultry. Proceedings, Second Intl. Congress of Food Sci. and Technol. Aug., Warsaw, Poland. Dept. of Food Technology, Iowa State University, Ames, IA 50011.

Barnes, E.M. 1956. Methods for isolation of fecal streptococci from bacon factories. J. Appl. Bacteriol. 19: 193.

Barnes, E.M. 1972. Food poisoning and spoilage bacteria in poultry processing. Vet. Rec. 90: 720.

Bladel, B.O. and Greenberg, R.A. 1965. Pouch method for isolation and enumeration of clostridia. Appl. Microbiol. 13: 281.

Debevere, J.M. and Voets, J.P. 1973. Influence of packaging materials on quality of fresh poultry. British Poultry Sci. 14: 17.

Dixon, J.M.S. and Pooley, F.E. 1961. The effect of chlorination of chicken carcasses infected with Salmonellae. J. Hygiene Camb. 59: 343.

Federal Register. 1978. Poultry Inspection Regulations 43, No. 65: 14043.

Galton, M.M., Boring, J.R., and Martin, W.T. 1968. Salmonellae in foods. Communic. Dis. Center, Public Health Service, U.S. Dept. of Health, Education and Welfare, Atlanta, GA.

Herman, L.G. and Morelli, F.A. 1960. The growth and isolation of coagulase positive staphylococci on medium. No. 110 fortified with egg yolk. Bacteriol. Proc. 102.

Kraft, A.A. and Ayres, J.C. 1961. Production of fluorescence on packaged chicken. Appl. Microbiol. 9: 549.

Patterson, J.T. 1968. Chlorination of water used for poultry processing. British Poultry Sci. 9: 129.

Ranken, M.D. 1973. Chlorination and hygienic operation of spin chillers. 4th European Poultry Conf. Proc., London.

Rey, C.R. and Kraft, A.A. 1971. Effect of freezing and packaging methods on survival and biochemical activity of spoilage organisms on chicken. J. Food Sci. 36: 454.

Shank, J.L. and Lundquist, B.R. 1963. The effect of packaging conditions on the bacteriology, color and flavor of table-ready meats. Food Technol. 17: 1163.

Thomson, J.E., Banwart, G.J., Sanders, D.H., and Mercuri, A.J. 1967. Effect of chlorine, antibiotics, ß-propiolactone, acids, and washing on Salmonella typhimurium on eviscerated fryer chickens. Poultry Sci. 46: 146.

Walker, H.W. and Ayres, J.C. 1956. Incidence and kinds of microorganisms associated with commercially dressed poultry. Appl. Microbiol. 4: 345.

Wells, F.E., Spencer, J.V., and Stadelman, W.J. 1958. Effect of packaging materials and techniques on shelf life of fresh poultry meat. Food Technol. 12: 425.

Ziegler, F. and Stadelman, W.J. 1955. Increasing shelf life of fresh chicken meat by using chlorination. Poultry Sci. 34: 1389.

Ms received 7/24/81; revised 19/19/81; accepted 19/22/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 1981.

Journal Paper No. J-10342 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2380.

The authors thank Linda Halleland for typing the manuscript.

Appreciation is also expressed to American Can Co., Neenah, Wisconsin for technical assistance and partial support of this work.

PRE & POSTRIGOR BEEF PREBLENDS FOR WIENER PRODUCTION . . . From page 379

Gornall, A.G., Bardwill, C.J. and David, M.M. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751.

Hadden, J.P., Ockerman, H.W., Cahill, V.R., Parrett, N.A., and Borton, R.J. 1975. Influence of sodium nitrite on the chemical and organoleptic properties of comminuted pork. J. Food Sci. 40: 626.

Hamm, R. 1977. Post-mortem breakdown of ATP and glycogen in ground muscle: A review. Meat Sci. 1: 15.

Lew, Y.T. and Tappel, A.L. 1956. Antioxidant and synergist inhibition of hematin-catalyzed oxidative fat rancidity. Food Technol. 10: 285.

Lin, H., Topel, D.G., and Walker, H.W. 1979. Influence of prerigor and posrigor muscle on the bacteriological and quality characteristics of pork sausage. J. Food Sci. 44: 1055.

Marshall, W.H., Dutson, T.R., Carpenter, Z.L. and Smith, G.C. 1975. A simple method for emulsion end-point determination. J. Food Sci. 40: 896.

Nordin, H.R. 1969. The depletion of added sodium nitrite in ham. Can. Inst. Food Technol. 2: 7.

Olson, D.G. and Rust, R.E. 1973. Oxidative rancidity in dry-cured hams: Effect of low pro-oxidant and antioxidant salt formulations. J. Food Sci. 38: 251.

Reagan, J.O., Pirkle, S.L., Campion, D.R., and Carpenter, J.A. 1981. Processing, microbial and sensory characteristics of cooler and freezer stored hot-boned beef. J. Food Sci. 46: 838.

Sales, C.A., Bowers, J.A., and Kropf, D. 1980. Consumer acceptability of turkey frankfurters with 0, 40 and 100 ppm nitrite. J. Food Sci. 45: 1060.

Sebranek, J.G., Schroeder, B.G., Rust, R.E., and Topel, D.G. 1977. Influence of sodium erythorbate on color development, flavor, and overall acceptability of frankfurters cured with reduced levels of sodium nitrite. J. Food Sci. 42: 120.

Sweet, C.W. 1973. Activity of antioxidants in fresh fish. J. Food Sci. 38: 1260.

Swift, C.E., Lockett, C., and Fryar, A.J. 1961. Comminuted meat emulsions. The capacity of meats for emulsifying fat. Food Technol. 15: 468.

Tarladgis, B.G., Watts, B.M., and Younathan, M.T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Waldman, R.C., Westerberg, D.O., and Simon, S. 1974. Influence of curing ingredients and storage time on the quality of preblended sausage meats and frankfurters. J. Food Sci. 39: 718.

Watts, B.M. 1954. Oxidative rancidity and discoloration in meat. Adv. Food Res. 5: 1.

Zaika, L.L., Zell, T.E., Smith, J.L., Palumbo, S.A., and Kissinger, J.C. 1976. The role of nitrite and nitrate in Lebanon bologna, a fermented sausaage. J. Food Sci. 41: 1457.

Zipser, M.W. and Watts, B.M. 1962. A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. Food Technol. 16: 102.

Ms received 6/4/81; revised 9/4/81; accepted 9/7/81.

Effects of Vacuum Mixing and Precooking on Restructured Steaks

W. R. WIEBE JR. and G. R. SCHMIDT

ABSTRACT

The effects of vacuum mixing and precooking on the binding strength and cook yield of restructured steaks were evaluated. Vacuum mixing was responsible for an increased binding strength of the restructured steak, but had no effect on the cook yield. Precooked, reheated steaks had a significantly higher binding strength, but a lower cook yield than steaks cooked from the frozen, raw state.

INTRODUCTION

THE RESTRUCTURING of meat pieces into a roll or steak is of great interest to the meat industry. The restructured steak or rolls serves as an outlet for meat trimmings which would normally be used for ground beef.

Maas (1963) patented a procedure using mechanical action to aid in the formation of a creamy, tacky surface exudate which functioned in the binding of red meat pieces. This creamy, tacky surface exudate which Maas (1963) noted after mechanical action was salt-soluble protein (Schnell et al. 1970). Since the time of this patent, the use of mechanical action to enhance protein extraction has been studied extensively. Many researchers have shown that mechanical action causes muscle tissue disruption which results in the release of their intracellular contents (salt-soluble proteins). The salt-soluble proteins coat the surface of the meat pieces and upon heating, they coagulate, giving rise to the characteristic bind (Maesso et al., 1970, Schnell et al., 1970; Vadehra and Baker, 1970). Maesso et al. (1970) also suggested that mechanical action increased the surface area of the tissue, thus exposing a greater amount of protein for binding with both protein and non-protein moieties.

Currently there is interest in the use of vacuum mixing and chopping as a means of improving the binding of meat pieces. Information on this subject is limited. Solomon and Schmidt (1980) investigated the effect vacuum mixing on protein extraction and functionality in a model system. There was a significant increase in the amount of crude myosin extracted under a vacuum, but the total amount of protein extracted was not affected. Siebert (1978) and Anon (1978) stated that 20–30% more protein is extracted in a vacuum chopper as opposed to an open bowl chopper. This enhanced protein extraction resulted in a product with a firmer texture, but the water holding capacity was not affected (Anon, 1978).

The effect of precooking on beef patties was studied by Cross et al. (1979). Precooked, reheated patties were found to be significantly firmer than patties cooked from a raw state. Cooking losses for patties that were precooked and reheated were from 10–15 percentage points greater than those for non-precooked patties.

This study evaluated effects of vacuum mixing, precooking and reheating on the binding strength and cook yield of restructured steaks.

Authors Wiebe and Schmidt are with the Dept. of Animal Sciences, Colorado State Univ., Fort Collins, CO 80523.

MATERIALS & METHODS

Product preparation

Beef inside rounds were obtained from a reputable supplier, dissected free of visible fat and connective tissue, stored at 2–4°C for 2 days, and then ground through a 2.54 cm plate.

Each treatment of 13.6 kg consisted of 13.0 kg (95.75%) of beef inside rounds, 136.0g (1.00%) of salt, 34.0g (0.25%) of Heller's Soluble Phosphate (WJ-0052) and 408g (3.00%) of water. Heller's Soluble Phosphate is composed of food grade sodium tripolyphosphate and sodium hexametaphosphate.

The experiment consisted of three 4-min mixing treatments with varying vacuum exposure in a Keebler Model No. 238 mixer (37.5 kg cap.) as presented in Table 1. Each treatment was replicated four times.

The salt, phosphate and water were added prior to mixing and the meat mixture temperature was between 3 and 5°C. Samples from each treatment were removed and analyzed for fat and moisture (AOAC, 1970). The raw meat mixture contained 3% fat and 73% water, with no difference between replicates.

After mixing each raw meat mixture was placed in an E-Z Pak water driven stuffer (45 kg cap.). Approximately 2.4 kg of meat mixture was stuffed into each of four Teepak moisture-impermeable, fibrous cellulose casings (10.2 cm in diameter, 45.7 cm in length). Two of the four rolls were frozen at −30°C and the remaining two were cooked in a Vortron Smokehouse (see smokehouse schedule in Table 2) to an internal temperature of 68°C and then chilled to 4°C in the cooler. The following day four steaks, 2 cm thick were sawed from each of the frozen rolls and six steaks, 2 cm thick, from each of the cooked rolls.

Binding strength determination

The binding strength (particle to particle cohesion) of the restructured steaks was measured using the Instron Universal Testing Machine and a breaking bar and bridge assemble similar to that described by Pepper and Schmidt (1975). Two (160g) steaks from each frozen roll and each cooked roll were grilled on a Farberware Electric broiler to an internal temperature of 68°C. All steaks were turned every 3 min until the proper internal temperature time was obtained. Steaks sawed from the frozen roll were grilled from the frozen state. The internal temperature of each steak was monitored

Table 1—Mixing treatments of coarse ground beef, water, salt and phosphate

Treatment A	4 min of mixing without the application of vacuum.
Treatment B	3 min of mixing without the application of vacuum followed by 1 min of mixing with the application of 635 mm Hg vacuum.
Treatment C	4 min of mixing with the application of 635 mm Hg vacuum.

Table 2—Smokehouse schedule

Time	Dry bulb temp (°C)	Wet bulb temp (°C)
1 hr	57	0*
1 hr	63	0
1 hr	68	0
3 hr	77	0

*No humidity was added to the smokehouse.

with an O.F. Ecklund needle-type thermocouple inserted at the geometric center. The thermocouples were attached to a Leeds and Northrup Speedomax H Recorder using 20 gauge copper-constantan wire. Two (160g) steaks from each cooked roll that received no grilling were also analyzed for binding strength. All steaks were placed in a polyethylene bag and held overnight at 4°C. The following day two slices 1 cm wide were cut from each of the six 2 cm thick steaks. The 2 cm side was laid flat on the breaking unit having a base with a bridge width of 4 cm. The breaking bar traveled at a rate of 20 cm/min. and the chart at 2 cm/min. The cross-sectional area of the slice was measured at the point where the break occurred. The binding strength of the slice was expressed as the maximum force per unit cross-sectional area (g/cm^2).

Cook yield

Four steaks from each treatment were analyzed for cook yield. The ratio of the weight of the steak after its final cook to that before cooking expressed as a percentage was used to indicate cook yield.

Chemical analyses

The raw meat mixture and four cooked steaks from each treatment were analyzed for fat and moisture. Fat was determined by extraction with ether (AOAC, 1970). Moisture was determined using the vacuum oven procedure (AOAC, 1970).

Statistical analyses

Analysis of variance of the binding strength, cook yield, fat and moisture content was determined in a split plot design. When F-values were significant, Fischer's Least Significant Difference was used to locate differences between treatment means (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

THE EFFECTS of mixing treatments on the binding strength and cook yield of the restructured steaks are shown in Table 3. Products mixed in the presence of vacuum for either amount of time exhibited a higher binding strength than products mixed in the absence of vacuum. One possible explanation for increased binding is the increased protein extraction due to mixing under a vacuum (Siebert, 1978; Solomon and Schmidt, 1980). Another possible explanation is the absence of air voids at the binding junction of the meat particles. Air voids at the binding junction would prevent necessary protein-protein interactions, resulting in a product with inferior binding strength. Vacuum mixing did not influence the cook yield, moisture or fat.

Table 4 shows the effect of precooking on restructured steaks. Precooked steaks were less tender than steaks grilled from the frozen, raw state. This is indicated by their higher binding strength. They also exhibited a signifcantly lower cook yield and moisture content. There was no difference in fat content between the two cooking treatments. These results closely correlate with those Cross et al. (1979) reported for beef patties.

The effect of vacuum mixing on each cooking treatment is presented in Table 5. When vacuum mixed (for either length of time) both the precooked/not grilled and raw/grilled steaks showed a higher binding strength. No significant difference in cook yield, moisture or fat content was evident. These results closely correlate with those reported in Table 3. One possible explanation for increased binding is the increased protein extraction due to mixing in the presence of vacuum (Siebert, 1978, Solomon and Schmidt, 1980). Another explanation is the absence of air voids at the binding junction of the meat particles. The precooked/grilled steaks had a significantly higher binding strength when nonvacuum mixed for 3 min followed by a 1 min vacuum mix than when either vacuum mixed for 4 min, or nonvacuum mixed. We can speculate that 3 min mixing in the absence of vacuum increases availability of muscle cells to be swelled by the application of vacuum. This swelling results in an increased surface area available for interaction with the extraction solution and/or mixer paddles. The result is increased protein extraction and bind. The cook yield, fat and moisture content were not significantly influenced by either mixing treatment.

—Continued on page 396

Table 3—Overall effect of mixing treatments on restructured steaks[a,b,c]

	Mixing treatments		
	No vacuum 4 min	No vacuum (3 min) Vacuum (635 mm Hg) 1 min	Vacuum (635 mm Hg) 4 min
Binding strength (g/cm^2)	441.5[d]	529.8[e]	504.2[e]
Cook yield (%)	79.3	79.0	79.7
Moisture (%)	66.0	66.4	67.3
Fat (%)	3.7	3.8	3.7

[a] Each value is the mean of four replicates averaged over cooking treatments, with 12 samples per replicate.
[b] Means of the same line with different superscripts were significantly different ($P<0.05$).
[c] Means of the same line without superscripts were not significantly different.

Table 4—Overall effect of precooking on restructured steaks[a,b,c]

	Cooking treatment	
	Precooked/ grilled	Raw/ grilled
Binding strength (g/cm^2)	615.1[e]	405.3[f]
Cook yield (%)	71.9[e]	79.8[f]
Moisture (%)	64.4[e]	66.2[f]
Fat (%)	3.8	3.8

[a] Each value is the mean of four replicates averaged over mixing treatments, with 12 samples per replicate.
[b] Means of the same line with different superscripts were significantly different ($P<0.05$).
[c] Means of the same line without superscripts were not significantly different.

Table 5—Effect of mixing treatment on restructured steaks[a,b,c]

	Mixing treatment		
	No vacuum 4 min	No vacuum (3 min) Vacuum (635 mm Hg) 1 min	Vacuum (635 mm Hg) 4 min
Precooked/Not Grilled			
Binding strength (g/cm^2)	393.9[d]	477.7[e]	493.7[e]
Cook yield (%)	86.9	85.5	86.7
Moisture (%)	69.3	69.3	68.8
Fat (%)	3.4	3.8	3.8
Precooked/Grilled			
Binding strength (g/cm^2)	577.3[d]	672.6[e]	595.4[d]
Cook yield (%)	72.2	71.1	72.4
Moisture (%)	63.3	63.8	66.1
Fat (%)	4.0	3.7	3.8
Raw/Grilled			
Binding strength (g/cm^2)	353.3[d]	439.1[e]	423.4[e]
Cook yield (%)	78.8	80.4	80.2
Moisture (%)	65.5	66.1	66.9
Fat (%)	3.9	4.0	3.6

[a] Each value is the mean of four replicates with 4 samples per replicate.
[b] Means of the same line with different superscripts were significantly different. ($P<0.05$)
[c] Means of the same line without superscripts were not significantly different.

Effect of Thermal Processing on the Survival of Foot-and-Mouth Disease Virus in Ground Meat

J. H. BLACKWELL, D. RICKANSRUD, P. D. McKERCHER, and J. W. McVICAR

ABSTRACT

Foot-and-mouth disease virus was examined for its stability during cooking in tissues from infected cattle. The 0_1 (CANEFA 2) serotype of foot-and-mouth disease virus survived in lymph node tissues after heating for 2 hr at 69°C, for 1 hr but not for 2 hr at 82°C, and for 15 min but not for 0.5 hr at 90°C. Incorporation of 1% NaCl into suspensions of infected lymph nodes enhanced viral survival after heating for 0.5 hr but not for 1 hr at 90°C. The virus did not survive in either ground beef or meatballs contaminated with infected lymph node tissue, when processed to internal temperatures of 93.3, 96.1 or 98.8°C using commercial thermal processing procedures. Accurate temperature measurements were achieved with a temperature sensitive indicator disc developed in this study.

INTRODUCTION

CHEMICAL CHANGES, especially the increase in hydrogen ion concentration (formation of sarcolactic acid), that occur as part of the aging process of meat enhance the gradual inactivation of foot-and-mouth disease (FMD) virus present in muscle tissue of infected cattle (Cottral et al., 1960). However, because lymph nodes, bones and blood clots are not affected by concomitant changes accompanying rigor mortis, acid does not develop to the concentration that would inactivate FMD virus (Henderson and Brooksby, 1948). The persistence of FMD virus in these tissues at 4°C has been reported by Cottral (1969) and Cottral et al. (1960, 1963), Gailiunas et al. (1969) and Auge de Mello et al. (1966). The virus has been reported to survive in lymph nodes from slaughtered cattle kept at 4°C for 70 days, in bone marrow for 210 days, and in blood clots for 60 days. Thus, contamination of muscle tissue with the above could provide a source of viable virus even after carcass aging (Cottral, 1969; Cox et al., 1961).

In a thermal process described by Heidelbaugh and Graves (1968), FMD virus resident in lymph nodes from infected cattle was inactivated by retort cooking for 2.5 hr at 69°C. In addition, McKercher et al. (1980) reported inactivation at the same temperature when lymph node tissues were heated in a water bath. Thus, many international animal health regulations require that importation of meat from countries in which the disease is present be restricted to meat which has been cooked at that temperature. Significant economic effects, such as the easement of restrictions against meat production in FMD affected countries, development of new commercial outlets, and maintenance of a disease-free state in existing livestock populations (de las Carreras, 1978) could result from the development of cooking technology that would inactivate FMD virus in contaminated ground beef. The incorporation of the principles of industrial food manufacture, such as the tube cooking of ground beef, may lend themselves to the satisfactory development of thermal process for the manufacture of ground meat products free from FMD virus (Dimopoullos et al., 1959).

Thus, objectives of study were to evaluate the effect of commercial cooking schema on the thermal stability of FMD virus in lymph nodes, bone marrow and clotted blood and in ground meat products containing these tissues.

Authors Blackwell, McKercher, and McVicar are with the Plum Island Animal Disease Center, U.S. Dept. of Agriculture, Greenport, NY 11944. Author Rickansrud is with the Campbell Institute for Research & Technology, Campbell Soup, Camden, NJ 08101.

MATERIALS & METHODS

Virus

Foot-and-mouth disease virus 0_1 serotype (strain CANEFA 2) was originally isolated from bovine tongue epithelium in a field outbreak in Argentina and was obtained from the Comision Asesora Nacional para la Eradicacion de Fiebre Aftosa (Canefa). The virus was subsequently passaged 8 times in primary bovine kidney (BK) cell culture before use (McVicar and Sutmoller, 1969).

Cattle

Grade Hereford steers, 1.5–3 years in aged and weighing 300-400 kg. were housed in animal isolation rooms with controlled air systems as described by Callis and Cottral (1968).

Preparation of virus-infected tissues

Previous data by McVicar and Sutmoller (1976) show that high concentrations of foot-and-mouth disease virus can be recovered from lymph nodes, bone marrow and blood of cattle 48 hr after infection. Thus, tissues were collected from infected steers killed 48 hr after instillation of 7.0 $\log_{10}$ plaque-forming units (PFU) ml of virus suspension into the ventral portion of each nasal passage through a 12 cm length of sterile latex tubing attached to a 5 ml syringe (McVicar and Sutmoller, 1969).

Lymph nodes. Atlantal, mandibular, parotid, suprapharyngeal, prescapular, prefemoral and popliteal lymph nodes were collected, trimmed of fat, minced, then ground through a 3.12 mm die plate (cutting plate) of a household chopper.

Bone marrow. Long bones (radius, tibia) were collected, muscle tissie removed, and the bones cracked with a cleaver. The marrow was removed with a spoon-type spatula, stored at 4°C for 18 hr, and then ground.

Blood clots. Blood was collected from the arteries, veins and vessel linings and decanted into a sterile specimen bottle, stored overnight at 4°C to allow blood to clot, then ground and further treated as described for lymph nodes.

In order to examine the stabilizing effect of salt on virus survival during heating, tissues were also prepared containing a final concentration of 1% NaCl. Salt (NaCl) concentrations of 1% and higher enhance FMD virus stability (Cottral, 1969; Farid et al., 1976; Fellowes, 1966) and thus a universal meatball formulation containing 3% NaCl was also used in the present study.

Examination of heated tissues for survival of FMD virus

One gram samples of ground tissues were added to each of thirty-five 13 x 100 mm glass tubes kept in an ice bath. Tubes were sealed with a rubber closure and stored at 4°C until used (no more than 24 hr).

A laboratory cooking vat consisted of a 20L heavy walled glass water bath, containing 12L of distilled water. Processing temperatures (69, 82, and 90°C) were achieved and maintained by the use of a circulating heater and wand-type immersion heater. Eleven chilled tubes containing the ground tissue were placed vertically into a 13 x 26 x 7.5 cm metal support rack and completely submerged under 12.5 cm of water. A temperature monitor tube was fabricated from one of the eleven tubes by forcing a 6 x 62 mm thermocouple

—Continued on next page

through a slit in the rubber closure and into the center of the sample mass. A portion of the 180 cm cable lead extended back from the thermocouple out through the tube to a 12 channel pyrometer. When the tissue sample in the monitor tube reached the desired temperature, duplicate tubes were removed from the hot water and immersed immediately into an ice bath (0–2°C). Additional duplicate tubes were removed at 0.25, 0.5, 1.0 and 2.0 hr. Processing of the second set of samples at the next highest temperature was begun after the 2 hr samples were removed from the water bath.

The chilled samples were then aseptically removed from the tubes, pooled, placed into Ten Broeck glass tissue grinders and homogenized as a 20% suspension in HEPES buffer (N-2 hydroxyethylpiperazine-N[1]-2 ethan sulfonic acid, (Calbiochem Behring Corp., LaJolla, CA) containing 0.5% lactalbumin hydrolysate and antibiotics at a concentration of 100 units of penicillin, 100 g of streptomycin and 50g fungizone/ml. A portion of this sample then was assayed for infectivity in secondary bovine kidney cell culture. Samples negative in cell culture were inoculated into susceptible cattle.

Temperature indicator disc

To provide an objective method for confirming that a given internal product temperature was achieved, a temperature recording disc, composed of five thermosensitive spots which irreversibly changed color from silver to black when the specified temperature was achieved, was used (Telatemp Corp., Fullerton, CA). The respective temperature sensitivities were 76.7°C, 82.2°C, 87.8°C, 93.3°C, and 98.8°C. Since the temperature indicator discs must be kept dry, the discs were sealed between two 1.5 cm diameter plexiglass circles of 0.8 mm thickness and glued with clear epoxy. Preliminary tests showed that the respective temperature sensitivities were within ± 0.7% of the designated temperature.

Preparation and thermal processing of virus contaminated ground beef products

Preparation of meat samples. The ground beef and meatball samples were prepared and formulated by the Campbell Institute for Research & Technology (CIRT). Samples were stored at 4°C and used within 96 hr after preparation.

Ground beef. Frozen beef trimmings (classified at 80% lean beef) were obtained from a local abattoir. The beef trimmings were cut into 2.5 cm strips and ground twice using a Hobart meat grinder, using respective die plates of 1.6 and 0.3 cm. The ground beef was packaged in polyethylene bags for cool storage. Proximate analysis and pH measurements of the ground beef samples are presented in Table 1.

Thermal processing conditions. Ground beef (1.23 kg) was handpacked into a 17.5 x 32 cm flexible nylon cooking tube of 80 μM thickness (MQ Plastics, Freehold, NJ) as seen in Fig. 1 then overlaid with a gauze swatch. The area just above the swatch, previously determined to be the zone containing the cold spot in the tube fully packed with ground beef, was packed with 260g of ground beef contaminated with 36g of FMD virus-infected lymph node material. A temperature indicator disc was placed into the center of the contaminated ground beef which was then covered by an additional gauze swatch. The center of the swatch was pierced by the tip of a thermocouple probe whieh was then positioned in the contaminated layer, perpendicular to the indicator disc. The remainder of the tube was packed with 0.9kg of ground beef. A weight was tied to the bottom of the tube to prevent floating and the entire unit placed in a 45 x 47.5 cm stainless steel vat of boiling water. Thermal processing was initiated within 1 hr after preparation of the contaminated layer. Water temperatures were maintained at 98–100°C by use of two circulating heat pumps (Haake, E-52, Fisher Scientific, Springfield, NJ), four quartz-type immersion heaters and flowing steam from a 18 kg service line. The meat was cooked to an internal temperature of 98.8°C, at which time it was removed, placed in an ice bath, and left undisturbed until the product temperature cooled to 70°C or lower. The contaminated layer was recovered by making a lengthwise cut through the nylon tube and congealed meat and removing the ground meat layer contained between the two gauze swatches. An approximate 50-g sample was removed from the center of the layer, homogenized as a 20% suspension in minimum essential medium (MEM) (F-15, GIBCO, NY) and clarified at 800 x *g* for 20 min at 4°C. The supernatant fluid was assayed for infectivity in bovine kidney cell cultures, then stored at −70°C. If cell culture was negative for the detection of virus, samples were inoculated into cattle.

Meatball preparation. Ground beef for meatball preparation was placed into a Seydelmann cutter and chopped at low speed while ingredients listed in Table 2 were added over a 2 min period. An additional 3 min at high speed was used to thoroughly chop and mix ingredients.

The mix was handformed into 10 ± 2g meatballs, of approximately 2.2 cm diameter, using a 3.2 cm diameter ice cream scoop. The meatballs were then placed in a wire mesh basket and case-hardened by submerging in boiling water for 10 sec. Case-hardening was necessary to prevent the meatballs from sticking together or changing shape during thermal processing.

Meatballs (),57 kg) were added to the cooking tube to a height of 10 cm (zone containing predetermined cold spot). Meatballs which served as monitors for virus inactivation were prepared as follows: 9g of meatball mixture of a universal formulation containing 3% NaCl (Table 2) were mixed with 1.0g FMD virus infected lymph node material containing 3% NaCl (Table 2). The case-hardened, monitor meatballs were then sliced in half and a tem-

Table 1—Proximate analysis of raw ground beef and raw meatball mix

	Raw ground beef, % Sample No.			Raw meatball mix, % Sample No.		
Component[a]	1	2	3	1	2	3
Solids	36.9	37.2	37.2	41.0	41.5	41.5
Protein	19.0	18.8	18.8	18.3	17.5	17.5
Fat	16.9	17.2	17.2	10.1	10.5	10.5
Ash	0.9	0.9	0.9	4.1	4.0	4.0
N free extract (NFE)[b]	0.1	0.3	0.3	8.5	9.5	9.5
Salt	<0.1	<0.1	<0.1	3.1	3.0	3.0
pH c	6.0	6.0	6.0	6.2	6.2	6.2

[a] Analysis by AOAC procedures
[b] NFE by difference
[c] pH measured on a blended slurry of 10g meat sample + 100 ml distilled water

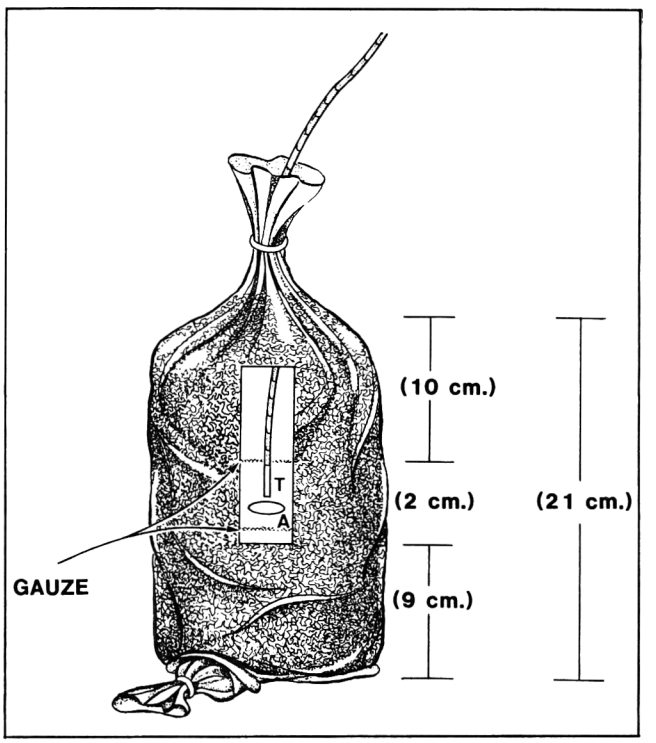


Fig. 1—Schematic of nylon cooking tube containing FMD virus-contaminated ground beef showing placement of temperature indicator disc (A) and thermocouples (T).

perature indicator disc placed in the center of the bottom one-half and both halves pressed together. A thermocouple probe was then inserted through the center of the upper half of the meatball until it touched the indicator disc perpendicularly. The monitor meatball was secured to the thermoucouple probe with a gauze square and tied with string (Fig. 2). The monitor meatball was positioned at the cold spot. Another monitor meatball was positioned one layer of meatballs above the first one. The remaining 1.7 kg meatballs were then added to the cooking tube. An additional meatball containing a thermocouple probe alone was placed at the top of the tube to measure temperature in that portion of the tube (Fig. 3). The meatballs then were covered with 1250 ml tap water. The tube was weighted down, tied off, placed into a vat and processed as described with ground meat. The meatballs were cooked to final internal "cold spot" temperatures of 93.3, 96.1 and 98.8°C respectively. After reaching the respective temperature, the tubes were immediately placed into an ice bath and left undisturbed until the temperature of the meatballs reached 70°C or lower. The two virus contaminated meatballs were removed, combined, homogenized as 20% suspension and assayed for infectivity as described below in detection of virus.

Detection of virus

Confluent cell culture monolayers of secondary bovine kidney cell culture were grown in MEM supplemented with 5% bovine serum in 6 well disposable plates (Linbro Plastics, Inc., Hamden, CT) at 37°C in a humidified 3% CO_2 environment.

Serial tenfold dilutions of 20% supernatant fluids of the heated samples were prepared in chilled MEM after thermal processing and clarification. The remainder of the sample was stored at −70°C. An inoculum of 0.05 ml of sample was adsorbed onto the plate culture monolayer for 1 hr at 37°C. The cells were then overlayered with 3 ml of 1.2% methylcellulose suspension, diluted with an equal volume of MEM and antibiotics as previously described, and then incubated at 37°C for 18 hr. Monolayers were then fixed and stained with crystal violet solution in 20% formalin, rinsed with distilled water and air-dried. Plaques were enumerated macroscopically and virus concentration recorded as PFU per ml. A minimum of three replicates per dilution sample were observed.

Table 2—Meatball formulation

Ingredient		Formula Weight Composition
Ground beef (80% beef trimmings)		65.0%
Farinaceous Material:		12.0%
a. soy concentrate	5.0%	
b. isolated soy protein	2.0%	
c. starch	5.0%	
Salt		3.0%
Seasonings:		3.5%
a. onion powder	2.0%	
b. pepper	0.2%	
c. garlic powder	0.2%	
d. parsley flakes	0.1%	
e. hydrolyzed vegetable protein	1.0%	
Caramel color		0.5%
Water		16.0%
TOTAL		100.0%

[a] Meatball formula based on the requirements that meatballs contain a minimum of 65% beef and not more than 12% farinaceous material.

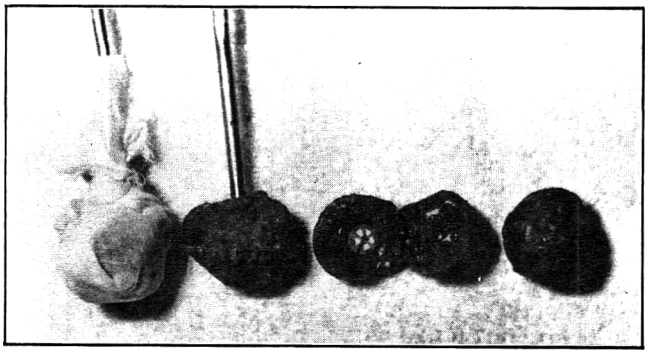


Fig. 2—Preparation of temperature monitor meatball showing right to left, intact meatball, placement of temperature indicator disc in sliced meatball, positioning of thermocouple perpendicular to disc and securing meatball and thermocouple with gauze.

Cattle inoculation

Those samples negative for detection of virus by cell culture assay were thawed rapidly in a 37°C water bath, and 2 ml were inoculated intradermally (ID) at 20 sites of the tongue and 5 ml were inoculated intramuscularly into the gluteal region of each of two steers. The animals were observed daily for development of vesicular lesions. When vesicular lesions developed, samples of material were harvested and typed serologically. Blood and esophageal-pharyngeal (EP) fluids (Sutmoller and Cottral, 1967) were collected daily for a period of 10 days for the presence of FMD virus.

The sera of cattle which remained clinically negative for 21 days were examined for the presence of antibody to virus infection associated (VIA) antigen (Cowan, 1968) and of virus neutralizing activity.

RESULTS

Presence of FMD virus in tissues of infected cattle

Foot-and-mouth disease virus was detected in lymph nodes, bone marrow and clotted blood of infected donor cattle. The mean virus titers (log_{10} PFU/ml found were: lymph node tissues (5.1), clotted blood (4.1) and the lowest in the bone marrow (2.34). Mean titer of tissues containing 1% NaCl were: lymph nodes (5.5), clotted blood (4.7) and bone marrow (1.6).

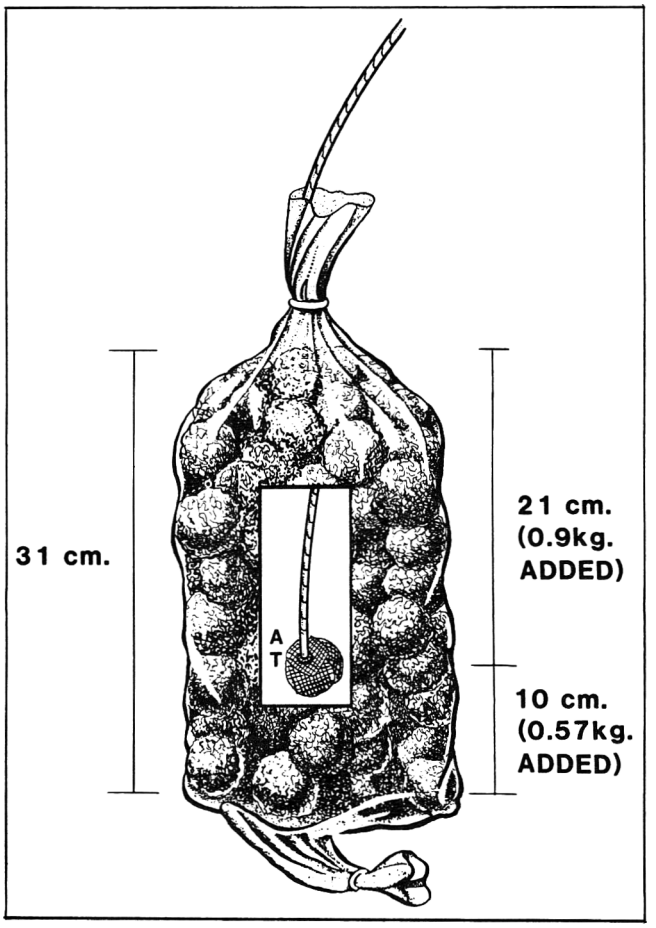


Fig. 3—Schematic diagram of nylon cooking tube containing FMD virus contaminated meatballs showing placement of temperature indicator disc (A) and temperature monitor meatball (T).

Survival of virus in tissues after thermal processing

Lymph node tissue, which had the highest infectivity titer of the 3 tissues examined, was used in the initial heating trials. Virus survival after a particular processing condition (temperature/exposure period) was considered as a positive finding for all tissues. However, all three tissues had to be tested and found negative before a given processing conditions was considered to be effective. Virus survival was detected only by cell culture infectivity assay in the clotted blood sample containing 1% NaCl, sampled immediately after coming to temperature at 69°C. As observed by animal inoculation, the virus survived heating for 2 hr at 69°C, for 1 hr at 82°C and for 0.25 hr at 90°C (Table 3). The virus survived heating at 90°C for 0.5 hr but not for 1.0 hr when 1% NaCl was incorporated into the tissues.

Survival of virus in contaminated ground beef after tube cooking

Complete correlation between thermocouple readings and temperature of activation of temperature sensitive paint in the indicator disc was observed.

Foot-and-mouth disease virus was not detected in ground beef or meatball preparations after cooking in nylon tubes to internal temperatures of 98.8°C (Fig. 4). Although FMD virus was not detected in the raw contaminated ground beef preparations by cell culture assay, FMD was produced in cattle by inoculation with two of three preparations (Table 4). The virus concentration of the lymph node contaminant in meatball preparations (3% salt) remained stable (Table 5).

The mean processing time to reach an internal temperature of 98.8°C in the ground beef was 3.4 hr. Approximately 1 hr of this time was spent at or above 90°C (Table 4). As seen in Fig. 4 and in Table 5, mean processing times for the meatball preparations were varied: 93.3°C – 16.3 min (3.3 min at or above 90°C); 96.1°C – 20.3 min (6.6 min at or above 90°C); 98.8°C – 39.6 min (19.6 min at or above 90°C).

None of the cattle inoculated with heated ground beef or meatball samples had virus in the blood or EP fluids nor developed clinical FMD, serum neutralizing activity or antibody against VIA antigen during the 21 day observation period.

DISCUSSION

THE EFFECTIVENESS of the thermal process in inactivating Type 0_1 (CANEFA 2) FMD virus was examined in tube cooking of virus-contaminated ground beef products.

In contrast to the ease of detection of FMD virus in ground infected LN material, the virus was not detected by cell culture assay in ground beef contaminated with this same infected tissue, sampled within 1 hr after preparation. Rapid inactivation of the virus in ground lymph node-muscle mixture was attributed to the acidic environment. This conclusion is supported by a report by Cottral (1969) on the inactivation of FMD virus in muscle meat.

In the meatball mixture, although the pH was only 0.2 of a pH unit higher (pH 6.2) than the ground beef mixture, virus was not inactivated by the low pH. The relatively high salt concentration (3%) of the mixture, in all likelihood was a major factor in stabilizing the virus present in the ground lymph node-meatball mixture (Cottral, 1969).

Exposure to an acidic environment, absence of stabilizing compounds and subsequent heating at high temperatures, contributed to the inactivation of the virus in ground beef.

The inactivation of FMD virus in meatballs was due solely to heat. The pH effect was negated by the presence of salt.

As shown by the cooking curves presented in Fig. 4, thermal processing time was greatly reduced by convection versus conduction heating.

Methods currently accepted for monitoring the cooking

Table 3–Survival[a] of FMD virus in infected lymph nodes[b] after thermal processing

Processing temp °C	Processing time (hr) 0.25	0.5	1	2
69	+	+	+	+
82	+	+	+	–
90	+	–[c]		

[a] + FMD produced in cattle; –FMD not produced in cattle.
[b] Mean infectivity titer of raw lymph node material (3 trials): 5:1 $\log_{10}$ PFU/g.
[c] Positive with 1% NaCl, negative in cattle after heating sample for 1 hr.

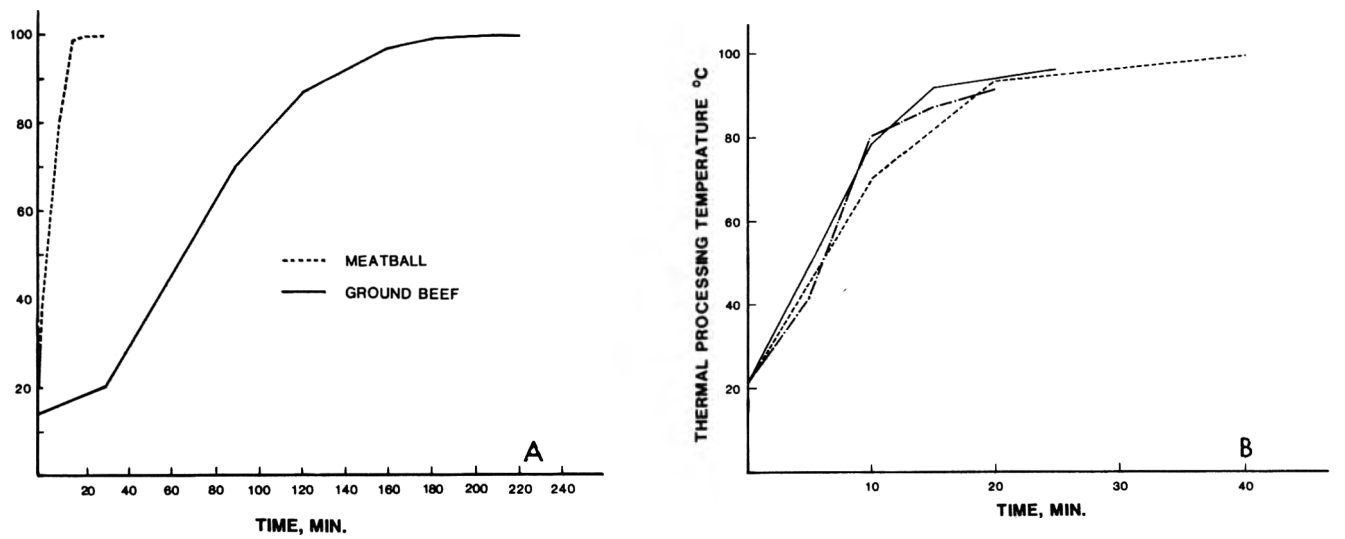


Fig. 4–Thermal processing curves of ground meat products: (A) Comparison of thermal processing curves of ground beef and meatballs plus water cooked to an internal temperature of 98.8°C; (B) Comparison of processing curves for meatballs cooked to internal temperatures of 93.3 (– · –), 96.1 (- · · -), and 98.8°C (——).

Table 4—Survival of FMD virus in ground beef after commercial thermal processing

Replicate No.	Time to reach internal Temp 98.8°C at cold spot hr	Elapsed cooking time from 90°C to 98°C & return to 90°C hr	Total elapsed cooking time from 14°C to 98.8°C & return to 70°C & below hr	Virus recovery log_{10} PFU/g		Response in animals	
				Raw lymph nodes	Lymph node contaminated ground beef	Unheated sample	Heated sample
1	3.5	1.08	4.0	4.6	<0.4[a]	2/2[b]	0/2
2	3.0	0.83	3.5	4.0	<0.4	0/2	NT
3	3.75	1.16	4.25	4.0	<0.4	2/2	0/2

[a] Plaques not observed in 0.15 ml of sample
[b] No. steers positive per No. steers inoculated

Table 5—Survival of FMD virus in meatballs (3% NaCl) after commercial thermal processing

Sample cooked to internal Temp, °C	Replicate no.	Time to reach desired internal temp at cold spot min	Elapsed cooking time from 90°C to desired temp & return to 90°C min	Total elapsed cooking time from 14°C to desired internal temp & return to 70°C and below min	Virus recovery log_{10} PFU/g		Response in animals
					Unheated sample	Heated sample	
93.3							
	1	19	4	20	3.0	<0.4[a]	0/2[a]
	2	14	2	15	3.0	<0.4	0/2
	3	16	4	17	3.0	<0.4	0/2
96.1							
	1	24	8	25	3.5	<0.4	0/2
	2	18	5	19	2.6	<0.4	0/2
	3	19	7	20	2.6	<0.4	0/2
98.8							
	1	49	19	55	3.0	<0.4	0/2
	2	32	19	35	3.0	<0.4	0/2
	3	38	21	43	3.0	<0.4	0/2

[a] Plaques not observed In 0.15 ml of sample.
[n] No. steers positive per No. steers inoculated.

process of meats for export in countries where FMD and other exotic diseases are endemic, are of a qualitative and highly subjective nature. Results of this study, however, demonstrate that quantifiable temperature measurements can be achieved with devices using temperature sensitive pigments.

Tube cooking of infected ground beef and ground beef products at the temperatures and conditions examined appears to be an adequate manufacturing schema for the inactivation of 0_1 (CANEFA 2) serotype of FMD virus.

The above report also provides an impetus for the development of methods to verify the time and temperature conditions necessary to inactivate FMD virus in meat products.

REFERENCES

Auge de Mello, P., Honigman, M.N., and Fernandes, M.V. 1966. Supervivencia en bovinas del virus modificado de la fiebre aftosa. Bull. Off. int. Epiz. 65: 2091.

Callis, J.J., and Cottral, G.E. 1968. Methods for containment of animal pathogens at the Plum Island Animal Disease Laboratory. In "Methods in Virology," Ed. K. Maramorosch and H. Koprowski, p. 465. Academic Press, New York, NY.

Carreras, de las, A.E. 1978. Foot-and-mouth disease and the world meat trade. Presented at XI Inter-American Meeting, at the ministerial level, on foot-and-mouth disease and zoonoses control, World Health Organization, Washington, DC, April 11—14.

Cottral, G.E. 1969. Persistance of foot-and-mouth disease virus in animals, their products and the environment. Bull. Off. int. Epiz. 71: 549.

Cottral, G.E., Cox, B.F., and Baldwin, D.E. 1960. The survival of foot-and-mouth disease virus in cured and uncured meat. Am. J. Vet. Res. 21: 288.

Cottral, G.E., Gailiunas, P., and Campion, R.L. 1963. Detection of foot-and-mouth disease virus in lymph nodes of cattle throughout the course of infection. Proc. 67th Ann. U.S. Livest. Assoc., p. 463.

Cowan, K.M. 1968. Immunochemical studies on FMD. IV. Preparation and evaluation of antisera specific for virus, virus protein subunit and the infection-associated antigen. J. Immun. 101: 1183.

Cowan, K.M. and Trautman, R. 1967. Immunochemical studies of foot-and-mouth disease. 1. Complement fixation reactions with isolated antigenic components. J. Immun. 99: 729.

Cox, B.F., Cottral, R.E., and Baldwin, D.E. 1961. Further studies on the survival of foot-and-mouth disease virus in meat. Am. J. Vet. Res. 22: 224.

Dimopoullos, G.T., Fellowes, O.N., Callis, J.J., Poppensiek, G.C., Edward, G.A., and Graves, J.H. 1959. Thermal inactivation and antigenicity studies of heated tissue supension containing foot-and-mouth disease virus. Am. J. Vet. Res. 20: 510.

Farid, A., Moussa, A.A.M., Daoud, A., and Moussa, A.Y. 1976. The influence of mono- and dicationic salts on the stability of foot-and-mouth disease virus at different temperatures. J. Egypt. Vet. Med. Assoc. 36: 108.

Fellowes, O.N. 1966. Influence of salts on foot-and-mouth disease virus. Appl. Microbiol. 14: 280.

Gailiunas, P., Cottral, G.E., and Scott, F.W. 1969. Survival of foot-and-mouth disease virus on meat packaging materials. Proc. 73rd Ann. Meeting U.S. Anim. Health Assoc. p. 425.

Heidelbaugh, N.D. and Graves, J.H. 1968. Effects of some techniques applicable in food processing on the infectivity of foot-and-mouth disease virus. Food Technol. 22: 120.

Henderson, W.M. and Brooksby, J.B. 1948. The survival of foot-and-mouth disease virus in meat offal. J. Hyg. 46: 394.

McKercher, P.D., Morgan, D.O., McVicar, J.W., and Shout, N.J. 1980. Thermal processing to inactivate viruses in meat products. Proc. 84th Annu. Meet. U.S. Anim. Health Assoc.

McVicar, J.W. and Sutmoller, P. 1969. The epizootiological importance of foot-and-mouth disease carriers. 2. The carrier status of cattle exposed to foot-and-mouth disease following vaccination with an oil adjuvant inactivated virus vaccine. Arch. ges. Virusforsch. 26: 217.

McVicar, J.W. and Sutmoller, P. 1976. Growth of foot-and-mouth disease virus in the supper respiratory tract of non-immunized, vaccinated and recovered cattle after intranasal inoculation. J. Hyg. Comb. 76: 467.

Sutmoller, P. and Cottral, R.E. 1967. Improved techniques for the detection of foot-and-mouth disease virus in carrier cattle. Arch. ges. Virusforsch. 21: 170.

Ms received 7/31/81; revised 9/25/81; accepted 9/28/81.

The authors acknowledge the encouragement of Drs. J.J. Callis and J. Roswurm, USDA in these studies and thank Ms. M. Hardy, Messrs. H. Kislow, W. Parrish, PIADC, and Ms. C. Luker, Food Research Institute, Univ. of Wisconsin, Madison, for their technical assistance.

Aroma, Color, and Lipid Oxidation of Turkey Muscle Emulsions

C. SUSAN TELLEFSON, JANE A. BOWERS, CECELIA MARSHALL, and ARTHUR D. DAYTON

ABSTRACT

Turkey emulsions were prepared with (1) no additives, (2) sodium chloride (NaCl), (3) sodium nitrite ($NaNO_2$), (4) sodium ascorbate (NaAsc), or (5) both $NaNO_2$ and NaAsc. Raw and cooked emulsions from each of the five treatments were stored (−18°C) and then evaluated before and after heating. Emulsions with $NaNO_2$ and NaAsc contained less malonaldehyde than those with NaCl or no additive and raw turkey emulsions generally contained less malonaldehyde than cooked. Nitrite was the additive that produced the major effect on color of heated emulsions. Generally, emulsions containing both $NaNO_2$ and NaAsc had the most meaty aroma and the least stale aroma. Emulsions with NaCl tended to have greater stale aroma.

INTRODUCTION

CURING MIXTURES for meat and poultry usually contain salt, nitrite, ascorbate or erythorbate, and spices. Combined, they contribute to cured-meat flavor and can influence oxidation rate and development of stale or rancid aroma and flavor. Their individual roles in promoting or inhibiting oxidation in turkey products have not been fully analyzed. Most research has dealt with pork, beef, and model systems.

Studying the effects of salt, Olson and Rust (1972) found that flavor of dry cured hams were preferred when a salt with low heavy metal content was used instead of regular salt, indicating that trace metals may have caused the prooxidant effects rather than the NaCl. Labuza (1971) theorized that NaCl may affect the water activity, and in turn the oxidative stability. Refrigerated cured pork was found to be more stable to lipid oxidation than uncured meat (Bailey and Swain, 1973; Zipser et al., 1964), but Zipser et al. (1964) concluded that NaCl promoted oxidation of frozen cured pork. Ellis et al. (1968) reported that $NaNO_2$ caused greater oxidation of frozen cured bacon than NaCl and concluded that the oxidative effects of NaCl and $NaNO_2$ were independent and additive.

Research with nitrite (MacDonald et al., 1980) indicated that nitrite in a model system can be a prooxidant at concentration greater thatn 25 ppm, but nitrite reduced oxidation rates when systems contained ferrous iron or ferrous iron-EDTA. Giddings (1977) stated that when the iron atom of the cured meat myoglobin had nitric oxide attached to the 6th position, the compound was very stable. Heat-denatured, cured myoglobin is probably a dinitrosylhemochrome (Cassens et al., 1979) which may affect oxidation differently than the nonheat-denatured pigment. Not all of the nitrite added to meat reacts with myoglobin; generally about 1−5% of added nitrite reacts with lipid (Cassens et al., 1977), particularly unsaturated fatty acids (Frouin et al., 1975). Warmed-over-flavor (WOF) development in meat was inhibited by nitrite (Sato and Hegarty, 1971).

Author Bowers, Marshall and Dayton are with the Departments of Foods & Nutrition and Statistics, Kansas State Univ., Manhattan, KS 66506. Author Tellefson formerly with Kansas State Univ., is now affilitated with Western Illinois Univ., Macomb, IL 61455.

While low levels of ascorbic acid promoted oxidation in model systems, higher levels inhibited oxidation in model systems and in beef (Sato and Hegarty, 1971). High levels of ascorbate inhibited oxidation in turkey emulsions also (Tellefson and Bowers, 1980). Sato and Hegarty (1971) concluded that ascorbic acid retarded autoxidation by upsetting the balance between Fe^{++} and Fe^{+++} or by O_2 scavenging.

Plate waste of bacon cured with or without nitrite was not different (Williams and Greene, 1979), but Sales et al. (1980) found that consumers preferred turkey frankfurters cured with nitrite to those without nitrite. Rancid aroma and flavor in turkey frankfurters decreased as nitrite concentration increased (Sales et al., 1980).

This experiment was conducted to compare malonaldehyde content (oxidation), color, and aroma of turkey muscle emulsions made with sodium chloride, sodium nitrite, and/or sodium ascorbate and subjected to cooking, frozen storage, and further heating.

EXPERIMENTAL

Sample preparation

Muscle and fat from one turkey breast were used per replication. Raw turkey emulsions were prepared using a Waring Blendor (6.45 cm plate) using 304g ground turkey, 80g ice water, and (1) no other additions, (2), 20,000 ppm sodium chloride (NaCl), (3) 62 ppm sodium nitrite ($NaNO_2$), (4) 520 ppm sodium ascorbate (NaAsc), or (5) 62 ppm $NaNO_2$ and 520 ppm NaAsc. The turkey emulsions were stuffed into synthetic casings using a Mirro cookie gun with a special attachment and then hand-tied into links. Half of the links from each of the five treatments were cooked at 176.5°C to an internal temperature of about 60°C and then cooled (4°C). The other links were not cooked. Both raw and cooked links were vacuum-packaged and stored for 5 wk at −18°C. After storage, emulsions were evaluated without heating and after heating by boiling in water (raw links for 5 min and cooked links for 3 min).

Lipid oxidation and color

Lipid oxidation of raw and cooked turkey muscle emulsions was determined immediately after processing, after 5 wk frozen storage (−18°C), and after storage and heating using a modification (Kuntapanit, 1978) of the extraction procedure (Witte et al., 1970) for malonaldehyde which used 2-thiobarbituric acid. Values for duplicate samples were reported as mg malonaldehyde/kg turkey emulsion.

Color of turkey muscle emulsions was determined with a Hunter-Lab Colorimeter immediately after processing, after 5 wk frozen storage, and after storage and heating. Values for the standardizing tile were Rd = 58.3, a = +5.00, b = +49.52.

Sensory evaluation

Raw and cooked links from each of the five treatments were thawed and evaluated at room temperature and after heating in boiling water. Heated links were split, put in covered glass petri dishes, and immediately placed on a warming tray and held warm while panelists judged them. Red lights were used to mask color differences. Five experienced panelists evaluated the turkey emulsions for meaty and stale aromas after 5 wk frozen storage by marking a 13-cm. structured scale to represent intensity of meatiness (raw meaty or cooked meaty) and staleness. The measurement in cm between the beginning of the scale and the mark was used for statistical analysis; larger numbers indicated a greater intensity.

—Continued on next page

Analysis of variance was completed using data from all five panelists and from all combinations of two to four panelists. To examine repeatability of each panelist, variances of individual panelist's scores by treatment were calculated. Values reported are those from the entire five-member panel and also those from a subgroup of three panelists who were selected on the basis of low variance and ability to detect differences.

Experimental design and data analysis

Five replications for sensory evaluation and four for malonaldehyde and color measurements were completed using a split-plot design:

Source of variation	Degrees of freedom: Sensory evaluation	Degrees of freedom: Color & Malonaldehyde measurements
Turkey (Rep)	4	3
Treatment	4	4
State (raw or cooked)	1	1
Treatment x State	4	4
Error (a)	36	27
Type (unstored, stored, and stored and heated)[a]	1	2
Treatment x Type	4	8
State x Type	1	2
Treatment x State x Type	4	8
Error (b)	40	60

[a] For sensory evaluation data, the types are stored and stored and heated.

When F values were significant, LSD's were computed (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

Malonaldehyde content

Treatment means are presented for malonaldehyde and color measurements and sensory scores in Tables 1–5. For some measurements, means are presented for each of the 20–30 treatment combinations. But if there were no significant three-way interactions, then significant two-way interaction or main effects treatment means are presented.

Cooked turkey muscle emulsions had more malonaldehyde (MA) than raw emulsions unless nitrite was present, in which cases there were no differences between the raw and cooked states (Table 1). Raw and cooked emulsions with no additives or with sodium chloride (NaCl) oxidized more than emulsions with sodium ascorbate (NaAsc) or sodium nitrite ($NaNO_2$), and emulsions with a combination of $NaNO_2$ and NaAsc had the lowest MA content. In raw products there was no difference in MA content between the emulsions containing $NaNO_2$ and those containing NaAsc, but cooked emulsions containing NaAsc had more MA than cooked emulsions with $NaNO_2$ or raw emulsions with NaAsc.

With added nitrite, alone or in combination with ascorbate, MA content among the types of emulsions (unstored, frozen storage, and stored and heated) were similar (Table 1). Stored and heated emulsions that did not contain nitrite had greater MA contents than unstored or frozen-stored emulsions. Unstored, frozen-stored, and stored-heated emulsions with both $NaNO_2$ and NaAsc generally had less MA than those receiving the other treatments.

After 5 wk of frozen storage, turkey emulsions containing NaAsc were similar in MA content to unstored emulsions with NaAsc and to frozen-stored emulsions with $NaNO_2$; however, when the stored emulsions were heated, those with NaAsc were oxidized more than those with $NaNO_2$. Emulsions containing either no additives or NaCl had similar MA contents; MA values increases with storage and with heating and tended to be greater than for treatments containing $NaNO_2$ and/or NaAsc.

There were no differences in MA content of turkey muscle emulsions with no additives and with added NaCl, which indicates that salt was not acting as a prooxidant. This is in agreement with the findings of Zipser et al. (1964) for uncured pork. In our study, $NaNO_2$ was not found to exhibit prooxidant effects during frozen storage as Ellis et al. (1968) reported with bacon, but rather produced antioxidant effects such as Sato and Hegarty (1971) found with beef. While NaAsc was as effective as $NaNO_2$ in preventing MA development in raw turkey muscle emulsions at the levels used, their combined effect in preventing oxidation was greater (possibly synergistic), and MA content of emulsions containing both NaAsc and $NaNO_2$ did not increase after 5 wk frozen storage or after being stored and heated.

Color

Cooking lightened (higher Rd value) the color of unstored and frozen-stored emulsions (Table 2). Raw emulsions stored frozen for 5 weeks were darker in color than those unstored. Rd values of cooked emulsions were not affected by frozen storage or heating.

Table 2—Mean HunterLab Colorimeter Rd and "b" values of turkey muscle emulsions made with NaCl, $NaNO_2$, and/or NaAsc[a]

Type	Raw	Cooked
Rd values		
Unstored	40.50a	47.94bc
5 wk frozen storage	35.77b	48.00c
Stored and heated	47.11c	47.75c
"b" values		
Unstored	15.30a	13.56b
5 wk frozen storage	15.36a	12.73c
Stored and heated	13.00c	12.92c

[a] Mean of 20 values averaged over treatment and replication; values in the same row or column not showing a common letter are significantly different ($P < 0.001$).

Table 1—Mean malonaldehyde content (mg/kg emulsion) of turkey muscle emulsions made with NaCl, $NaNO_2$ and/or NaAsc[a]

Treatment	State[b]: Raw	State[b]: Cooked	Type[c]: Unstored	Type[c]: 5 wk frozen storage	Type[c]: Stored and heated
No additives	2.66a	3.43[b]	2.22ab	2.84c	4.07d
20,000 ppm NaCl	2.97a	3.76[b]	2.73a	3.38c	3.98d
62 ppm $NaNO_2$	1.34bc	1.60c	1.60bc	1.41b	1.40b
520 ppm NaAsc	1.54c	2.66[e]	1.34cef	1.55bf	3.41d
62 ppm $NaNO_2$ and 520 ppm NaAsc	0.69d	0.62[d]	0.69e	0.66e	0.62e

[a] Values in the same row or column under State or Type not showing a common letter are significantly different ($P < 0.01$).
[b] Means averaged over type and replication
[c] Means averaged over state and replication

Table 3—Mean HunterLab Colorimeter "a" values of turkey muscle emulsions made with NaCl, $NaNO_2$ and/or NaAsc[a]

Treatment	Raw			Cooked		
	Unstored	After 5 wk frozen storage	Stored and heated	Unstored	After 5 wk frozen storage	stored and heated
No additive	7.63a	5.93b	0.29cgh	2.09dj	2.01dei	0.24ck
20,000 ppm NaCl	6.22b	4.17c	0.08dg	1.70aj	1.26ad	0.36dk
62 ppm $NaNO_2$	3.57ci	6.28bd	2.96fi	2.07ejk	2.81efi	3.03f
520 ppm NaAsc	7.89ag	7.85g	1.11h	2.01hijk	2.26i	1.35h
62 ppm $NaNO_2$ and 520 ppm NaAsc	4.09ce	9.04a	4.53e	2.88bdk	4.38cg	4.69eg

[a] Mean of four replications; values in the same row or column not showing a common letter are significantly different ($P < 0.001$).

Cured color as determined by "a" (redness) values was influenced by treatment, state and type (Table 3). With all treatments, cooked unstored and frozen-stored turkey muscle emulsions were less red (lower "a" values) than raw ones. Stored-heated emulsions, whether stored raw or cooked, had similar "a" values within a treatment. Frozen-stored and stored-heated emulsions containing both $NaNO_2$ and NaAsc were redder than frozen-stored and stored/heated emulsions of other treatments. When both $NaNO_2$ and NaAsc were added, raw and cooked emulsions were redder after 5 wk frozen-storage. With no additives and with added NaCl, unstored raw emulsions were redder than frozen-stored ones, and heating after storage caused further decreases in redness. Raw emulsions with NaAsc retained redness during frozen-storage, but redness decreased with heating. The antioxidant properties of ascorbate promotes its function as a meat color stabilizer by reducing metmyoglobin. Rikert et al. (1957) reported that ascorbic acid stabilized ground meat color.

In the raw unstored emulsions, links stuffed with the emulsions containing NaCl appeared very splotchy (pink), but during storage the color became more uniform. The presence of nitrite was generally the major factor affecting the color of heated emulsions.

Determinations of "b" color values indicated no differences in unstored turkey muscle emulsions due to treatment (Table 4), but cooked emulsions had lower "b" values (less yellowness) than raw emulsions except after storage and heating (Table 2). Raw and cooked emulsions that were stored and heated were less yellow than unstored emulsions (Table 2). Heating after storage decreased yellowness with all treatments (Table 4).

Table 4—Mean HunterLab Colorimeter "b" values of turkey muscle emulsions made with NaCl, $NaNO_2$ and/or NaAsc[a]

Treatment	Unstored	After 5 wk frozen storage	Stored and heated
No additives	14.45a	14.29a	13.68bc
20,000 ppm NaCl	14.47a	14.20ab	13.23cd
62 ppm $NaNO_2$	14.39a	14.05ab	12.44de
520 ppm NaAsc	14.58a	14.34a	13.64bc
62 ppm $NaNO_2$ and 520 ppm NaAsc	14.13a	13.34b	11.83e

[a] Mean of eight values averaged over state and replication. Means in the same row or column not showing a common letter are significantly different ($P < 0.01$).

Aroma evaluation

Meaty aroma. When significant differences were detected, samples containing both $NaNO_2$ and NaAsc generally had the most meaty aroma (Table 5). Meaty aroma of unheated turkey emulsions stored raw was not affected by additives. If raw turkey emulsion was heated after storage, samples with both $NaNO_2$ and NaAsc were found by the 3 member sensory panel to have more meaty aroma than those with NaCl, $NaNO_2$, or NaAsc, but not more than samples with no additives. The three-member sensory panel observed that unheated turkey emulsions cooked prior to frozen storage

Table 5—Mean meaty and stale aroma scores of turkey muscle emulsions made with NaCl, $NaNO_2$ and NaAsc[a]

	Unheated						Heated					
	No Additives	20,000 ppm NaCl	62 ppm $NaNO_2$	62 ppm $NaNO_2$ 520 ppm NaAsc	520 ppm NaAsc	(LSD)	No Additives	20,000 ppm NaCl	62 ppm $NaNO_2$	320 ppm NaAsc	62 ppm $NaNO_2$ 520 ppm NaAsc	(LSD)
Stored Raw												
Meaty												
3 panelists	5.4	5.4	3.8	3.4	3.7	NS	7.1	5.7	6.0	6.4	8.4	**(1.6)
5 panelists	5.3	5.2	4.2	3.9	3.9	NS	6.6	6.2	6.8	6.4	7.5	NS
Stale												
3 panelists	5.4	4.3	3.9	3.9	2.2	**(1.6)	5.0	6.8	6.1	4.9	3.5	***(1.4)
5 panelists	6.4	6.2	3.9	4.2	2.3	***(1.5)	5.9	7.3	5.1	4.8	3.7	***(1.1)
Stored Cooked												
Meaty												
3 panelist	3.9	4.7	4.4	5.1	6.3	*(1.6)	4.6	4.9	5.6	6.6	7.7	***(1.3)
5 panelists	5.1	5.5	5.6	4.9	6.1	NS	4.8	5.0	5.5	6.4	7.0	*(1.6)
Stale												
3 panelists	6.6	7.8	6.0	6.4	4.9	*(1.6)	6.8	7.7	5.0	5.6	4.3	***(1.3)
5 panelists	6.7	8.2	5.9	7.8	4.2	**(1.9)	6.6	7.8	4.3	4.9	4.0	***(1.1)

[a] Mean of five replications. Larger numbers indicate a more intense meaty or stale aroma
* ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$)

had more meaty aroma if $NaNO_2$ and NaAsc were present than did emulsions subjected to any other treatment except NaAsc; upon further heating, samples with either NaAsc or $NaNO_2$ plus NaAsc had similar meaty aromas which were greater than those of samples with no additives or with NaCl.

Stale aroma. Turkey emulsions containing $NaNO_2$ plus NaAsc that were stored raw for 5 wk had less stale aroma (had less oxidative rancidity) than any other treatment (Table 5). Turkey emulsions stored raw were less stale if they contained ascorbate compared with no additives or NaCl, according to the 5-member panel. Unheated and heated emulsions with $NaNO_2$ and NaAsc that were cooked prior to frozen storage had less stale aroma than those with no additives or with NaCl. Emulsions with NaCl tended to have greater stale aroma than other treatments, especially if they were heated after storage. The prooxidant properties of NaCl on meat had been recognized by others (Ellis et al., 1968). Three-member vs five-member panel.

Three-member vs five-member panel

Data presented in Table 5 indicated that the three-member sensory panel detected more significant differences among treatments applied to the turkey muscle emulsions than the five-member sensory panel. Both panels found differences in stale aroma for stored raw and cooked emulsions evaluated either unheated or heated, but the five-member panel was less successful in detecting differences in meaty aroma. Both panels tended to score treatments similarly and similar trends were found. Since the three-member panel was able to detect a greater number of significant differences, perhaps panelists' variability should be considered more carefully than is often done in research and attempts made to find the best set of panelists for a particular study.

We found that a combination of $NaNO_2$ and NaAsc in turkey emulsions generally resulted in the least stale and most meaty aroma as well as being reddest in color and having the lowest MA values. This was true whether samples were stored raw or cooked.

REFERENCES

Bailey, M.E. and Swain, J.W. 1973. Influence of nitrite on meat flavor. Proc. Meat Ind. Res. Conf. 29.

Cassens, R.G., Greaser, M.L., Ito, T., and Lee, M. 1979. Reactions of nitrite in meat. Food Technol. 33: 46.

Cassens, R.G., Woolford, G., Lee, S.H., and Goutefongea, R. 1977. Fate of nitrite in meat. [From Tinbergen, B.J. and Krol, B. (1977). "Proceedings of the Second International Symposium on Nitrite in Meat Products."] PUDOC, Wageningen, The Netherlands.)

Ellis, R., Currie, G.T., Thornton, F.E., Bollinger, N.C., and Gaddis, A.M. 1968. Carbonyls in oxidizing fat. 11. The effect of the pro-oxidant activity of sodium chloride on pork tissue. J. Food Sci. 33: 555.

Frouin, A., Jondeau, D., and Thenot, M. 1975. Studies about the state and availability of nitrite in meat products for nitrosamine formation. 21st European Meet., Meat Res. Workers, Berne, Switzerland.

Giddings, G.G. 1977. The basis of color in muscle foods. J. Food Sci. 42: 288.

Kuntapanit,C. 1978. Beef muscle and adipose lipid deterioration as affected by nutritional regime, vacuum aging, display and carcass conditioning. Ph.D. thesis, Kansas State Univ., Manhattan, KS.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. Critical Reviews in Food Technol. 2: 355.

MacDonald, B., Gray, J.I., and Gibbins, L.N. 1980. Role of nitrite in cured meat flavor: Antioxidant role of nitrite. J. Food Sci. 45: 893.

Olson, D.G. and Rust, R.E. 1973. Oxidative rancidity in dry-cured hams: effect of low pro-oxidant and antioxidant salt formulations. J. Food Sci. 38: 251.

Rikert, J.A., Bressler, L., Ball, C.O., and Stier, E.F. 1957. Factors affecting quality of prepackaged meat. Food Technol. 11: 567.

Sales, C.A., Bowers, J.A., and Kropf, D. 1980. Consumer acceptability of turkey frankfurters with 0, 40, and 100 ppm nitrite. J. Food Sci. 45: 1060.

Sato, K. and Hegarty, G.R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36: 1098.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. Iowa State University Press, Ames.

Tellefson, C.S. and Bowers, J.A. 1981. Effects of ascorbate and nitrite concentrations in turkey frankfurter-type products. Poultry Sci. 60: 579.

Williams, J.C. and Greene, B.E. 1979. Plate waste of bacon cured with and without sodium nitrite. J. Food Sci. 44: 1260.

Witte, V.C., Krause, G.F., and Bailey, M.E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. 35: 582.

Zipser, M.W., Kwon, T.W., and Watts, B.M. 1964. Oxidative changes in cured and uncured frozen cooked pork. J. Agr. Food Chem. 12: 105.

Ms received 8/3/81; revised 10/9/81; accepted 10/31/81.

Contribution No. 81-125-j, Dept. Foods & Nutrition. Kansas Agricultural Experiment Station. Manhattan, KS 66506.

MIXING AND PRECOOKING OF RESTRUCTURED STEAKS . . . From page 387

CONCLUSIONS

VACUUM MIXING for either length of time resulted in a steak with a higher binding strength. There was no significant difference in cook yield due to mixing treatment. Steaks precooked and then grilled exhibited a higher binding strength, but a lower cook yield than those grilled from the frozen, raw state. It is doubtful that the advantage of a firmer texture is sufficient to offset the disadvantages of increased cooking losses and energy expenditures necessary to precook, cool and reheat the product.

REFERENCES

Annonymous. 1978. Advantages of vacuum chopping in sausage manufacturing. Meat Industry 24(10): 65.

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

Cross, H.R., Muse, D., and Green, E.C. 1979. Effects of precooking beef patties on palatability, cooking properties and storage stability. J. Food Sci. 44: 869.

Maas, R.H. 1963. Processing meat. U.S. Pat. 3,076,713.

Maesso, E.R., Baker, R.C., Bourne, M.C., and Vadehra, D.V. 1970. Effect of some physical and chemical treatments on the binding quality of poultry loaves. J. Food Sci. 34: 440.

Pepper, F.H. and schmidt, G.R. 1975. Effect of blending time, salt, phosphate and hot-boned beef on binding strength and cook yield of beef rolls. J. Food Sci. 40: 227.

Schnell, P.G., Vadehra, D.V., and Baker, R.C. 1970. Mechanism of binding chunks of meat. 1. Effect of physical and chemical treatments. Can. Inst. Food Tech. J. 3(2): 44.

Siebert, H. 1978. A look at the plusses of vacuum cutting. Food in Canada. 38(3): 26.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods." 6th ed. The Iowa State University Press, Ames, IA.

Solomon, L.W. and Schmidt, G.R. 1980. The effect of vacuum and mixing time on the extractability and functionality or pre and postrigor beef. J. Food Sci. 45: 283.

Vadehra, D.V. and Baker, R.C. 1970. The mechanism of heat initiated binding of poultry meat. Food Technol. 24: 766.

Ms received 5/4/81; revised 11/4/81; accepted 11/4/81.

This investigation was supported by a research grant from The Old West Regional Commission.

Comparison of Subcutaneous Fat Thickness, Marbling and Quality Grade for Predicting Palatability of Beef

H. G. DOLEZAL, G. C. SMITH, J. W. SAVELL and Z. L. CARPENTER

ABSTRACT

Beef from yearling steers (n = 254) which were fed either grass only or high-concentrate diets was used to study subcutaneous fat thickness as an alternative method for grading beef carcasses. Assigning carcasses to three expected-palatability groups based on fat thickness was at least equivalent to, and perhaps slightly more precise than, the use of USDA quality grades for grouping the carcasses according to expected palatability. There were progressive increases in palatability of cooked beef as fat thickness of carcasses from cattle fed 90–160 days increased from less than 2.53 mm up to 7.61 mm, but quantities greater than 7.61 mm did not further improve palatability.

INTRODUCTION

SOME RESEARCHERS have reported close associations between USDA quality grade and cooked beef palatability (McBee and Wiles, 1967; Covington et al., 1970; Jennings et al., 1978; Davis et al., 1979); others have concluded that USDA quality grades (Romans et al., 1965; Berry et al., 1974; Campion et al., 1975; Garcia-de-Siles et al., 1977) or marbling scores (Tuma et al., 1962; Breidenstein et al., 1968; Parrish et al., 1973) provide little assurance that beef will be palatable. Recently, there have been efforts to identify alternative systems for segmenting the beef supply into expected palatability groupings. Smith (1980) suggested that a specific time-on-feed period and/or subcutaneous fat thickness may be possible adjuncts to, or substitutes for, the present method (maturity, marbling, quality grade) of predicting beef palatability.

Adams et al. (1977), Harrison et al. (1978), Tatum et al. (1980) and Dolezal et al. (1982) reported that beef from cattle that have been fed a high-concentrate diet for a specified period of time will be acceptable in palatability, irrespective of its quality grade. Although the time-on-feed concept appears to be valid, complications would arise in monitoring such a system which would greatly limit its usefulness for grading or palatability prediction on a national basis.

Fattening is one of the consequences of feeding a high-energy diet to cattle. For centuries, fattening has been thought to improve the palatability of beef. The mechanism by which fattening improved tenderness was partially clarified when Smith et al. (1976) found that increased thickness of subcutaneous fat on lamb caused carcasses to chill more slowly, increased enzyme activity, lessened sarcomere shortening and improved meat tenderness. Subsequent investigations have substantiated and partially characterized the relationship between tenderness and subcutaneous fat thickness in beef (Dutson et al., 1975; Bowling et al., 1977, 1978; Meyer et al., 1977; Tatum, 1978; Lochner et al., 1980; Marsh and Lochner, 1981; Tatum et al., 1982). That research has generally shown that 6–10 mm of subcutaneous fat thickness is sufficient to retard the postmortem chilling process in order to assure that beef from young cattle will be tender; however, the usefulness of this carcass trait for grading purposes has not been fully explored. As compared with the present system of quality grading in which marbling is used as the basis for reflecting differences in fatness, an approach which gives consideration to fatness based on thickness of subcutaneous fat could (1) be more easily and uniformily applied and (2) would be easier to relate to grades for slaughter cattle.

The population of rib steaks used by Dolezal et al. (1982) to examine effects of time-on-feed on beef palatability offered an opportunity to determine the validity of the hypothesized relationship of subcutaneous fat thickness of carcasses to palatability of beef in a population of cattle for which exact feeding history was known. In the present study, usefulness of subcutaneous fat thickness, marbling and USDA quality grade for predicting palatability of rib steaks was examined using the complete population of steaks and subsequently, using only those steaks from cattle in specified time-on-feed strata.

EXPERIMENTAL

A PART OF THE POPULATION of rib steaks used by Dolezal et al. (1982) were used in the present study. Yearling steers (n = 254) of multiple breed-origin that had never been fed grain or that had been fed grain for 30–160 days were obtained from a number of sources (Dolezal et al., 1982); included were cattle of Brahman, British (Shorthorn, Angus, Hereford), continental European (Charolais, Maine-Anjou, Simmental, Limousin) and dairy (Holstein, Jersey, Brown Swiss) breeding and crosses of most of these breeds. Grass-fed steers (0 day time-on-feed) were from two sources (La. Agr. Expt. Sta., Homer, LA; Texas Agr. Expt. Sta., Overton, TX) and were maintained entirely on millet-bermuda grass or coastal bermuda grass pastures until slaughter. Grain-fed steers (30, 60, 90, 100, 130, 160 days time-on-feed) were from four sources (La. Agr. Expt. Sta., Homer, LA; Texas Agr. Expt. Sta., Overton, TX; Monfort of Colorado, Gilchrest, CO; Harrell Cattle Company, Gonzales, TX) and were fed high-concentrate finishing diets of generally similar energy-density for the designated periods of time (Table 1).

—*Continued on next page*

Table 1—Diet energy levels for steers from each group in the study

N	Time-on-feed (days)	Diet energy level[a] NEm, Mcal/kg	NEg, Mcal/kg
39	0	—	—
29	30	1.54	.98
20	60	1.58	.85
38	90	1.57	.89
40	100	1.52	.95
43	130	1.52	.96
45	160	1.52	.96

[a] Expressed on a 100% dry matter basis; values for diet energy levels were calculated from actual diets fed to each group of animals using values from NRC (1976).

All authors were affiliated with the Meats & Muscle Biology Section, Dept. of Animal Science, Texas Agricultural Experiment Station, Texas A&M Univ., College Station, TX 77843 at the time this study was conducted. Author Dolezal is presently employed by the Dept. of Animal Science, Colorado State Univ., Fort Collins, CO 80523.

Upon termination of each feeding period, steers were slaughtered conventionally in two different plants with similar chilling conditions (temperatures and times) and, at 24 hr postmortem, yield and quality grade (USDA, 1975) data were obtained. Ribs were aged for 14–16 days postmortem at 2 ± 1°C and then fabricated such that two steaks (3.2 cm thick) were obtained from the 9th–11th rib section. Steaks were wrapped in polyethylene-coated freezer paper, frozen at −34°C, stored at −20°C for 4–6 months, thawed at 2°C and broiled to an internal temperature of 70°C on Farberware Open-Hearth broilers. Samples from the 9th rib steaks were served, while warm, to an experienced 8-member sensory panel for evaluations of palatability using 8-point descriptive scales (8 = extremely juicy, extremely tender, no detectable connective tissue, extremely desirable flavor and extremely desirable overall palatability; 1 = extremely dry, extremely tough, abundant connective tissue, extremely undesirable flavor and extremely undesirable overall palatability). The 10th rib steaks were cooked to 70°C, allowed to cool to room temperature (23°C) and then cored (1.27 cm in diameter) for Warner-Bratzler shear force determinations.

Data were analyzed by analysis of variance (Snedecor and Cochran, 1967) and mean separation analysis (Kramer, 1956, modification of the Duncan, 1955, multiple range test) with the Statistical Analysis System package (Barr et al., 1979).

Table 2–Means, standard deviations and coefficients of variation for carcass characteristics and palatability attributes

Item	Mean	Std dev.	Coefficient of variation
Carcass characteristic			
USDA maturity score[a]	147.1	22.8	15.5
USDA marbling score[b]	243.2	142.9	58.8
USDA quality grade[c]	336.4	82.3	24.5
Fat thickness, mm	8.3	5.5	66.6
Longissimus muscle area, cm^2	74.2	13.4	18.0
Estimated kidney, pelvic and heart fat, %	1.9	.7	38.1
Carcass weight, kg	276.8	71.6	25.9
USDA yield grade	2.3	.9	39.5
Palatability attribute			
Juiciness[d]	5.0	.7	13.8
Myofibrillar tenderness[e]	5.5	1.1	20.3
Connective tissue amount[f]	6.6	.7	10.0
Overall tenderness[e]	5.4	1.1	21.1
Flavor desirability[g]	5.4	.7	12.9
Overall palatability[g]	5.1	1.0	19.4
Shear force, kg	5.4	2.4	44.4

[a] Maturity score: 100—199 = A; 200—299 = B.
[b] Marbling score: 0—99 = practically devoid; 100—199 = traces; 200—299 = slight; 300—399 = small; 400—499 = modest; 500—599 = moderate; 600—699 = slightly abundant; 700—799 = moderately abundant; 800—899 = abundant.
[c] Quality grade (USDA, 1975): 200—299 = Standard; 300—399 = Good; 400—499 = Choice; 500—599 = Prime.
[d] 8 = extremely juicy; 1 = extremely dry.
[e] 8 = extremely tender; 1 = extremely tough.
[f] 8 = none; 1 = abundant.
[g] 8 = extremely desirable; 1 = extremely undesirable.

RESULTS & DISCUSSION

MEANS, STANDARD DEVIATIONS and coefficients of variation for carcass characteristics and palatability attributes are presented in Table 2. Marbling score and fat thickness were the most variable of the carcass characteristics, while shear force was the most variable palatability attribute.

In this population, all but five carcasses received scores of "A" for overall maturity (these five were in the "B" maturity group) therefore, no maturity adjustment was made for analyses involving marbling score. Data presented in Table 3 indicate that, in general, sensory panel ratings increased and shear force values decreased as marbling score increased. Similar results were reported by McBee and Wiles (1967), Jennings et al. (1978) and Tatum et al. (1980). Steaks from carcasses with "modest" or more marbling received higher ($P < 0.05$) ratings for juiciness, tenderness, flavor desirability and overall palatability and had lower ($P < 0.05$) shear force values than did steaks with "slight-minus", "traces" or "practically devoid" degrees of marbling. Tatum et al. (1980) reported that palatability ratings were similar for steaks ranging in marbling score from "typical-slight" to "small-plus." In the present study, no significant differences were observed in any of the palatability attributes among steaks from carcasses with "small," "slight-plus," or "slight-typical" degrees of marbling.

USDA quality grade and cooked beef palatability have been reported to be closely associated by McBee and Wiles (1967), Covington et al. (1970), Jennings et al. (1978) and Davis et al. (1979). Data presented in Table 4 show that steaks from Standard carcasses received the lowest ($P < 0.05$) ratings for all of the palatability attributes except juiciness. Steaks from Choice carcasses received the highest ($P < 0.05$) ratings for juiciness, flavor desirability and overall palatability and had the lowest ($P < 0.05$) shear force values; however, steaks from Good carcasses were comparable to steaks from Choice carcasses in tenderness and organoleptically detectable connective tissue. Steaks from Prime carcasses were deleted from this analysis because there were only four in the population.

Steaks from Choice carcasses received the highest percentages of "very desirable" ratings and the lowest percentages of "undesirable" ratings for overall tenderness,

Table 3–Mean values for palatability attributes stratified according to marbling group

Palatability attribute	USDA marbling score group[a]							
	8	7	6	5	4	3	2	1
Number of observations	41	37	22	20	20	61	31	22
Juiciness[b]	5.31^{f}	5.08^{fg}	4.89^{gh}	5.16^{fg}	4.77^{gh}	4.75^{h}	4.95^{gh}	4.86^{gh}
Myofibrillar tenderness[c]	6.08^{f}	5.77^{f}	5.67^{fg}	5.94^{f}	5.22^{gh}	5.29^{g}	4.72^{h}	5.07^{gh}
Connective tissue amount[d]	6.75^{f}	6.76^{f}	6.80^{f}	6.77^{f}	6.53^{fg}	6.45^{fg}	6.15^{g}	6.28^{g}
Overall tenderness[c]	5.96^{f}	5.71^{f}	5.57^{fg}	5.84^{f}	5.08^{gh}	5.15^{g}	4.55^{h}	4.94^{gh}
Flavor desirability[e]	5.83^{f}	5.72^{fg}	5.54^{fgh}	5.60^{fgh}	5.39^{ghi}	5.28^{hij}	4.98^{ij}	5.00^{ij}
Overall palatability[e]	5.77^{f}	5.44^{f}	5.30^{f}	5.49^{f}	4.82^{g}	4.84^{g}	4.50^{g}	4.54^{g}
Shear force, kg	3.91^{f}	4.54^{fg}	5.17^{gh}	5.23^{gh}	5.39^{gh}	6.11^{hi}	6.83^{i}	6.35^{hi}

[a] 8 = modest and higher; 7 = small; 6 = slight-plus; 5 = slight-average; 4 = slight-minus; 3 = traces-average and traces-plus; 2 = practically devoid-plus and traces-minus; 1 = practically devoid-average and lower (USDA, 1975).
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant.
[e] 8 = extremely desirable; 1 = extremely undesirable.
[f,g,h,i,j] Means in the same row bearing a common superscript letter are not significantly ($P > 0.05$) different.

flavor desirability and overall palatability (Table 5). The highest percentages of "undesirable" ratings for the same three palatability attributes were observed for steaks from the lowest quality (Standard) carcasses. Assignment of USDA quality grades to this population of carcasses created three groups of rib steaks of significantly different palatability.

It has been postulated that categorizing beef carcasses according to subcutaneous fat thickness might serve as effectively as quality grading for segregating beef into palatability groups (Smith, 1980). To test the validity of that hypothesis, data of the present study were examined using the complete population of rib steaks and, subsequently, using only those steaks from cattle in specified time-on-feed strata. For the complete population (n = 254), sensory panel ratings generally increased and shear force values generally decreased as fat thickness of the carcass from which the steaks were obtained increased; however, no significant improvement in palatability of steaks was observed in association with fat thicknesses greater than 12.69 mm (Table 6). Steaks from carcasses with at least 10.16 mm of subcutaneous fat thickness received higher ($P < 0.05$) ratings for tenderness, connective tissue amount, flavor desirability and overall palatability and had lower ($P < 0.05$) shear force values than did steaks from carcasses that had less than 5.08 mm of fat thickness. Steaks from carcasses with less than 2.54 mm of fat thickness received the lowest ($P < 0.05$) ratings for connective tissue amount, overall tenderness, flavor desirability and overall palatability and had the greatest ($P < 0.05$) resistance to shear force.

Among carcasses (n = 97) with at least 10.16 mm of subcutaneous fat, 96%, 99% and 90% were rated at least "desirable" in overall tenderness, flavor, and overall palatability, respectively (Table 7). Almost 63% of the steaks from carcasses with less than 2.54 mm of fat thickness were rated "undesirable" in overall palatability while about 10% of the steaks from carcasses with at least 10.16 mm of subcutaneous fat received "undesirable" overall palatability ratings.

In general, results presented in Table 6 segmented this population of carcasses into three fat thickness categories—5.07 mm or less; 5.08–10.15 mm; 10.16 mm or greater—with regard to palatability of cooked rib steaks. Therefore, additional analyses were conducted to determine the magnitude of palatability differences among steaks from carcasses in these fat thickness groups (Table 8). Except for juiciness ratings, consistent and statistically significant sensory panel and shear force differences were observed among steaks from carcasses in the three fat thickness groups. In addition, steaks from carcasses with at least 10.16 mm of subcu-

Table 4—Mean values for palatability attributes stratified according to USDA quality grade

Palatability attribute	USDA quality grade[a]		
	Choice	Good	Standard
Number of observations	74	61	115
Juiciness[b]	5.18[f]	4.94[g]	4.82[g]
Myofibrillar tenderness[c]	5.93[f]	5.60[f]	5.11[g]
Connective tissue amount[d]	6.74[f]	6.70[f]	6.34[g]
Overall tenderness[c]	5.84[f]	5.49[f]	4.96[g]
Flavor desirability[e]	5.74[f]	5.51[g]	5.15[h]
Overall palatability[e]	5.59[f]	5.20[g]	4.70[h]
Shear force, kg	4.20[f]	5.29[g]	6.33[h]

[a] USDA (1975).
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant.
[e] 8 = extremely desirable; 1 = extremely undesirable.
[f,g,h]Means in the same row bearing a common superscript letter are not significantly ($P > 0.05$) different.

Table 5—Frequency percentages of steaks within each of three levels of overall tenderness, flavor desirability and overall palatability stratified according to USDA quality grade

Item	USDA quality grade[a]		
	Choice	Good	Standard
Number of observations	74	61	115
Overall tenderness[b]			
"Very desirable"	50.0	41.0	23.5
"Desirable"	45.9	41.0	46.9
"Undesirable"	4.1	18.0	29.6
Flavor desirability[b]			
"Very desirable"	44.6	24.6	11.3
"Desirable"	52.7	65.6	71.3
"Undesirable"	2.7	9.8	17.4
Overall palatability[b]			
"Very desirable"	36.5	24.6	8.7
"Desirable"	52.7	54.1	56.5
"Undesirable"	10.8	21.3	34.8

[a] USDA (1975).
[b] "Very desirable" = mean sensory panel ratings of 6.00 or higher; "Desirable" = mean sensory panel ratings of 4.50—5.99; "Undesirable" = mean sensory panel ratings lower than 4.50.

Table 6—Mean values for palatability attributes of steaks from grass-fed steers and from steers fed a high-concentrate diet for 30, 60, 90, 100, 130 or 160 days stratified according to subcutaneous fat thickness group

Palatability attribute	Subcutaneous fat thickness group[a]							
	1	2	3	4	5	6	7	8
Number of observations	32	40	40	45	41	22	14	20
Juiciness[b]	5.08[fg]	4.84[g]	4.79[g]	4.83[g]	5.02[fg]	5.29[f]	5.31[f]	5.03[fg]
Myofibrillar tenderness[c]	4.73[h]	5.23[gh]	5.52[fg]	5.28[g]	5.91[f]	5.89[f]	6.10[f]	5.78[fg]
Connective tissue amount[d]	6.00[i]	6.33[h]	6.68[fg]	6.46[gh]	6.87[f]	6.79[fg]	6.81[fg]	6.75[fg]
Overall tenderness[c]	4.53[h]	5.07[g]	5.39[fg]	5.17[g]	5.87[f]	5.77[f]	5.99[f]	5.66[fg]
Flavor desirability[e]	4.63[i]	5.13[h]	5.43[g]	5.45[g]	5.80[fg]	5.85[f]	5.81[fg]	5.74[fg]
Overall palatability[e]	4.25[j]	4.71[i]	5.10[ghi]	5.01[hi]	5.58[fg]	5.60[fg]	5.75[f]	5.50[fgh]
Shear force, kg	7.89[i]	6.46[h]	5.43[g]	5.12[fg]	4.28[f]	4.28[fg]	4.17[f]	4.58[fg]

[a] 1 = 2.53 mm or less; 2 = 2.54—5.07 mm; 3 = 5.08—7.61 mm; 4 = 7.62—10.15 mm; 5 = 10.16—12.69 mm; 6 = 12.70—15.23 mm; 7 = 15.24—17.77 mm; 8 = 17.78 mm or greater.
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant.
[e] 8 = extremely desirable; 1 = extremely undesirable.
[f,g,h,i,j]Means in the same row bearing a common superscript letter are not significantly ($P > 0.05$) different.

Table 7—Frequency percentages of steaks within each of three levels of overall tenderness, flavor desirability and overall palatability stratified according to subcutaneous fat thickness group

Item	Subcutaneous fat thickness group[a]							
	1	2	3	4	5	6	7	8
Number of observations	32	40	40	45	41	22	14	20
Overall tenderness[b]								
"Very desirable"	15.6	30.0	35.0	31.1	51.2	50.0	57.2	35.0
"Desirable"	40.6	45.0	40.0	46.7	43.9	45.5	35.7	65.0
"Undesirable"	43.8	25.0	25.0	22.2	4.9	4.5	7.1	–
Flavor desirability[b]								
"Very desirable"	–	7.5	17.5	20.0	46.4	45.5	57.1	45.0
"Desirable"	62.5	70.0	72.5	75.6	51.2	54.5	42.9	55.0
"Undesirable"	37.5	22.5	10.0	4.4	2.4	–	–	–
Overall palatability[b]								
"Very desirable"	6.2	5.0	20.0	13.3	39.0	50.0	42.9	20.0
"Desirable"	31.3	65.0	50.0	71.1	48.8	36.4	50.0	75.0
"Undesirable"	62.5	30.0	30.0	15.6	12.2	13.6	7.1	5.0

[a] 1 = 2.53 mm or less; 2 = 2.54—5.07 mm; 3 = 5.08—7.61 mm; 4 = 7.62—10.15 mm; 5 = 10.16—12.69 mm; 6 = 12.70—15.23 mm; 7 = 15.24—17.77 mm; 8 = 17.78 mm or greater.
[b] "Very desirable" = mean sensory panel ratings of 6.00 or higher; "Desirable" = mean sensory panel ratings of 4.50—5.99; "Undesirable" = mean sensory panel ratings lower than 4.50.

Table 8—Mean values for palatability attributes stratified according to three subcutaneous fat thickness groups

Palatability attribute	Subcutaneous fat thickness group[a]		
	1	2	3
Number of observations	72	85	97
Juiciness[b]	4.94[fg]	4.81[g]	5.12[f]
Myofibrillar tenderness[c]	5.01[h]	5.39[g]	5.91[f]
Connective tissue amount[d]	6.18[h]	6.56[g]	6.82[f]
Overall tenderness[c]	4.83[h]	5.27[g]	5.82[f]
Flavor desirability[e]	4.91[h]	5.44[g]	5.80[f]
Overall palatability[e]	4.50[h]	5.05[g]	5.59[f]
Shear force, kg	7.10[h]	5.27[g]	4.32[f]

[a] 1 = 5.07 mm or less; 2 = 5.08—10.15 mm; 3 = 10.16 mm or greater.
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant.
[e] 8 = extremely desirable; 1 = extremely undesirable.
[f,g,h] Means in the same row bearing a common superscript letter are not significantly ($P > 0.05$) different.

Table 9—Frequency percentages of steaks within each of three levels of overall tenderness, flavor desirability and overall palatability stratified according to three subcutaneous fat thickness groups

Item	Subcutaneous fat thickness group[a]		
	1	2	3
Number of observations	72	85	97
Overall tenderness[b]			
"Very desirable"	23.6	33.0	48.5
"Desirable"	43.1	43.5	47.4
"Undesirable"	33.3	23.5	4.1
Flavor desirability[b]			
"Very desirable"	4.2	18.8	47.4
"Desirable"	66.6	74.1	51.6
"Undesirable"	29.2	7.1	1.0
Overall palatability[b]			
"Very desirable"	5.6	16.5	38.1
"Desirable"	50.0	61.2	51.6
"Undesirable"	44.4	22.3	10.3

[a] 1 = 5.07 mm or less; 2 = 5.08—10.15 mm; 3 = 10.16 mm or greater.
[b] "Very desirable" = mean sensory panel ratings of 6.00 or higher; "Desirable" = mean sensory panel ratings of 4.50—5.99; "Undesirable = mean sensory panel ratings lower than 4.50.

taneous fat thickness received the highest percentages of "very desirable" ratings and the lowest percentages of "undesirable" ratings for overall tenderness, flavor desirability and overall palatability, whereas opposite results were observed for steaks from carcasses with less than 5.08 mm of subcutaneous fat (Table 9).

A question regarding the ability of USDA quality grades to categorize beef, assigning it to groups that will be homogeneous in palatability, regards the rationale of that system's reliance on differences in marbling as the best measure of differences in fatness of beef; it seems possible that relative fatness might better be equated by use of other traits—like subcutaneous fat thickness—that might be more effective predictors of cooked beef palatability. Data presented in Tables 4 and 8 suggest that the use of subcutaneous fat thickness to assign carcasses to three expected palatability groups, using fat thickness categories of 5.07 mm or less, 5.08—10.15 mm and 10.16 mm or greater, was at least equivalent to, and perhaps slightly more precise than, the use of quality grades (Choice, Good or Standard) for grouping the carcasses of the present population according to expected eating quality of their rib steaks; similar results are evident in Tables 5 and 9. That the two methods of grouping carcasses were similar in segmentation accuracy was not unexpected since the simple correlation between marbling score and fat thickness was relatively high ($r = 0.63$). Steaks from carcasses with at least 10.16 mm of fat thickness were similar in palatability characteristics to steaks from Choice carcasses, while steaks from carcasses with 5.08—10.15 mm of fat thickness were generally equivalent to palatability to steaks from Good grade carcasses and steaks from carcasses with 5.07 mm or less fat thickness were similar in palatability to steaks from Standard grade carcasses. While the 5.08—10.15 mm and the 10.16 mm or more fat thickness groups correctly identified larger percentages of "desirable" and "very desirable" steaks than did the Good and Choice grades, the Standard grade identified relatively more of the carcasses with "undesirable" steaks than did the group with 5.07 mm or less fat thickness. These data suggest that use of subcutaneous fat thickness as a grade-determining factor might

Table 10—Mean values for palatability attributes of steaks from steers fed a high-concentrate diet for 90, 100, 130 or 160 days stratified according to subcutaneous fat thickness group

Palatability attribute	Subcutaneous fat thickness group[a]							
	1	2	3	4	5	6	7	8
Number of observations	3	10	28	33	37	21	14	20
Juiciness[b]	5.00[f]	4.91[f]	4.90[f]	4.95[f]	5.06[f]	5.29[f]	5.31[f]	5.03[f]
Myofibrillar tenderness[c]	3.87[h]	5.02[g]	5.75[f]	5.66[f]	6.01[f]	5.98[f]	6.10[f]	5.78[f]
Connective tissue amount[d]	5.87[g]	6.25[g]	6.69[f]	6.66[f]	6.90[f]	6.82[f]	6.81[f]	6.75[f]
Overall tenderness[c]	3.77[g]	4.86[g]	5.60[f]	5.57[f]	5.97[f]	5.84[f]	5.99[f]	5.66[f]
Flavor desirability[e]	5.17[fg]	4.93[g]	5.53[f]	5.55[f]	5.80[f]	5.86[f]	5.81[f]	5.74[f]
Overall palatability[e]	3.77[g]	4.49[g]	5.26[f]	5.32[f]	5.63[f]	5.67[f]	5.75[f]	5.50[f]
Shear force, kg	7.82[i]	6.13[h]	4.85[g]	4.60[fg]	4.11[f]	4.23[fg]	4.17[fg]	4.58[fg]

[a] 1 = 2.53 mm or less; 2 = 2.54—5.07 mm; 3 = 5.08—7.61 mm; 4 = 7.62—10.15 mm; 5 = 10.16—12.69 mm; 6 = 12.70—15.23 mm; 7 = 15.24—17.77 mm; 8 = 17.78 mm or greater.
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant.
[e] 8 = extremely desirable; 1 = extremely undesirable.
[f,g,h,i] Means in the same row bearing a common superscript letter are not significantly ($P > 0.05$) different.

enhance palatability-prediction accuracy of a quality grading system even if there are carcasses in the population from steers that have been fed high-concentrate diets and carcasses from steers that have only been fed grass.

It has been reported that once cattle have been fed a high-concentrate diet for a certain period of time, they produce steaks of highly acceptable palatability, regardless of their marbling amounts or quality grades (Tatum et al., 1980). To determine the usefulness of carcass fat thickness for predicting palatability in a population of carcasses from cattle of similar feeding history, data from cattle fed 90–160 days were stratified according to fat thickness ranges (Table 10). Steaks from carcasses with at least 5.08 mm of fat thickness were superior ($P < 0.05$) to steaks from carcasses with less subcutaneous fat (5.07 mm or less) for all palatability attributes. Steaks from carcasses with less than 2.54 mm of external fat received the lowest ($P < 0.05$) sensory panel ratings for myofibrillar tenderness and had the highest ($P < 0.05$) shear force values. As fat thickness of carcasses from cattle fed 90–160 days increased from less than 2.53 mm up to 7.61 mm, there were progressive increases in palatability of cooked beef; however, deposition of subcutaneous fat in quantities greater than 7.61 mm did not further improve cooked beef palatability. The data of this population of cattle, carcasses and steaks support the theory that subcutaneous fat thickness could be used as an alternative to use of the present USDA quality grading system for predicting beef palatability.

REFERENCES

Adams, N.J., Smith, G.C., and Carpenter, Z.L. 1977. Carcass and palatability characteristics of Hereford and crossbred steers. J. Anim. Sci. 46: 438.

Barr, A.J., Goodnight, J.H., Sall, J.P., Blair, W.H., and Chilko, D.M. 1979. SAS User's Guide. SAS Institute, Raleigh, NC.

Berry, B.W., Smith, G.C., and Carpenter, Z.L. 1974. Beef carcass maturity indicators and palatability attributes. J. Anim. Sci. 38: 507.

Bowling, R.A., Smith, G.C., Carpenter, Z.L., Dutson, T.R. and Oliver, W.M. 1977. Comparison of forage-finished and grain-finished beef carcasses. J. Anim. Sci. 45: 209.

Bowling, R.A., Riggs, J.K., Smith, G.C., Carpenter, Z.L., and Butler, O.D. 1978. Production, carcass and palatability characteristics of steers produced by different management systems. J. Anim. Sci. 46: 333.

Breidenstein, B.B., Cooper, C.C., Cassens, R.G., Evans, G., and Bray, R.W. 1968. Influence of marbling and maturity on the palatability of beef muscle. 1. Chemical and organoleptic considerations. J. Anim. Sci. 27: 1532.

Campion, D.R., Crouse, J.D., and Dikeman, M.E. 1975. Predictive value of USDA beef quality grade factors for cooked meat palatability. J. Food Sci. 40: 1225.

Covington, R.C., Tuma, H.J., Grant, D.L., and Dayton, A.D. 1970. Various chemical and histological characteristics of beef muscle as related to tenderness. J. Anim. Sci. 30: 191.

Davis, G.W., Smith, G.C., Carpenter, Z.L., Dutson, T.R., and Cross, H.R. 1979. Tenderness variations among beef steaks from carcasses of the same USDA quality grade. J. Anim. Sci. 49: 103.

Dolezal, H.G., Smith, G.C., Savell, J.W., and Carpenter, Z.L. 1982. Effect of time-on-feed on the palatability of rib steaks from steers and heifers. J. Food Sci. (In press).

Duncan, D.B. 1955. New multiple range and multiple F tests. Biometrics 11: 1.

Dutson, T.R., Smith, G.C., Hostetler, R.L., and Carpenter, Z.L. 1975. Postmortem carcass temperature and beef tenderness. J. Anim. Sci. 41: 289 (Abstr.).

Garcia-de-Siles, J.L., Ziegler, J.H., and Wilson, L.L. 1977. Effects of marbling and conformation scores on quality and quantity characteristics of steer and heifer carcasses. J. Anim. Sci. 44: 36.

Harrison, A.R., Smith, M.E., Allen, D.M., Hunt, M.C., Kastner, C.L., and Kropf, D.H. 1978. Nutritional regime effects on quality and yield characteristics of beef. J. Anim. Sci. 47: 383.

Jennings, T.G., Berry, B.W., and Joseph, A.L. 1978. Influence of fat thickness, marbling and length of aging on beef palatability and shelf-life characteristics. J. Anim. Sci. 46: 658.

Kramer, C.Y. 1956. Extension of multiple range test to group means with unequal numbers of replications. Biometrics 12: 307.

Lochner, J.V., Kauffman, R.G., and Marsh, B.B. 1980. Early-postmortem cooling rate and beef tenderness. Meat Sci. 4: 227.

Marsh, B.B., and Lochner, J.V. 1981. New approaches to beef quality. Proc. National Beef Grading Conf., Iowa State Univ., Ames p. 98.

McBee, J.L. and Wiles, J.A. 1967. Influence of marbling and carcass grade on the physical and chemical characteristics of beef. J. Anim. Sci 26: 701.

Meyer, R.M., Young, A.W., Marsh, B.B., and Kauffman, R.G. 1977. Effect of backfat in preventing cold shortening and maintaining tenderness in beef. J. Anim. Sci. 45(Suppl. 1): 70 (Abstr.).

NRC. 1976. "Nutrient Requirements of Beef Cattle," No. 4, 5th rev. ed. National Research Council-National Academy of Sciences, Washington, DC.

Parrish, F.C. Jr., Olson, D.G., Miner, B.E., and Rust, R.E. 1973. Effect of degree of marbling and internal temperature of doneness on beef rib steaks. J. Anim. Sci. 37: 430.

Romans, J.R., Tuma, H.J., and Tucker, W.L. 1965. Influence of carcass maturity and marbling on the physical and chemical characteristics of beef. 1. Palatability, fiber diameter and proximate analysis. J. Anim. Sci. 24: 681.

Smith, G.C. 1980. Grades for the future: what, why and how? Proc. Recip. Meat Conf. 33: 89.

Smith, G.C., Dutson, T.R., Hostetler, R.L., and Carpenter, Z.L. 1976. Fatness, rate of chilling and tenderness of lamb. J. Food Sci. 41: 748.

Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods (6th Ed.), Iowa State Univ. Press, Ames, IA.

Tatum, J.D. 1978. Effects of management history, breed-type and carcass characteristics on palatability attributes of beef. Ph.D. dissertation, Texas A&M Univ., College Station, TX.

Tatum, J.D., Smith, G.C., Berry, B.W., Murphey, C.E., Williams, F.L., and Carpenter, Z.L. 1980. Carcass characteristics, time on feed and cooked beef palatability attributes. J. Anim. Sci. 50: 833.

Tatum, J.D., Smith, G.C., and Carpenter, Z.L. 1982. Interrelationships between marbling, subcutaneous fat thickness and cooked beef palatability. J. Anim. Sci. (In press).

Tuma, H.J., Henrickson, R.L., Stephens, D.F., and Moore, R. 1962. Influence of marbling and animal age on factors associated with beef quality. J. Anim. Sci. 21: 848.

USDA. 1975. Official United States standards for grades of carcass beef. USDA-AMS, Washington, DC.

Ms received 7/10/81; revised 9/14/81; accepted 10/6/81.

T.A. 16665 of the Texas Agricultural Experiment Station. This study was partially supported by King Ranch, Inc., Kingsville, TX and by the Texas Cattle Feeders Association, Amarillo, TX and contributes to the TAES Expanded Research Program on Lean Beef Production.

Potato Starch and Flour in Frankfurters: Effect on Chemical and Sensory Properties, and Total Plate Counts

A. A. BUSHWAY, P. R. BELYEA, R. H. TRUE, T. M. WORK,
D. O. RUSSELL and D. F. McGANN

ABSTRACT

Potato starch and flour were examined as possible constituents in frankfurter formulations. Frankfurters manufactured with 3% potato starch or 1.5% potato starch plus 1.5% potato flour in place of 3% wheat flour normally used as a binder, and stored at 4–5°C for 28 days did not differ significantly in percent shrink, water, protein, fat, ash or salt. Residual nitrite levels decreased during the storage period. Total plate counts reflect residual nitrite levels since the frankfurters (3% starch) with the lowest nitrite concentration also had the greatest number of bacteria (6.4 x 10^6/g) on day 28. Frankfurters formulated with 1.5% potato starch plus 1.5% potato flour were rated more tender and more juicy than those made with 3% potato starch ($P \leq 0.05$ and $P \leq 0.01$) and 3% wheat flour ($P \leq 0.01$ and $P \leq 0.01$), respectively. Fresh potato starch and wheat flour franks did not differ from each other in either characteristic but after one week's storage the potato starch samples were rated more tender ($P \leq 0.05$) and more juicy ($P \leq 0.01$). Less force was required to shear the frankfurters formulated with 3% potato starch.

INTRODUCTION

THE USE OF EXTENDERS in processed meats has been well documented (Sofos and Allen, 1977; Inklaar and Fortuin, 1969; Pearson et al., 1965; Smith et al., 1973). Common ingredients which are used in sausage manufacture include cereal flours, starches and corn syrup or corn syrup solids (Forrest et al., 1975). These extenders may be added to processed meat to increase the water binding capacity and/or to provide protein which can act as an emulsifier.

One vegetable which can furnish extenders for processed meat manufacture is potato. Potatoes can be processed to provide starch, flour and protein (Satterlee, 1981; Meuser and Smolnik, 1979; Forrest et al., 1975) which can be used in processed meat to increase the water binding capacity of the emulsion.

From the nutritional standpoint, potato protein has been reported to have a biological value of 80 when compared to egg protein which was given a value of 100 (Kofranyi and Jekat, 1965; Markakis, 1975; Meister and Thompson, 1976; Satterlee, 1981). Potato proteins are relatively high in lysine, but are low in methionine (Markakis, 1975; Meister and Thompson, 1976).

Flour and starch from potatoes have been used in the manufacture of bakery (Harris et al., 1952; Knorr, 1977) and meat (Wyler, 1971; Leest et al., 1971) products.

This study was conducted to determine the effects of replacing wheat flour with potato starch (particle size from 15–150 microns) and with a combination of potato starch plus potato flour ("fines" remaining after potato flake manufacture) on the chemical and sensory properties and total plate counts of frankfurter-type sausage.

Authors Bushway, Belyea, True, Work and McGann are affiliated with the Dept. of Food Science, Holmes Hall, Univ. of Maine, Orono, ME 04469. Author Russell is with COLBY Co-Operative Starch Company, Caribou, ME 04736.

EXPERIMENTAL

POTATO STARCH and flour were obtained from COLBY Co-Operative Starch Company, Caribou, ME and Potato Service, Inc., Presque Isle, ME, respectively. All other ingredients were those used by W.A. Bean & Sons in the manufacture of their natural casing frankfurter.

Frankfurter formulation and processing

The control frankfurter formulation consisted of the following: lean beef, beef heart, pork fat, ice, spices, corn syrup solids, non-fat dry milk, sodium nitrite, sodium erythrobate and wheat flour. Experimental franks contained the same ingredients as the control except for the 3% wheat flour which was replaced with 3% potato starch or with a combination of 1.5% potato starch plus 1.5% potato flour.

Frankfurters were manufactured at W.A. Bean & Sons, Bangor, ME using standard commercial practices. The ingredients were mixed in 50 lb batches and stuffed into natural casings prior to cooking, chilling and packing in boxes. Boxes containing 10 lb of franks were stored at 4–5°C for 28 days.

Chemical analyses

Four links were randomly selected from each batch and analyzed for proximate composition (moisture, fat, protein and ash) according to procedures outlined by AOAC (1980). Salt content was also determined according to AOAC methods (1980) after 1 day of storage.

Residual nitrite was determined after 1, 12, 19 and 26 days of storage using the Griess method as outlined in AOAC (1980).

Total plate counts

Microbiological analyses were performed on days 0, 5, 18 and 28 of storage. Four frankfurters were randomly selected from each batch and homogenized in a sterile Waring Blendor. Appropriate serial decimal dilutions were prepared with 0.1% peptone and used for inoculating triplicate pour plates of Plate Count Agar (Difco). All inoculated plates were incubated at room temperature (22°C) for up to 5 days at which time the colonies were counted.

Sensory quality evaluation

Frankfurters were evaluated fresh and after 1 wk's storage at 4–5°C for quality by sensory panels of 20 members. Fourteen of the panelists in Test 1 and 17 in Test 2 were experienced in the evaluation of flavor and texture of other commodities. The testing was conducted in individual booths.

For each replication, 12 frankfurters of each formulation were placed in stainless steel pans containing 2L of boiling water. The pans were covered, removed from the source of heat and allowed to stand for 4 min. The frankfurters were sliced into sections ca 2.5 cm long and the end portions were discarded. The samples were coded and two slices of each were presented in a randomized complete block design with four replications (Cochran and Cox, 1950) to the sensory panel.

The panel members were asked to rank the samples for texture and for flavor according to preference, for moisture according to juiciness, and to check the term which best described the texture.

Scores of +1, 0 and −1 were assigned to the ranked data. For texture characterization: +2 = tough, +1 = firm, 0 = tender, −1 = soft, −2 = mushy. The data were analyzed by the variance method (Steel and Torrie, 1960) using the treatment x judge interaction for the error term (Bliss, 1960) to test for a significant treatment F ratio. Duncan's Multiple Range Test (Duncan, 1955) was used to determine significant differences among the treatment means and the LSD was used to compare the mean of the control sample with

Table 1—Chemical composition of frankfurters manufactured with 3% wheat flour, 3% potato starch and 1.5% potato starch plus 1.5% potato flour

Sample	%[a]				
	H_2O	Protein	Fat	Ash	Salt
3% Wheat flour	54.9 ± 0.19	13.5 ± 0.25	26.0 ± 0.34	2.25 ± 0.02	1.8
3% Potato starch	53.9 ± 0.06	13.1 ± 0.06	27.1 ± 0.13	2.50 ± 0.02	1.9
1.5% Potato starch + 1.5% potato flour	54.2 ± 0.16	12.7 ± 0.10	25.7 ± 0.16	2.80 ± 0.02	1.9

[a] All values with standard deviations represent the mean of four samples. All other values are the average of duplicate samples.

Table 2—Residual nitrite levels in 3% wheat flour, 3% potato starch and 1.5% potato starch plus 1.5% potato flour frankfurters

Day analyzed[a]	NO_2^- (ppm)		
	3% Wheat flour	3% Potato starch	1.5% Potato starch + 1.5% potato flour
1	30 ± 1.20	24 ± 1.40	38 ± 0.83
12	24 ± 0.67	15 ± 1.64	35 ± 0.41
19	18 ± 0.45	7 ± 0.16	25 ± 0.52
26	11 ± 0.68	5 ± 0.17	22 ± 0.35

[a] All samples were stored at 4—5°C. All values with standard deviations represent the mean of three samples.

Table 3—Total plate counts on frankfurters manufactured with 3% wheat flour, 3% potato starch and 1.5% potato starch plus 1.5% potato flour

Day analyzed[a]	Total plate counts (bacteria/g frankfurter)		
	3% Wheat flour	3% Potato starch	1.5% Potato starch + 1.5% potato flour
0	1.3×10^4	1.4×10^4	1.3×10^4
5	2.2×10^4	2.3×10^4	2.4×10^4
18	2.8×10^4	4.8×10^4	3.0×10^4
28	7.8×10^4	6.4×10^6	1.2×10^5

[a] All samples were stored at 4—5°C. All values represent the mean of nine plates.

those of the other two treatments. Correlation coefficients were calculated (Steel and Torrie, 1960) to determine the interrelationships of texture preference to tenderness to juiciness.

Shear force measurement

For shear force measurements, four links from each treatment were placed in 1 liter of boiling water, covered, removed from the source of heat, allowed to stand for 4 min and drained. The ends were removed from each frank and the remaining link sliced into four 2.5 cm slices. A center core was removed from each slice using a No. 10 cork borer. Each section was weighed, cooled for 20 min and the force required to shear the sample was measured using a 5 lb transducer and a single blade cell.

RESULTS & DISCUSSION

Chemical and microbiological analyses

The proximate composition of the three types of frankfurters is given in Table 1. There was no significant difference in the precent water, protein, fat or ash content of the three formulations. The percent shrink after processing was 12.0, 10.9 and 9.1 for the control, 3% potato starch and 1.5% potato starch plus 1.5% potato flour, respectively. Statistical analysis showed no significant difference in shrink among the three treatments which would indicate that those franks manufactured with potato starch did not bind more water than the wheat flour franks.

Residual nitrite levels decreased with storage time at 4–5°C (Table 2). Of particular note were the lower initial nitrite and final nitrite levels in the 3% potato starch frankfurters. Total plate counts reflect residual nitrite levels since the frankfurters (3% starch) with the lowest nitrite concentration also had the greatest number of bacteria (6.4×10^6/g) on day 28 (Table 3). Residual nitrite levels in the control franks and the 1.5% potato starch plus 1.5% potato flour were greater than 10 ppm throughout the study and bacterial counts were less than 2×10^5/g on day 28 (Tables 2 and 3). It should be noted that the shelf-life of the three types of frankfurters was at least 18 days at 4–5°C. Also, these frankfurters were bulk packaged and would have a shorter shelf-life than vacuum packaged frankfurters.

Sensory quality

The mean scores for sensory quality of frankfurters formulated with 1.5% potato starch plus 1.5% potato flour, 3% potato starch, and 3% wheat flour are presented in Table 4.

Preferences for texture and flavor of the fresh samples, as determined by a sensory panel of 20 members, were not significant at the 5% level of detection. Frankfurters formulated with 1.5% potato starch plus 1.5% potato flour were rated more tender than those made with 3% potato starch ($P \leq 0.05$) and with 3% wheat flour ($P \leq 0.01$) and were more juicy than both the starch and wheat samples ($P \leq 0.01$). The potato starch and wheat flour franks did not differ from each other in either characteristic.

Following 1 wk's storage at 4–5°C, there also was no significant preference for flavor. However, the texture of frankfurters formulated with potato starch plus potato flour was significantly preferred ($P \leq 0.01$) to that of the control sample. Franks containing 3% starch did not differ from the other two formulations at the 5% level of significance. Samples of the starch plus flour combination were characterized as more tender than those with 3% starch ($P \leq 0.05$) and with 3% wheat ($P \leq 0.01$). Frankfurters containing 3% starch also were more tender than the control ($P \leq 0.05$). For juiciness, differences between the adjacent means were significant at the 1% level with the formulation of starch plus flour being rated the most juicy and the wheat flour control least juicy.

It was demonstrated in these studies that 1.5% potato starch plus 1.5% potato flour or 3% potato starch alone may successfully be used as an extender in the formulation of frankfurters. For all of the sensory quality attributes examined, the experimental products were equal to or superior to the control sample (3% wheat flour) which is currently marketed through normal channels.

Although Meuser and Smolnik (1979) reported that potato protein incorporated into foods as a protein extender may impart undesirable flavor and textural characteristics unless extensive purification steps were taken, it was unlikely, due to the low level of potato protein used and to the addition of spices, that the flavor and texture of frankfurters would be adversely affected.

—Continued on next page

Table 4—Mean scores for sensory quality of frankfurters formulated with 1.5% potato starch plus 1.5% potato flour, 3% potato starch, and 3% wheat flour[a]

Extender	Fresh				Held 1 wk at 4–5°C			
	Texture			Flavor	Texture			Flavor
	Preference	Tenderness[b]	Juiciness[c]	preference	Preference[c]	Tenderness[b]	Juiciness[c]	preference
1.5% Potato starch + 1.5% potato flour	+0.18	−0.06 f **	+0.46 e	+0.16	+0.26 e	−0.40 g **	+0.39 e	+0.18
3% Potato starch	+0.01	+0.20 e	−0.11 f	+0.06	−0.01 ef	+0.26 f	+0.02 f	+0.02
3% Wheat flour (Control)	−0.19	+0.36 e	−0.35 f	−0.22	−0.25 e	+0.48 e	−0.41 g	−0.20
LSD 0.05	NS	0.23	0.29	NS	0.34	0.21	0.26	NS
0.01		0.31	0.39		0.46	0.28	0.35	

[a] Means for four replications, 20 judges (16 judges for flavor preference, fresh). Texture and flavor ranked for preference: +1 = like best, 0, −1 = like least. Juiciness ranked in order of juiciness: +1 = most juicy, 0, −1 = least juicy. Tenderness: +2 = tough, +1 = firm, 0 = tender, −1 = soft, −2 = mushy.
[b] Means followed by similar letters do not differ significantly at P≤0.05 using Duncan's Multiple Range test.
[c] Means followed by similar letters do not differ significantly at P≤0.01 using Duncan's Multiple Range test.
[d] ** Mean differs significantly from the Control at P≤0.01.

Significant differences in tenderness were found among the samples with those containing the potato based extenders being rated more tender; however, all samples were of acceptable quality.

For juiciness, the differences among the mean scores were of a larger magnitude than those for the other quality attributes. Although this would indicate that the contrasts were more apparent and more easily detected by the panelists, it should be mentioned that a higher degree of agreement among the judges is expected when a product is ranked for intensity of a specific property as opposed to being ranked according to personal preference.

The property causing the sensation of juiciness apparently is not related to moisture or fat content as the composition of the samples did not differ (Table 1). There also was no difference in the water holding capacity of the heated frankfurters.

The correlation coefficients for textural properties of frankfurters, fresh and after 1 wk's storage at 4–5°C, are shown in Table 5. For the fresh samples, the relationships of preference to tenderness and to juiciness were not significant. The low correlation coefficients may be attributed to the fact that there were no differences among the treatment means for texture preference as determined by analysis of variance (Table 4). The remaining correlation coefficients for the interrelationships of preference to tenderness to juiciness were significant at the 1% level.

Shear data

The shear data demonstrated that the 1.5% potato starch plus 1.5% potato flour and the 3% wheat flour frankfurters were firmer than those formulated with 3% potato starch (Table 6). These results seem to differ from those of the sensory panel which perceived the 1.5% potato starch plus 1.5% potato flour as being more tender (Table 4). It should be noted that the panelists did not describe any of the frankfurters as being tough and the mouth feel on chewing may have influenced their rating.

SUMMARY

THESE STUDIES demonstrated that 1.5% potato starch plus 1.5% potato flour or 3% potato starch may be used as an extender in the formulation of frankfurters without changing the chemical and sensory properties of the finished product. For all of the sensory quality attributes examined, the experimental products were equal to or superior to the control sample (3% wheat flour) which is currently marketed.

Table 5—Correlation coefficients of textural properties of frankfurters

	Tenderness		Juiciness	
	Fresh	1 wk	Fresh	1 wk
Preference	−0.11	−0.75**	0.32	0.84**
Tenderness			−0.71**	−0.79**

** significant at P≤0.01.

Table 6—Shear force, standard error of the mean (S $\bar{x}$), and coefficient of variability (CV) of frankfurters formulated with 1.5% potato starch plus 1.5% potato flour, 3% wheat flour, and 3% potato starch

Extender	Shear $\bar{x}$ (g/g)*	S $\bar{x}$ (g)	CV (%)
1.5% Potato starch + 1.5% potato flour	109.8 a	± 8.2	15
3% Wheat flour (Control)	100.2 a	± 6.4	13
3% Potato starch	75.0 b	± 7.2	19
LSD 0.05	23.4		

* Mean of 16 values. Means followed by similar letters do not differ significantly using Duncan's Multiple Range Test at P≤0.05.

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.
Bliss, C.I. 1960. Some statistical aspects of preference and related tests. Appl. Statistics 9(1): 8.
Cochran, W.G. and Cox, G.M. 1950. "Experimental Designs." John Wiley & Sons, Inc., New York.
Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1.
Forrest J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D., and Merkel, R.A. 1975. In "Principles of Meat Science," Ed. Schweigert, B.S. W.H. Freeman & Co., San Francisco, CA.
Harris, R.H., Sibbitt, L.D., and Banasik, O.J. 1952. Effect of potato flour on bread quality and changes with age. Cereal Chem. 29: 133.
Inklaar, P.A. and Fortuin, J. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. Food Technol. 23: 103.
Knorr, D. 1977. Protein recovery from waste effluent of potato processing plants. J. Food Tech. 12: 563.
Kofranyi, E. and Jekat, F. 1965. "Die biologische Wertigkeit von Kartoffel-proteinen." Westdeutscher Verlag. Koeln und Opladen, Germany.
Leest, J.A., Baal, J.P. W. van, and Male, J.P. van. 1971. Effect of PSE condition of pork on stability of luncheon meat during sterilization. In "Proceedings of the 2nd International Symposium on Condition and Meat Quality of Pigs," p. 287.
Markakis, P. 1975. The nutritive value of potato protein. In "Protein Nutritional Quality of Foods and Feeds," Part 2, Ed. Friedman, M. Marcel Deckker, Inc., New York.

—Continued on page 408

Incorporation of Blood Proteins into Sausage

H. A. CALDIRONI and H. W. OCKERMAN

ABSTRACT

The possibility of introducing beef plasma and decolorized globin proteins as ingredients in meat emulsions of cooked sausages was evaluated. The emulsifying capacity (EC) of plasma proteins was similar to that of meat, but globin proteins showed significantly lower EC values. The latter could be enhanced when plasma proteins were added in proportions ranging from 10 to 65%. Combinations of meat, plasma and globin proteins, containing up to 20% blood proteins, yielded acceptable EC values. These values were affected by the fat content of the meat in the emulsions and especially by the total content of proteins. Up to 12% of plasma protein and different combinations of plasma:globin proteins, containing up to 5% of the latter (12% of total protein replaced), was used in the preparation of cooked sausages. This yielded an acceptable product as ranked by a sensory panel.

INTRODUCTION

THE USE OF PROTEIN fractions isolated from blood has been suggested as a source of high quality dietary proteins (Tybor et al., 1973; 1975). Although isoleucine and methionine are in limited percentages in plasma proteins (Young et al., 1973; Swingler et al., 1978) and globin proteins (De Vuono et al., 1979; Young et al., 1973), addition of these proteins to meat products could improve their physical and nutritional properties, yielding an economical, nutritionally acceptable product.

An important parameter to assess the ability of proteins for form and maintain a stable sausage preparation is the emulsifying capacity (EC). Satterlee et al. (1973) studied the functional properties of blood and many animal tissue protein powders to evaluate the possibility of their utilization in emulsified meat products. Whole blood protein powders gave improved EC values when compared to those of animal tissue powders but blood powders were objectionable because of the dark color they imparted to sausages. On the other hand, plasma and globin proteins separated from blood and decolorized exhibited excellent functional properties (Tybor et al., 1973) and the possibility of using them in commercial sausage operation was suggested (Tybor et al., 1975).

The purpose of this work was to evaluate the effect of adding different proportions of plasma and decolorized globin proteins to comminuted meat, and to determine the emulsifying properties of the resulting mixtures. Some of these proportions were then used in the production of cooked sausages and the subjective acceptability of the product was evaluated.

Author Ockerman is affiliated with the Dept. of Animal Science, The Ohio State Univ., Columbus, OH 43210, and The Ohio Agricultural Research & Development Center, Wooster, OH 44691. Author Caldironi is Assoc. Professor of Biological Chemistry at Nacional del Centro, Facultad de Agronomia, Azul (Argentina) and a member of the Instituto de Investigaciones Bioquimicas, (UNS-CONICET) 8000 Bahia Blanca - Argentina.

EXPERIMENTAL

PLASMA AND RED CELLS from bovine blood were separated following basically the method of Tybor et al. (1973). Beef blood was collected directly from the jugular vein during slaughter and mixed with an equal volume of 0.9% sodium chloride solution containing 0.5% sodium citrate to prevent coagulation.

After cooling for 24 hr at 4°C, plasma and red cells were separated by centrifugation (red cells precipitated after 10 min. at 3,000 x *g*).

The red cells were diluted with water (1:1) and chloroform was added to the solution (0.25% v/v). The stroma was separated by settling and the decanted supernatant was mixed with 2 volumes of ascorbic acid (2%) solution which resulted in the mixture having a pH of 4 or less.

By bubbling air through the solution, the hemoglobin was changed into choleglobin by oxidation. The heme group was solubilized and the protein fraction was precipitated by adding methanol (or acetone) 20% v/v, instead of the four volumes of acetone used in the Tybor procedure. Raterman et al. (1980) showed that treating diluted blood samples with these quantities of solvents was very effective for removing the proteins. This technique was used to separate both the globin and the plasma proteins from their respective solutions. Preliminary research proved the most effective pH for globin precipitation was 5.7 (no adjustment was necessary) while plasma proteins were precipitated at pH 3.0 (obtained by adding 1N HCl). After centrifugation (10 min at 3,000 x *g* at 4°C) the globin fraction yielded a red-brown paste which contained some solvent, easily removable by vacuum. The mean and standard deviation of the globin (6 samples) protein and moisture content was 22.1±2.8% and 73.9±0.9%, respectively. Plasma proteins gave a white creamy suspension containing 9.0±2.1 % protein, and 88.5± 1.3% moisture.

Protein, moisture and fat contents and the emulsifying capacity (EC) were determined according to methods described by Ockerman (1980).

To determine EC, 50 ml of solutions containing 0.10, 0.20 and 0.25g of proteins were prepared in 1N NaCl (Swift et al., 1963) and the pH was adjusted to 5.7 when necessary, with diluted HCl or NaOH. Blendor jars containing the solutions were cooled in crushed ice for 10 min. before EC determinations. Temperatures were as follows: initial, 3.3±0.5°C; final, 9.6±0.7°C; room and oil, 22.0± 1.0°C (15 determinations). Protein solutions were blended at high speed (17,000 rpm) in a Waring Blendor and refined soybean oil was added at a rate of 60 ml/min. (Swift et al., 1961). The emulsion collapse was determined by a sudden increase in the electric resistance. At this point the addition of oil was stopped and the total amount of oil added was recorded. Lean beef meat samples (19.4% protein, 72.6% moisture, 6.9% fat and 5.7 pH) were used for EC determinations. Plasma and globin proteins were evaluated at the same pH. Each EC determination was repeated 4 times and reported as an average.

The basic sausage formula was 36% (weight) of lean (6.9% fat) beef trimmings, 36% regular (35.5% fat) pork trimmings, 24% of crushed ice, 0.23% white pepper, 0.10% ground coriander, 0.05% ground sage, 0.05% nutmeg, 0.03% garlic powder, 0.10% mace, 0.04% sodium nitrate, 0.01% sodium nitrite, 2.3% sodium chloride, 0.35% sucrose and 0.04% sodium ascorbate. Ground lean beef, cure and seasoning and blood protein plus one-third of the ice were chopped for 1.5 min. Salt and one-third of the ice were added and chopped for an additional period of 0.5 min. The ground pork, ascorbate and the remainder of the ice were mixed and chopping was continued for 2 min (maximum temperature 10°C).

Known quantities of meat were replaced in the basic formulation with blood proteins, maintaining a constant moisture and total protein values in the product. Percentages and proteins evaluated are specified in Table 1.

—Continued on next page

Table 1—Sensory evaluation panel results[a] for various combinations of meat, plasma and globin proteins

	(Control)									
Meat proteins %	100	88	88	88	80	80	80	90	87.5	85
Plasma proteins %	–	12	–	6	20	–	10	5	10.0	10
Globin proteins %	–	–	12	6	–	20	10	5	2.5	5
Flavor[b/f]	5.9	4.5	3.5	5.3	5.0	4.7	4.7	4.0	5.5	3.3
	(1.3)	(2.0)	(2.0)	(1.5)	(1.4)	(1.2)	(0.9)	(1.6)	(1.0)	(1.7)
Texture[c/f]	5.6	4.3	4.7	5.0	4.5	5.5	5.7	4.5	4.8	4.5
	(1.4)	(0.9)	(1.2)	(0.8)	(0.6)	(0.6)	(1.2)	(0.6)	(0.5)	(1.0)
Odor[d]	6.2	5.3	3.0[g]	5.5	5.5	4.5	5.0	5.5	5.3	4.7
	(1.4)	(0.9)	(0.8)	(1.3)	(1.0)	(2.0)	(1.4)	(1.9)	(0.9)	(1.3)
Color[e]	5.7	4.7	6.7	6.0	3.8[h]	9.0[h]	8.5[h]	7.0	6.3	7.3[h]
	(0.6)	(0.5)	(1.2)	(0.8)	(0.5)	(0.0)	(0.6)	(0.8)	(0.5)	(0.5)

[a] Values represent mean vlaues (SD) from at least 4 determinations. Controls consisted of 12 evaluations.
[b] Flavor: 9 = intensive bologna flavor, 5 = moderate, 1 = off flavor.
[c] Texture: 9 = hard, 5 = moderate, 1 = soft.
[d] Odor: 9 = intensive bologna odor, 5 = moderate, 1 = off odor.
[e] Color: 9 = dark, 5 = moderate/normal, 1 = light.
[f] Flavor values and texture values were not different from their control.
[g/h] Significantly different from their respective control.

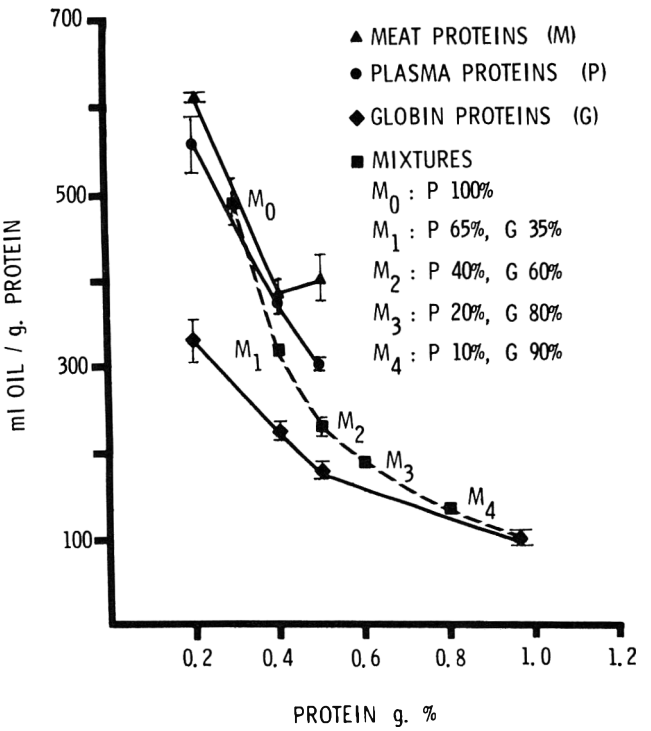


Fig. 1—Emulsifying capacity of meat, plasma and globin proteins and mixtures of plasma and globin protein. Protein g% is the total protein content in the slurry.

A panel of experienced (average 7 yr of evaluation) members evaluated in duplicate odor, color, flavor and texture in accordance with the scale shown in Table 1.

RESULTS & DISCUSSION

FIG. 1 SHOWS the EC values obtained for meat, plasma, globin proteins as well as mixtures of the latter. Meat and plasma proteins displayed significantly higher EC values than globin. Mixtures of plasma and globin proteins gave EC values between those of the pure components approximating the other curves depending on the proportions of each in the sample. EC of meat and plasma proteins were similar below 0.4 g% of total protein concentrated but above this value, meat proteins seemed to maintain a high EC whereas the plasma proteins continued to decrease. At concentrations below 0.4 g% protein, mixtures containing globin and plasma proteins were not significantly different in EC values from those obtained for plasma proteins. Mixtures containing more than 0.4 g% protein (35% or more of globin) more nearly approximated the globin curve.

Plasma and globin proteins, either alone or mixed, were added to meat suspensions substituting known amounts of blood proteins for equal quantities of meat proteins and the mixtures were then evaluated for EC and the results are shown in Fig. 2. Meat:plasma:globin (protein/protein/protein) mixtures in the ratios 80:10:10, 60:20:20, 80:20:00 and 80:00:20 showed EC values similar to or higher than meat at low concentrations (below 0.4 g% protein) of total proteins. However, the EC values for all mixtures were lower than those of meat at high (0.6 g% protein) protein concentrations or at all protein levels when the proportions of exogenous protein incorporated were higher than 40%. Thus, the EC behavior of emulsion mixtures can be changed by using convenient proportions of different proteins but the EC is very dependent upon the quantity of proteins.

If the fat present in the solutions obtained from the meat tissue is considered, it is interesting to note that the EC decreases as sample fat content decreases (Fig. 3). However, fat content has a less significant effect on the EC than total protein concentration in the solution. Thus, b, d, and g mixtures (Fig. 3) at 0.2 g% of total protein, show little variation in EC despite the different fat content and the different combinations of protein qualities. For a given fat content, different mixtures show similar EC values at the same total protein concentration. Moreover, samples of similar protein composition and fat content, displayed an inverse relationship when EC was compared to protein concentration. The EC values of meat protein are included in Fig. 3 for comparison purposes, and these values are approximately on the protein-content linear lines. Comparing the EC of proteins from various red meat sources, Borton et al. (1968) observed that tissues with different fat content had different EC values. They speculated that this might be due to the protein being more widely distributed and thus more easily solubilized. Satterlee et al. (1973) using different tissue powders showed that lower EC values are obtained with tissues of low fat content. Moreover, they also found that tissue powders whose soluble proteins possess the greatest emulsion capacity produce emulsions with the smallest globule size. It, therefore, seems likely that with tissues containing higher proportion of fat, the protein-protein interaction would be lower than in lean tissues and proteins would be "loose." In such a case there would be more "protein available" to form fat-globules and to emulsify the oil.

From the EC studies it was determined that the most appropriate mixtures would allow up to 20% of blood proteins to be incorporated into meat products. Various proportions of plasma and/or globin proteins were combined in meat sausage mixtures, up to this percentage as shown in Table 1. The sensory evaluation panel judged very acceptable (not significantly different from control in flavor, texture, odor and color) sausages with 10% plasma proteins + 2.5% of globin proteins and also acceptable sausages including 5% and 6% of both plasma and globin proteins and product with up to 12% plasma proteins.

Plasma proteins can be incorporated into sausage mixtures in higher proportion than globin and result in an acceptable quality product. Suter et al. (1976) showed that the incorporation of 1% and 2% plasma proteins into cooked ground beef patties, resulted in a marked increase of the binding strength. Terrell et al. (1979) found that plasma protein isolate (PPI) is "functional" in conventionally manufactured frankfurters. Thus meat + 1% PPI and meat + 5% PPI did not differ in texture, elasticity and strength from all-meat frankfurters.

Incorporation of more than 12% of plasma proteins yielded a pale colored product. However, after 1 month of storage at 0–4°C, sausages containing plasma proteins were visually superior to the controls, since they maintained a light pink color while the controls turned grey, possibly by oxidation.

More limiting is the percentage of globin protein that can be incorporated because even the decolored protein retains a reddish cast and this color is transferred to the sausages. However, it is possible to combine a mixture of blood proteins, 3:1 and 4:1 (plasma:globin) to balance the color. Neither plasma nor globin proteins, at the concentrations tested, showed significant differences in flavor or texture.

Among animal tissues, blood, and particularly plasma, are some of the best sources of soluble proteins. Moreover, they excel any other protein in their ability to emulsify fat (Satterlee et al., 1973). The amount of soluble protein in a sausage will, therefore, give an enhanced product stability and appearance.

CONCLUSION

PLASMA PROTEINS showed very acceptable emulsifying properties, but compromised the color of the product when included in high proportions. Even though decolorized globin proteins displayed poorer emulsifying capacity, they have high nutritional value. These proteins can be combined to maintain good functional properties and a desirable colored product.

REFERENCES

Borton, R.J., Webb, N.B., and Bratzler, L.J. 1968. Emulsifying capacities and emulsion stability of dilute meat slurries from various meat trimmings. Food Technol. 22: 506.

DeVuono, C., Penteado, M., Lajolo, F.M., and Pereira dos Santos, N. 1979. Function and nutritional properties of isolated bovine blood proteins. J. Sci. Food Agric. 30: 809.

–Continued on next page

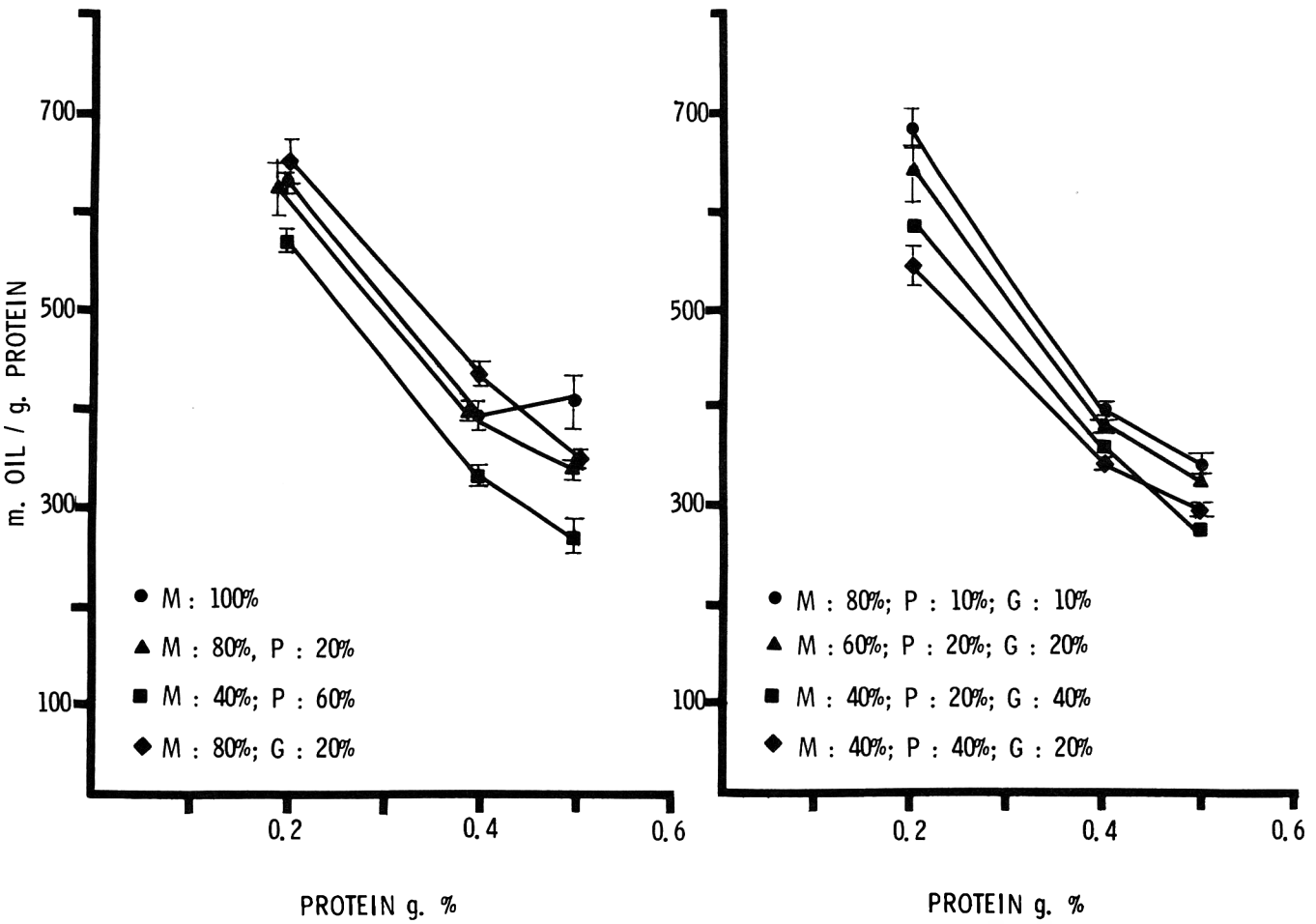


Fig. 2–Emulsifying capacity of meat proteins (M) and meat proteins mixed with plasma (P) and globin (G) protein individually and combined. Protein g% is the total protein content in the slurry.

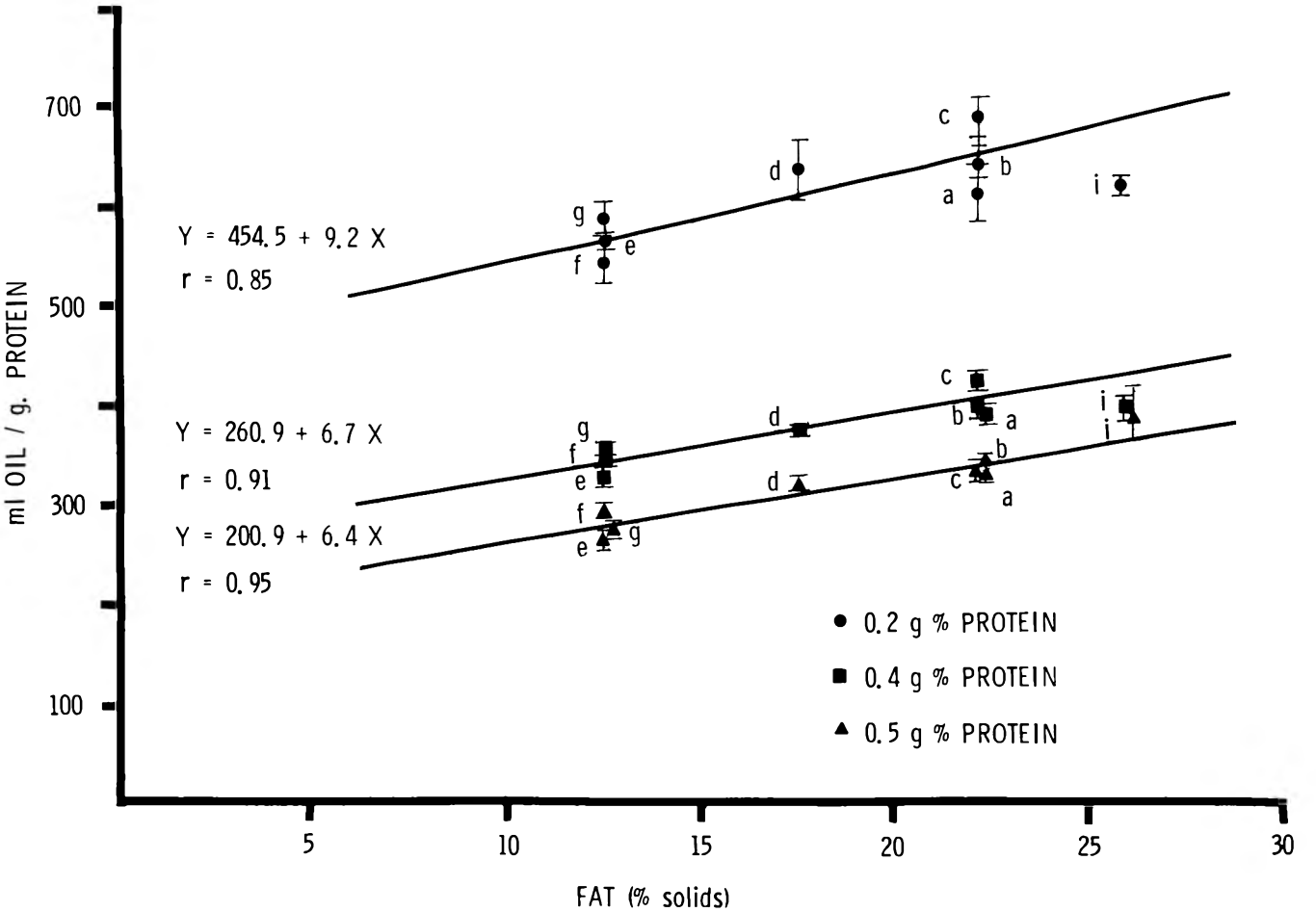


Fig. 3—Emulsifying capacity plotted against percent fat of the meat for various protein levels: M—Meat proteins; P—Plasma proteins; G—Globin proteins. (a) M: 80%, P: 20%; (b) M: 80%, G: 20%; (c) M: 80%, P: 10%, G: 10%; (d) M: 60%, P: 20%, G: 20%; (e) M: 40%, P: 60%; (f) M: 40%, P: 40%, G: 20%: (g) M: 40%, P: 20%, G: 40%; (i) M: 100%.

Ockerman, H.W. 1980. "Quality Control of Postmortem Muscle Tissue." 11th ed., Dept. of Animal Science, OSU, and The Ohio Agricultural R&D Center.
Ratermann, A.L., Burnett, H.W., and Vandegrift, V. 1980. Chemical coagulation of industrial animal blood using aluminum sulfate, zinc sulfate, methanol and acetone. J. Agric. Food Chem. 28: 438.
Satterlee, L.D., Free, B., and Levin, E. 1973. Utilization of high protein tissue powders as a binder/extender in meat emulsions. J. Food Sci. 38: 306.
Suter, D.A., Sustek, E., Dill, C.W., Marshall, W.H., and Carpenter, Z.L. 1976. A method for measurement of the effect of blood protein concentrates on the binding forces in cooked ground beef patties. J. Food Sci. 41: 1428.
Swift, C.E., Lockett, C., and Fryar, A.J. 1961. Comminuted meat emulsions. The capacity of meats for emulsifying fats. Food Technol. 15: 468.
Swift, C.E. and Sulzbacher, W.L. 1963. Comminuted meat emulsions. Factors affecting meat proteins as emulsion stabilizers. Food Technol. 17: 224.
Swingler, G.R., Neale, R.J., and Lawrie, R.A. 1978. Nutritive value of proteins isolates and fibers from meat industry by-products. Meat Sci. 2: 31.
Terrell, R.N., Weinblatt, P.J., Smith, G.C., Carpenter, Z.L., Dill, C.W. and Morgan, R.G. 1979. Plasma protein isolate effects on physical characteristics of all-meat and extended frankfurters. J. Food Sci. 44: 1041.
Tybor, P.T., Dill, C.W., and Landmann, W.A. 1973. Effect of decolorization and lactose incorporation on the emulsification capacity of spray-dried blood protein concentrates. J. Food Sci. 38: 4.
Tybor, P.T., Dill, C.W., and Landmann, W.A. 1975. Functional properties of proteins isolated from bovine blood by a continuous pilot process. J. Food Sci. 40: 155.
Young, C.R., Lewis, R.W., Landmann, W.A., and Dill, C.W. 1973. Nutritive value of globin protein fractions from bovine blood. Nutr. Rep. Intl. 8: 211.
Ms received 7/27/81; revised 9/14/81; accepted 9/17/81.

Journal Article 119-81 by the Ohio Agricultural Research and Development Center, Wooster, OH 44691.

POTATO STARCH AND FLOUR IN FRANKFURTERS . . . From page 404

Meister, E. and Thompson, N.R. 1976. Protein quality of precipitate from waste effluent of potato chip processing measured by biological methods. J. Agric. Food Chem. 24: 924.
Meuser, F. and Smolnik, H.D. 1979. Potato protein for human use. JAOCS 56: 449.
Pearson, A.M., Spooner, M.E., Hegarty, G.R., and Bratzler, L.J. 1965. The emulsifying capacity and stability of soy sodium proteinate, potassium caseinate and nonfat dry milk. Food Technol. 19: 1841.
Satterlee, L.D. 1981. Proteins for use in food. Food Technol. 35: 53.
Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Company, Inc., New York.
Smith, G.C., Juhn, H., Carpenter, Z.L., Mattil, K.F., and Carter, C.M. 1973. Efficacy of protein additives as emulsion stabilizers in frankfurters. J. Food Sci. 38: 849.
Sofos, J.N. and Allen, C.E. 1977. Effects of lean meat source and levels of fat and soy protein on the properties of wiener-type products. J. Food Sci. 42: 849.
Wyler, O.D. 1971. Additives and their influence upon technology and quality of meat products. IFST Proceedings 4: 167.
Ms received 7/17/81; revised 9/21/81; accepted 9/24/81.

This research was supported by a grant from COLBY Co-Operative Starch Company. The authors thank W.A. Bean and Sons for the use of their plant and personnel.

Effects of Mixing and Thermal Processing on Shelf-Stable Sectioned and Formed Beef

W. R. WIEBE JR. and G. R. SCHMIDT

ABSTRACT

Sectioned and formed roast beef, vacuum mixed and heat processed in either retortable pouches or cans, had a lower cook yield than its nonvacuum mixed counterpart. Vacuum mixing had no significant influence on increasing particle-to-particle cohesion (binding strength). Sectioned and formed roast beef cooked in retortable pouches at 121°C (F_0 = 6.0) had a higher cook yield and binding strength than product processed at 110°C (F_0 = 6.0). Processing the canned product at 121°C (F_0 = 6.0) as opposed to 110°C (F_0 = 6.0) resulted in no improvement of cook yield or binding strength. Overall, product processed in retortable pouches had a higher binding strength, but a lower cook yield than product processed in cans.

INTRODUCTION

THIS STUDY concentrated on improving the binding strength of a shelf-stable sectioned and formed roast beef product. The term "binding" will refer to the cohesion of meat pieces into a unit system.

Maesso et al. (1970) showed that mechanical action, salt and phosphate enhances the extraction of salt-soluble protein and results in a significant increase in binding of meat pieces into a unit system. Currently there is interest in using vacuum mixing as a means of improving the binding ability of meat pieces. Information about the effectiveness of these systems is scarce. The effect of vacuum mixing on protein extraction and functionality was investigated in a model system by Solomon and Schmidt (1980). There was a significant increase in the amount of crude myosin extracted under a vacuum, but the total amount of protein extracted was not affected. Solomon and Schmidt (1980) also found no effect of vacuum on the binding ability of the freeze-dried crude myosin binders. Siebert (1978) stated that 20–30% more protein is extracted in a vacuum chopper as opposed to an open bowl chopper. This enhanced protein extraction resulted in a product with a firmer texture, but there was no improvement of water holding capacity (Siebert, 1978).

Acton (1972) evaluated the effect of heat processing on the binding strength and cook loss of poultry meat loaves. There was no appreciable binding below 35°C, and above 82°C the binding strength decreased. Bard and Tischer (1951) examined the extent of changes in tenderness, drained juice, moisture content and pH of beef during processing to commercial sterility at 107°, 116° and 124°C. A high temperature, short time process was shown to result in a smaller loss of quality as compared with a process of equal sterilizing value carried out at a lower temperature.

The retortable pouch as a container for shelf-stable low acid products has a development history of 20 yr and was reviewed by Mermelstein (1976). Because the pouch has a thinner profile than the can, it takes less time to reach lethal temperature at the center of the food in the pouch than in cans (Guedez and Bates, 1975). Consequently, the product at the periphery is not overcooked, as it is in cans and the quality is better. The product is truer in color, and firmer in texture and most likely has less nutrient loss. Most work on the pouch has dealt with vegetables and fruit.

The objectives of this study were to determine the effects of vacuum mixing, thermal processing and precooking on the binding strength and cook yield of shelf-stable sectioned and formed roast beef. The effect of processing to commercial sterility in a pouch as well as a can was also examined.

Authors Wiebe and Schmidt are with the Dept. of Animal Sciences, Colorado State Univ., Fort Collins, CO 80523.

MATERIALS & METHODS

Product preparation

Beef inside rounds were obtained from a reputable supplier, dissected free of visible surface and seam fat and connective tissue, stored at 2–4°C for 2 days, and then ground through a 2.54 cm plate.

Each treatment of 20.4 kg consisted of 19.6 kg (95.75%) of beef inside rounds, 204g (1.00%) of salt, 51g (0.25%) of Heller's Soluble Phosphate (WJ-0052) and 613g (3.00%) of water. Heller's Soluble Phosphate is composed of food grade sodium tripolyphosphate and sodium hexametaphosphate.

The experiment consisted of three 4-min mixing treatments in a Keebler (Chicago, IL) Model No. 238 Mixer (37.5 capacity) as presented in Table 1. Each treatment was replicated three times. All ingredients were added prior to mixing and mixing temperature was between 3° and 5°C. Samples were removed and analyzed for fat and moisture (AOAC, 1970). The raw mixture contained 3% fat and 73% water with no difference between replicates.

Portions of each treatment were placed in a Hely Joly Stuffer, France. Equal portions of meat mixture (approximately 3.0 kg) were stuffed into two Teepak (Chicagok IL) moisture impermeable, fibrous cellulose casing (10.2 cm in diameter, 70 cm in length). One of the tw rolls was frozen at −30°C and the other was cooked in an Alkar Smokehouse, Lodi, WI (see smokehouse schedule in Table 2) to a final internal temperature of 68°c and chilled over-

Table 1—Mixing treatments of coarse ground beef, water, salt and phosphate

Treatment A	4 min of mixing without the application of vacuum.
Treatment B	3 min of mixing without the application of vacuum followed by 1 min of mixing with the application of 635 mm Hg vacuum.
Treatment C	4 min of mixing with the application of 635 mm Hg vacuum.

Table 2—Smokehouse schedule

Time	Dry bulb temp (°C)	Wet bulb temp (°C)
1 hr	55	0*
1 hr	63	0
1 hr	71	0
2 hr	77	57

*A zero indicates that no humidity was added to the smokehouse.

night at 4°C. Sixteen rectangular 314 x 202 x 304 cans were also stuffed, each with 340g of meat mixture and sealed under a vacuum (635 mm Hg). Eight of the 16 cans were chilled to 4°C, the other eight were processed in a 74°C water bath to an internal temperature of 68°C and then chilled to 4°C. All samples were held overnight. The following day, eight steaks, 2 cm thick, were sawed from each of the rolls. Each of the 16 steaks (approximately 160g each) were randomly placed in 14 cm wide x 18 cm long x 1.9 cm thick retort pouches (Ludlow Corporation, Lombard, Ill.) labeled and vacuum sealed (635 mm Hg) using a Multivac West Germany (Type AG-500) vacuum sealer. After pouching the frozen steaks were allowed to that (to 15°C).

Before being placed in the stationary retort, the pouches were placed in a retort pouch rack similar to the one used by Davis et al. (1972). The rack spacing established the maximum thickness the pouch will attain during heat processing. This enabled the establishment of a sterilization process based on a known maximum thickness. All pouched and canned products were processed in the stationary retort to an F_0 of 6.0 at 110° and 121°C. See Table 3 for time-temperature relationships necessary to achieve an $F_0 = 6.0$.

The procedure followed in conducting heat penetration studies of cans were those discussed by Alstrand and Ecklund (1952). The data from these studies were analyzed by the "formula" method of Ball (1923, 1928 and Ball and Olson 1957). Procedures used to determine the appropriate thermal process for pouches differ from the procedures for cans only in the way the thermocouples were located. Thermocouples in the cans were located according to Alstrand and Ecklund (1952); the pouches required a special thermocouple mounting bracket (O.F. Ecklund, Inc., Cape Coral, FL). This required a 6 mm diameter hole through the pouch. The hole was punched through the flange area with a 6 mm paper punch. Seal was effected by an "O" ring inserted on the inside of the pouch. The outer end of the receptacle assured that the thermocouple was located in the center of the 1.9 cm thick pouch. For further assurance of proper location, rubber spacer discs center drilled by 1.9 cm diameter by 3 mm thick were placed on the thermocouple. The heat penetration data was analyzed by the "graphical" method of Bigelow et al. (1920).

Table 3—Time-temperature relationships required for $F_0 = 6.0$

Container	Retort temp (°C)	Process time (min)	Initial temp (°C)
Pouch	110	90	11
	121	10	12
Can	110	145	13
	121	68	10

Table 4—Overall effect of mixing treatments on the physical and chemical properties of shelf-stable sectioned and formed roast beef[a,b,c]

	Mixing treatment		
	No vacuum 4 min	No vacuum 3 min + Vacuum (635 mm Hg) 1 min	Vacuum (635 mm Hg) 4 min
Pouch			
Binding strength (g/cm^2)	94.6	100.9	104.7
Cook yield (%)	67.9[d]	66.9[e]	66.3[e]
Moisture (%)	64.4	63.5	63.7
Fat (%)	3.0	2.4	3.1
CAN			
Binding strength (g/cm^2)	68.6	69.5	70.8
Cook yield (%)	76.2[d]	75.1[e]	74.7[e]
Moisture (%)	67.1	66.3	66.6
Fat (%)	3.3	3.7	3.5

[a] Each value is the mean of 3 replicates averaged over processing temperatures and precooking treatments, with 4 samples per replicate.
[b] Means of the same line without superscripts were not significantly different.
[c] Means of the same line with different superscripts were significantly different ($P < 0.05$).

Binding strength determination

The particle to particle cohesion (binding strength) of the loaves and steaks was measured using the Instron Universal Testing Machine (Canton, MA) and a breaking bar and bridge assembly similar to that of Pepper and Schmidt (1975). Two slices 1 cm wide were cut from each 2 cm thick steak (product from pouches), and two slices 1 cm wide were cut from each 5 cm thick loaf (product from cans). The 2 cm or 5 cm side was laid flat on the breaking unit having a base with a bridge width of 4 cm. The breaking bar traveled at a rate of 20 cm/min and the chart at a rate of 2 cm/min. The cross-sectional area of the slice was measured at the point where the break occurred. The binding strength of the slice was expressed as the maximum force per unit cross-sectional (g/cm^2).

Cook yield

The ratio of the weight of the seak or loaf after its final cook to that before cooking expressed as a percentage was used to indicate cook yield.

Chemical analyses

The raw meat mixture and the cooked steaks and loaves were analyzed for moisture and fat. Moisture was determined using the vacuum oven procedure (AOAC, 1970). Fat was determined by extraction with ether (AOAC, 1970).

Statistical analyses

Analysis of variance of the binding strength, cook yield, moisture and fat was determined in a split plot design. When F-values were significant, Fischer's Least Significant Difference was used to locate differences between treatment means (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

THE EFFECT OF MIXING treatments on the binding strength and cook yield of canned and pouched sectioned and formed roast beef is given in Table 4. Products mixed in the absence of vacuum showed a higher cook yield than products mixed in the presence of vacuum for either length of time. A possible explanation for this observation is that air voids in the nonvacuum mixed products may retain water released from the protein matrix. There was no effect of mixing treatment on the binding strength, moisture or fat content of these products.

Table 5 shows that the pouched product exhibited a higher binding strength and cook yield when processed to

Table 5—Overall effect of processing temperature on the physical and chemical properties of shelf-stable sectioned and formed roast beef[a,b,c]

	Processing temperature (°C)	
	110	121
Pouch		
Binding strength (g/cm^2)	88.3	111.8*
Cook yield (%)	66.2	67.8*
Moisture (%)	63.8	64.0
Fat (%)	3.4	2.9*
CAN		
Binding strength (g/cm^2)	66.1	73.2
Cook yield (%)	75.7	75.1
Moisture (%)	66.8	66.5
Fat (%)	3.4	3.6

[a] Each value is the mean of 3 replicates averaged over mixing and precooking treatments, with 6 samples per replicate.
[b] Means of the same line without asterisks were not significantly different.
[c] Means of the same line with an asterisk were significantly different ($P < 0.05$).

an F_o = 6.0 (commercial sterility) at 121°C rather than 110°C. This seems to indicate that a shorter time of exposure at higher temperatures results in an improved product. The moisture content was not significantly affected by thermal process, but the fat content was higher in pouched product processed to commercial sterility at 110°C. The binding strength, cook yield, fat and moisture content of the canned product was not significantly affected by processing to commercial sterility at either 110°C or 121°C. This indicates that, in the case of the can, time of exposure at 121°C as well as 110°C is excessive and there is no improvement in quality.

Results in table 6 indicate that precooking the sectioned and formed beef to 68°C, chilling it to 4°C, and processing to commercial sterility in a pouch the following day at either 110°C or 121°C, resulted in a product with a higher binding strength and lower cook yield. The precooked, pouched product also exhibited a higher moisture content, but a lower fat content. There is some question as to whether these results could be the same if the beef steak has been precooked to 68°C inside rather than outside the pouch. Product in the thinly profiled pouch would have cooked faster than product processed in a 10.2 cm diameter casing. The shorter cooking time in the pouch would probably produce a higher quality product. Precooking to 68°C in a sealed can and then processing to commercial sterility at either 110°C or 121°C did not significantly influence the binding strength, cook yield, moisture or fat content of the canned product. It was hoped that precooking and chilling would stabilize the canned products towards further processing, but this did not occur. A further study is needed to evaluate what effect precooking and immediate processing to commercial sterility would have on the quality of shelf-stable beef. The higher initial temperature would result in a shorter process time and an improvement in product quality.

Table 7 shows, considering all treatments, the pouched product exhibited a higher binding strength, but a lower cook yield, fat and moisture content than the canned product. Since the pouch has a thinner profile than the can, it takes less time to reach commercial sterility, producing an improvement in binding strength. The lower cook yield can be explained by the fact that product in the thinly profiled pouch has a larger surface area and smaller volume relative to the canned product. This large ratio of surface area to volume would minimize the entrapment of fat and water within the product and maximize release of these substances.

CONCLUSIONS

THE FOLLOWING CONCLUSIONS can be drawn from this study:

(1) vacuum mixing of canned and pouched product resulted in a lower cook yield and no significant enhancement of binding.

(2) processing pouched product to commercial sterility at 121°C as opposed to 110°C resulted in an increased binding strength and cook yield.

(3) processing temperature used did not significantly influence the binding strength and cook yield of the canned product.

(4) precooking the pouched product resulted in a higher binding strength, but a lower cook yield.

(5) precooking had no significant influence on the binding strength and cook yield of the canned product.

This study suggests that in shelf-stable meat products, changes induced by thermal processing dominate any effects which may be due to vacuum pretreatment. However, there may be benefits gained from vacuum processing which are beyond the scope of this study.

Table 6—Overall effect of precooking on the physical and chemical properties of shelf-stable sectioned and formed roast beef[a,b,c]

	Precooking treatment	
	Raw	Precooked
Pouch		
Binding strength (g/cm^2)	91.9	108.2*
Cook yield (%)	67.9	66.1*
Moisture (%)	63.6	64.2*
Fat (%)	3.3	3.0*
CAN		
Binding strength (g/cm^2)	68.4	70.9
Cook yield (%)	75.2	75.5
Moisture (%)	66.6	66.8
Fat (%)	3.5	3.5

[a] Each value is the mean of 3 replicates averaged over processing temperature and mixing treatments, with 6 samples per replicate.
[b] Means of the same line without asterisks were not significantly different.
[c] Means of the same line with an asterisk were significantly different ($P < 0.05$).

Table 7—Overall effect of container on the physical and chemical properties of shelf-stable sectioned and formed roast beef[a,b]

	Container	
	Pouch	Can
Binding strength (g/cm^2)	100.0	69.7*
Cook yield (%)	67.0	75.3*
Moisture (%)	63.9	66.7*
Fat (%)	3.1	3.5*

[a] Each value is the mean of 3 replicates averaged over processing temperatures, mixing and precooking treatments, with 12 samples per replicate.
[b] Means of the same line with an asterisk were significantly different ($P < 0.05$).

REFERENCES

Acton, J.C. 1972. Effect of heat processing on extractability of salt-soluble protein, tissue binding strength and cooking loss in poultry meat loaves. J. Food Sci. 37: 244.

Alstrand, D.V. and Ecklund, O.F. 1952. The mechanics and interpretation of heat penetration tests in canned foods. Food Technol. 6(5): 185.

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

Ball, C.O. 1923. Thermal process time for canned food. Bul. Nat. Res. Council, 7, Part 1, No. 37.

Ball, C.O. 1928. Mathematical solution of problems on thermal processing of canned foods. Univ. of California Pub. in Pub. Health, 1(2): 15.

Ball, C.O. and Olson, F.C.W. 1957. "Sterilization in Food Technology." McGraw-Hill Book Co., Inc., New York.

Bard, J.C. and Tischer, R.G. 1951. Objective measurement of changes in beef during heat processing. Food Technol. (7): 296.

Bigelow, W.D., Bohart, G.S., Richardson, A.C., and Ball, C.O. 1920. Heat penetration in processing canned foods. Nat. Canners Assoc. Bull. 16L.

Davis, R.B., Long, F.E. and Robertson, W.F. 1972. Engineering considerations in retort processing of flexible packages. Food Technol. 26(8): 65.

Guedez, O. and Bates, R.P. 1975. A laboratory procedure for the pressure processing of flexible pouches. J. Food Sci. 40: 724.

Maesso, E.R., Baker, R.C., Bourne, M.C., and Vadehra, D.V. 1970. Effect of some physical and chemical treatments on binding quality of poultry loaves. J. Food Sci. 35: 440.

Mermelstein, N.H. 1976. An overview of the retort pouch in the U.S. Food Technol. 30(2): 28.

Pepper, F.H. and Schmidt, G.R. 1975. Effect of blending time, salt, phosphate and hot-boned beef on binding strength and cook yield of beef rolls. J. Food Sci. 40: 227.

Siebert, H. 1978. A look at the plusses of vacuum cutting. Food in Canada. 38(3): 26.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. Iowa State Univ. Press, Ames, IA.

Solomon, L.W. and Schmidt, G.R. 1980. The effect of vacuum and mixing time on the extractability of pre- and postrigor beef. J. Food Sci. 45: 283.

Ms recieved 5/4/81; revised 11/4/81; accepted 11/4/81.

Supported by the Colorado State Univ. Experiment Station and published as Scientific Series Paper No. 2681.

The authors would like to thank the following companies for their assistance: Armour and Company, Armour Research Center, Scottsdale, AZ; Ludlow Packaging Corporation, Lombard, IL; Teepak, Incorporated, Chicago, IL; and the Old West Regional Commission for their financial assistance.

Water-Holding Capacity and Textural Acceptability of Precooked, Frozen, Whole-Egg Omelets

SALLIE WILLIARD O'BRIEN, ROBERT C. BAKER, LAMARTINE F. HOOD, and MYRA LIBOFF

ABSTRACT

Precooked, frozen omelets were analyzed for moisture loss, expressible moisture, shear force, and sensory evaluation to determine water-holding capacity and textural acceptability. Addition of 0.1% xanthan gum, application of moist heat in cooking, and cryogenic freezing with liquid carbon dioxide or nitrogen minimized moisture loss and shear force. Sodium carboxymethylcellulose (CMC), pregelatinized tapioca starch and sodium tripolyphosphate additives performed satisfactorily, but omelets containing xanthan gum were consistently rated highest in sensory evaluation of several treatments, including fresh and untreated control omelets. Steaming omelets for five minutes combined with cryogenic freezing produced a desirable omelet, requiring no additives. Steamed omelets were rated comparable to baked omelets in most sensory parameters.

INTRODUCTION

PRECOOKED, FROZEN OMELET products have recently gained the interest of the fast-food trade as highly nutritious, convenient, food items. Limitations to widespread institutional and commercial use involve mainly textural problems, resulting in the loss of water-holding capacity as weepage or syneresis occurs after two heating processes and freezing.

The texture of a desirable omelet is dependent upon the formation of a stable three-dimensional gel structure entrapping water. Gelation results from heat application to the native egg proteins which are subsequently denatured, producing long chain molecules of protein which are no longer in their native helical conformation (Golding, 1963). Segments of the molecules associate to form a three-dimensional network held together by the correct balance of attractive and repulsive forces (Ferry, 1948). The formation of crosslinks increases the rigidity of the gel and the amount of free and bound water in the gel (Golding, 1963). Physical factors such as freezing and reheating may alter the protein gel by disruption and reassociation of the necessary attractive forces. As the gel structure is altered, water may be freed from within the protein network, resulting in a more associated, less tender texture after freezing and reheating.

Taborsky (1970) did extensive research of the freezing and thawing effects on the conformation of phosvitin, an egg yolk protein. Upon suppression of ionization of its phosphomonester groups, phosvitin's conformation changed from a disordered complex to a highly ordered, beta-pleated, protein layer. This may account for a number of protein denaturations and subsequently restrict protein-water interactions in the frozen egg gel structure. Dowell et al. (1962) found that rapid freezing rates minimized ice crystal growth and formation and, therefore, cellular rupture.

Additives which may increase the water-holding capacity of frozen, reheated omelets include modified starches, gums, and polyphosphates. The colloidal properties and polymeric molecular weight of gums result in their water-binding capacity, freezing rate alteration, and modification of ice crystal size (Dinzis et al., 1970) through conformational change. Xanthan gum consists of ordered helices which unwind into random coils, increasing effective hydrodynamic volume and viscosity (Rees, 1972). Shemwell and Stadelman (1976) demonstrated that beneficial effects of adding 1% sodium carboxymethylcellulose (CMC) to reduce weepage of frozen, baked spinach souffle; but found that rapid freezing with liquid nitrogen or dichlorodifluoromethane (Freon 12) did not reduce weepage more than conventional freezing.

Upon heat application, native starch molecules imbibe water, forming a three-dimensional gel structure. Depending on the temperature, and size and shape of the starch molecules, retrogradation may occur (Osman, 1972). Retrogradation is the progressive association of the gel structure to a more orderly, partially crystalline state, resulting in syneresis. Incorporation of acetyl or hydroxypropyl groups in a cross-linking process prevents retrogradation during the freeze-thaw cycle (Greenwood, 1976).

Calcium-reduced skim milk (CRSM) has been developed for use in sausage manufacture, to promote water binding in the emulsion, increasing the juiciness of the product (Western Dairy Products, 1962).

Polyphosphates are used extensively in meat, poultry, and fish products to increase water-holding capacity and control "drip" loss. In muscle proteins, polyphosphates promote the complete hydration of proteins through their ability to cause dissociation of the actomyosin complex and partially complex divalent cations (Ellinger, 1972).

The following experiments were designed to evaluate the effects of heat application in cooking, freezing rate, and the use of additives such as starches, gums, and polyphosphates on the water-holding capacity and textural acceptability of precooked, frozen omelets.

EXPERIMENTAL

Experiment A

Several starches and gums were initially tested to determine their effect on the water-holding capacity and texture of omelets. They included pregelatinized tapioca starch (1.0%), modified waxy maize starch (1.0%), modified tapioca starch (1.0%), pregelatinized potato starch (1.0%), xanthan gum (0.1%), sodium carboxymethylcellulose (0.1%), and karaya gum (0.1%).

Three omelet formulations were prepared: (1) omelet with no additives, (2) omelet with modified starch, and (3) omelet with gum solution. The ingredients included 76.5–77.4% whole egg (382.5–387.0g), 20% water (100g), 2% nonfat dry milk (10g), 0.5% sodium chloride (2.5g), and 1% starch (5.0g) or 0.1–0.2% gum (0.5–1.0g).

Preparation of omelets containing starch and omelets with no additives consisted of blending the eggs and water a few seconds (Waring Blendor, model 11-183; speed two) with subsequent addition of the combined dry ingredients to the blender vortex. Blending time totaled 20–60 sec. In preparing omelets with added gum, a slurry of 0.5–1.0g gum and 2.0–3.5g vegetable oil was dispersed in 100g water, blending 7–10 min on speed two to insure hydration of the gum. The eggs and combined dry ingredients were subsequently added as above, blending an additional 20–60 sec.

Authors O'Brien and Baker are affiliated with the Dept. of Poultry and Avian Sciences and Authors Hood and Liboff are affiliated with the Dept. and Institute of Food Science, Cornell Univ., Ithaca, NY 14853.

For baking, nine aliquots of each formulation, 50g each, were poured into 7.6 cm circular tins presprayed with Pam release agent. The samples were baked at 177°C for 12 min. The omelets were cooled 10–20 min, refrigerated, and frozen within a few hours by (1) conventional freezing methods, (2) partial immersion in liquid Freon 12, or (3) contact with sprayed liquid carbon dioxide or liquid nitrogen. Conventional methods involved packaging three omelets per polyethylene bag and freezing at −15°C still air, −23°C still air, and −23°C forced air. Cryogenic methods involved immersing one side of the omelet in liquid Freon 12 for 5–7 min until nucleate boiling ceased, repeating on the opposite side. Liquid carbon dioxide and liquid nitrogen were sprayed under pressure into an insulated freezing chamber where three omelets were suspended in a wire mesh support.

Product evaluation included moisture loss and expressible moisture determinations, and shear force measurements. The omelets were reheated in aluminum containers with 11 cm Whatman #42 filter paper beneath, and Whatman #52 paper above the sample; the complete assembly tightly covered with foil, heated at 177°C for 17–20 min, and cooled for 10 min. Weighing of the container assembly, with and without sample, before and after reheating, determined moisture loss.

Expressible moisture, or the amount of water released upon application of a physical force, was determined by centrifugation in a Sorvall RC2-B centrifuge using a SS-34 head. Triplicate samples of 1.5–2.5g were placed in filter paper cones, three outer 4 cm Whatman #3 papers and one inner 7.5 cm Whatman #50, and centrifuged at 8000 revolutions per min. The weight difference of the cones before and after centrifugation, divided by the total moisture contents, as determined in a Boekel 2000 watt drying oven, was used as a measure of expressible moisture.

An Allo-Kramer Shear Press (model S2HE) and Varian strip chart recorder (model G-11A) were used at 2% of scale to determine pounds force per gram sample required to shear the reheated and cooled omelets.

For sensory evaluation, 12.5g portions were served immediately after reheating to a trained panel of 8 to 12 members. They scored intensity of color, texture, tenderness, flavor, and overall desirability on a scale from 1 (poor quality) to 9 (excellent quality), except for tenderness whose optimum rating was 5, with 1 = too tough and 9 = too tender.

Experiment B

The same formulations and procedures were followed as in Experiment A except for the substitution of 2% CRSM for NFDM in the formulation of selected omelet treatments. Starch and gum additions were limited to pregelatinized tapioca starch and xanthan gum. Moisture loss and texture evaluations were done as described above, with the exception of moisture loss comparisons between frozen and refrigerated omelets. In that comparison only frozen omelets were reheated, while refrigerated omelets were held at 2°C without reheating.

Sensory evaluation involved five treatments: (1) fresh, unfrozen control, (2) xanthan gum, (3) pregelatinized tapioca starch, (4) xanthan gum plus 2% CRSM, and (5) pregelatinized tapioca starch plus 2% CRSM. The samples were frozen with liquid carbon dioxide. Scoring was done in three replicated sessions. Analyses of variances were computed and least significant differences among treatments determined.

Experiment C

The effects of several polyphosphates on water-holding capacity of omelets were evaluated. Sodium tripolyphosphate, sodium hexametaphosphate, and Kena (a patented product combining 90% sodium tripolyphosphate and 10% sodium hexametaphosphate) were added at 0.5% by weight to omelet formulations, using the same procedures as described for modified starch and gum additions. Sensory evaluation consisted of six comparisons: 1) fresh – unfrozen control, 2) frozen control, 3) xanthan gum – frozen, 4) pregelatinized tapioca starch – frozen, 5) sodium tripolyphosphate – frozen, and 6) sodium hexametaphosphate – frozen. pH of the raw and baked polyphosphate omelets was monitored over a 21 day period with a Metrohm/Brinkman 103 pH Meter.

Experiment D

Steaming as a means of moist heat application was evaluated. Raw omelets were held for 5 min, within a covered kettle on stove-top, containing a few inches of water generating steam. Cooling, packaging, and freezing were as described previously.

Experiment E

Omelet ultrastructure was analyzed with scanning electron microscopy. Frozen omelets were prepared by freeze drying and then broken into pieces to expose interior regions. Pieces were mounted on stubs coated with silver paint. Stubs were placed in a direct current diode sputtering device and coated with gold/palladium for 2–3 min. Materials were examined with an AMR-1000 and micrographs were taken at 20 K.V.

RESULTS & DISCUSSION

THE RESULTS of Experiment A are summarized in Tables 1 and 2. None of the starch additives consistently minimized moisture loss in predictable trends; however,

Table 1—Percent moisture loss of frozen, reheated omelets formulated with modified starches and gums

Addition	Moisture Loss (% by wt) −15°C Still air	−23°C Still air	−23°C Forced air
Pregelatinized tapioca starch	5.3 ± 0.58^{ab}*	4.1 ± 1.66^{ab}	3.4 ± 1.37^{abc}
Modified waxy maize starch	4.7 ± 0.50^{ab}	4.1 ± 0.96^{abc}	3.6 ± 1.63^{bc}
Modified tapioca starch	5.5 ± 0.23^{abc}	2.4 ± 1.07^{a}	3.9 ± 1.30^{c}
Pregelatinized waxy maize starch	5.9 ± 0.61^{bcd}	4.2 ± 1.11^{abc}	3.5 ± 0.87^{bc}
Modified potato starch	6.9 ± 0.87^{d}	3.2 ± 0.10^{ab}	2.9 ± 0.95^{abcd}
Pregelatinized potato starch	4.5 ± 0.79^{ae}	4.0 ± 0.96^{ab}	1.9 ± 0.66^{abd}
Xanthan gum	3.3 ± 0.06^{e}	2.6 ± 0.50^{a}	1.2 ± 0.64^{d}
Na Carboxymethyl-cellulose	6.7 4.3 ± 1.13^{ae}	5.0 ± 0.23^{bc}	1.3 ± 0.25^{d}
Karaya gum	6.7 ± 0.35^{cd}	7.0 ± 1.04^{d}	1.5 ± 0.53^{ad}
No addition	7.1 ± 0.96^{d}	6.0 ± 0.62^{cd}	3.9 ± 1.25^{c}

* Treatment mean and standard error of values evaluated in triplicate. a,b,c,d, and e differ significantly ($P \leq 0.05$)

Table 2—Shear force values of frozen, reheated omelets formulated with modified starches and gums

Addition	Shear force (lb/g sample) −15°C Still air	−23°C Still air	−23°C Forced air
Pregelatinized tapioca starch	0.86 ± 0.40^{a}*	1.03 ± 0.20^{ab}	0.55 ± 0.00^{a}
Modified waxy maize starch	1.17 ± 0.13^{b}	1.21 ± 0.11^{b}	1.02 ± 0.02^{b}
Modified tapioca starch	1.32 ± 0.00^{b}**	1.05 ± 0.01^{ab}	0.99 ± 0.01^{b}
Pregelatinized waxy maize starch	1.29 ± 0.06^{b}	0.85 ± 0.08^{ac}	0.88 ± 0.05^{c}
Modified potato starch	1.32 ± 0.00^{b}**	1.14 ± 0.16^{b}	1.21 ± 0.01^{d}
Pregelatinized potato starch	1.22 ± 0.10^{b}	1.05 ± 0.01^{ab}	0.95 ± 0.06^{e}
Xanthan gum	1.08 ± 0.05^{ab}	0.75 ± 0.12^{c}	0.99 ± 0.03^{b}
Na Carboxymethyl-cellulose	1.25 ± 0.07^{b}	1.02 ± 0.08^{ab}	1.08 ± 0.05^{f}
Karaya gum	1.32 ± 0.00^{b}**	0.65 ± 0.31^{c}	1.00 ± 0.01^{b}
No addition	1.32 ± 0.00^{b}**	1.05 ± 0.04^{ab}	1.23 ± 0.03^{d}

* Treatment mean and standard error of values evaluated in triplicate. a, b, c, d, e and f differ significantly ($P \leq 0.05$)
** Indicates a shear force greater than recorded by the chosen range, 2% of scale.

Table 3—Percent moisture loss and shear force values of frozen reheated omelets and omelets held in refrigerated storage (2°C)

Addition	−23°C Forced air	Freon 12	Liquid CO_2	Liquid N	Refrigerated
Moisture Loss (% by weight)					
Xanthan gum	3.3 ± 0.79*	2.5 ± 0.99	2.4 ± 0.38	1.75 ± 0.54	3.0 ± 0.46
Xanthan gum plus 2% CRSM	3.2 ± 1.13	4.6 ± 1.08	2.3 ± 0.89	2.9 ± 0.81	2.7 ± 0.26
Pregelatinized tapioca starch	3.8 ± 0.47	2.8 ± 1.02	2.2 ± 0.84	2.5 ± 0.44	3.6 ± 0.40
Pregelatinized tapioca starch plus 2% CRSM	5.2 ± 0.21	2.6 ± 0.32	3.0 ± 1.35	3.2 ± 0.61	N/A
Shear Force (lb/g sample)					
Xanthan gum	0.99 ± 0.11*	0.95 ± 0.16	0.98 ± 0.17	0.74 ± 0.05	0.80 ± 0.04
Xanthan gum plus 2% CRSM	0.85 ± 0.18	1.25 ± 0.08	0.86 ± 0.11	0.86 ± 0.08	0.87 ± 0.08
Pregelatinized tapioca starch	0.70 ± 0.11	1.10 ± 0.00	1.05 ± 0.10	0.61 ± 0.03	0.63 ± 0.09
Pregelatinized tapioca starch plus 2% CRSM	0.87 ± 0.16	1.22 ± 0.09	1.14 ± 0.14	0.66 ± 0.09	N/A

* Treatment mean and standard error of values evaluated in triplicate.
N/A - not available, sample destroyed.

*Table 4—Sensory evaluation of liquid CO_2 frozen, reheated omelets prepared with modified starch, gum and/or CRSM**

Addition	Sensory parameter** Overall desirability	Color	Texture	Tenderness	Flavor
Xanthan gum	7.08[a]	7.56[a]	7.11[a]	5.07[a]	7.37[a]
Xanthan gum plus 1% CRSM	5.88[b]	7.12[a]	6.22[b]	5.48[a]	5.96[b]
Pregelatinized tapioca starch	6.19[b]	6.96[b]	6.07[b]	4.70[b]	6.48[b]
Pregelatinized tapioca starch plus 1% CRSM	6.35[b]	6.88[b]	5.85[b]	4.78[b]	6.85[b]
No addition, not frozen	6.04[b]	6.07[b]	5.56[b]	5.96[a]	6.52[b]

*The treatment means given are from three replicated sessions of nine panelists. a and b differ significantly ($P \leq 0.05$).
**All sensory parameters were scored on an intensity scale of 1 through 9, with 9 optimum; except for tenderness where 5 = optimum and 9 = too tender and 1 = too tough.

most did lessen moisture loss as compared to the no addition treatment. Hydrocolloid additives were more effective and consistent in decreasing moisture loss as illustrated by the significant differences in Table 1. In general, freezing effects on moisture loss were of greater magnitude and more consistent than additive effects, with the exception of the xanthan gum additive.

Pregelatinized tapioca starch and xanthan gum performed most satisfactorily in minimizing shear force (Table 2). Preliminary sensory evaluation correlated well with these findings, as xanthan gum and pregelatinized tapioca starch-containing omelets were rated highest as compared to a freshly baked control omelet. Xanthan gum was selected for subsequent testing as a result of its performance in moisture loss determinations and sensory evaluation. Pregelatinized tapioca starch was selected on the basis of preliminary shear force values and sensory evaluation.

Experiment B is summarized in Tables 3 and 4. Rapid freezing with cryogenics resulted in substantially less moisture loss and lower shear values, producing a more tender product than could be achieved with conventional freezing. CRSM apparently increased moisture loss, as much as twice the control value, in conventionally frozen omelets with a slight increase in shear force. Adding pregelatinized tapioca starch and xanthan gum improved water-holding capacity and tenderness of the CRSM omelets.

Table 3 compares unfrozen, refrigerated omlets held at 2°C with conventionally and cryogenically frozen omelets. Upon reheating, the rapidly frozen omelets demonstrated up to 1.25% less moisture loss as shown by the liquid nitrogen freezing treatment. But freezing resulted in a tougher product, as illustrated by shear force values. The omelets containing xanthan gum resulted in the least moisture loss, but the most tender product was produced by addition of pregelatinized tapioca starch, as determined from shear force values.

As illustrated by the previous results, the addition of CRSM has no consistent beneficial effects on water-holding capacity. This may be a result of CRSM's intended use as a moisture binding agent in emulsified, comminuted meat products, such as sausage (Western Dairy Products, 1962). Its apparent function in these products is to disrupt the calcium-protein micellar interactions present in milk, releasing the proteins for solubilization and subsequent binding of free water in the system. But, because an omelet is not an emulsion, the CRSM proteins apparently react differently with egg proteins and function differently on the omelets.

Obvious variations and inconsistencies existed in the reported moisture loss and shear force values of Experiments A and B. These variations may have arisen from differences in the length of frozen storage time. Samples in Experiment A were held 24 hr in frozen storage, while those in Experiment B were held 7–8 days. This may explain why moisture losses were lower in Experiment A. No attempt was made to hold frozen storage time constant among experiments, unless the results were meant to be compared directly.

Other variables such as small variations in oven temperature, baking or reheating times or frozen storage temperatures, which were difficult to hold exactly constant, may have also resulted in the observed variation within data from different experiments.

Sensory evaluation data (Table 4) showed xanthan gum-containing omelets to be consistently highest in overall desirability. They differed significantly from other treatments in color, texture, flavor, and overall desirability; including the freshly baked, untreated control omelet. Tenderness was the only parameter where the fresh omelet did not differ significantly from the omelet with xanthan gum.

Results of Experiment C are presented in Table 5. Comparing moisture loss data (Table 5) of omelets with polyphosphates with those containing modified starch and gum (Table 3) showed that similar or slightly lower moisture loss resulted from the addition of polyphosphates combined with cryogenic freezing. Conventional freezing pro-

duced fairly equivalent results regardless of which additives were used. Shear force values of polyphosphate omelets were similar to modified starch and gum-containing omelets when cryogenically frozen, but were slightly higher when conventionally frozen.

In sensory evaluations which compared polyphosphates with modified starch and gum additives (Table 6), significant differences were found in texture, tenderness, and overall desirability; while frozen control omelets were rated highest in tenderness, although not differing significantly from omelets with xanthan gum. The scores for xanthan gum-containing omelets and the frozen, control omelets did not differ significantly in any parameters, although the xanthan gum omelet scores were consistently higher. This may indicate that with cryogenic freezing, additives may not be necessary.

Polyphosphates produced few, if any, detrimental effects on color or flavor, although they did affect texture and tenderness. Both polyphosphate omelets were found to be too tender or "slightly mushy" by the taste panel, despite their performance as moisture-holders in objective tests.

Expressible moisture data of polyphosphate omelets, as compared to starch and gum omelets (Table 7), demonstrate the effectiveness of polyphosphates in holding onto water when omelets are subjected to a physical force intended to release water. Although starch and gum apparently hold similar amounts of water within the protein gel structure, they are less effective than polyphosphates in keeping it there. This may be a result of the pH in both raw and baked polyphosphate omelets (Fig. 1 and 2). Sodium tripolyphosphate, which raised omelet pH from 7.0 to approximately 8.5 in raw omelet, was most effective in maintaining the water-holding capacity of omelets, regardless of which freezing method was employed. Cryogenic freezing resulted in the lowest amount of expressible moisture, lower than omelets held in refrigerated storage at 2°C for less than 1 wk.

Table 7 illustrates the effects of Experiment D, steaming, on water-holding capacity and textural acceptability. Expressible moisture determinations of steamed omelets, when compared to those of baked omelets, were higher—at least 10% more in omelets with no additives. This may have been a result of moist heat promoting greater incorporation of water in the developing protein gel structure. Since this additional water was not tightly bound, it was released upon centrifugation, increasing expressible moisture values. Sensory evaluation of steamed omelets (Table 8) showed that most sensory parameters were rated comparable to baked omelets. The frozen control omelets were

Table 5—Percent moisture loss and shear force values of frozen, reheated omelets with polyphosphates

Addition	Moisture loss (% by wt)		Shear force (lb/g sample)	
	−23°C Forced air	Liquid CO_2	−23°C Forced air	Liquid CO_2
Tripoly-phosphate	3.6 ± 1.25*	0.9 ± 0.24	0.97 ± 0.09	0.93 ± 0.07
Kena	3.9 ± 0.47	1.9 ± 0.54	1.00 ± 0.08	0.68 ± 0.10
Hexameta-phosphate	4.0 ± 0.63	1.8 ± 0.40	1.03 ± 0.09	0.74 ± 0.14
No addition	3.9 ± 0.65	2.4 ± 0.50	1.10 ± 0.08	0.73 ± 0.13

*Treatment mean and standard error of eight values.

*Table 6—Sensory evaluation of liquid N_2 and liquid CO_2 frozen, reheated omelets formulated with modified starch, gum, and polyphosphates; stored 6 wk**

Addition	Sensory Parameter				
	Color	Texture	Tenderness**	Flavor	Overall Desirability
Pregelatinized tapioca starch	6.67[a]	5.67[a]	4.42[a]	6.58[a]	6.17[a]
Xanthan gum	7.08[a]	5.83[a]	5.96[bc]	6.92[a]	5.58[ab]
Tripolyphosphate	7.67[a]	5.67[a]	3.56[c]	7.22[a]	5.33[bc]
Hexametaphosphate	6.04[a]	4.42[b]	3.50[d]	6.29[a]	5.08[b]
Not frozen, no addition	6.79[a]	5.29[ab]	6.67[c]	6.58[a]	4.79[b]
Frozen, no addition	6.67[a]	5.38[ab]	5.38[b]	6.46[a]	5.67[ab]

* Values given are treatment mean responses of three replicated sessions of eight panelists. Liquid CO_2 frozen omelets were evaluated in two sessions, while liquid N_2 omelets were evaluated in the third session. a, b, c and d differ significantly ($P \leq 0.05$)
**Optimum tenderness = 5, too tender = 9, too tough = 1.

Table 7—Expressible moisture as a percentage of total moisture of frozen, baked and steamed omelets

Addition	% Expressible Moisture			
	Refrigerated	−23°C Forced air	Liquid CO_2	Liquid N_2
Baked				
Tripolyphosphate	19.92 ± 3.38*	20.64 ± 3.13	17.97 ± 1.14	18.66 ± 2.50
Hexametaphosphate	22.83 ± 4.34	29.60 ± 2.69	20.89 ± 5.66	30.14 ± 1.23
Xanthan gum	34.34 ± 6.37	31.81 ± 2.63	33.16 ± 1.17	30.46 ± 4.51
Pregelatinized tapioca starch	31.99 ± 4.60	38.41 ± 2.26	41.65 ± 1.36	29.86 ± 6.67
CRSM	26.67 ± 4.49	28.99 ± 2.09	33.13 ± 6.85	35.53 ± 5.49
No addition	29.76 ± 3.16	36.23 ± 3.34	41.50 ± 3.00	26.06 ± 3.16
Steamed				
Tripolyphosphate	21.24 ± 5.49	42.21 ± 4.43	22.35 ± 3.03	27.96 ± 2.52
Hexametaphosphate	27.66 ± 5.28	51.18 ± 1.00	30.22 ± 4.46	30.11 ± 2.54
Xanthan gum	32.67 ± 3.51	44.40 ± 2.48	41.62 ± 1.53	42.39 ± 2.33
Pregelatinized tapioca starch	34.09 ± 0.78	46.35 ± 1.08	34.83 ± 4.27	31.93 ± 5.30
No addition	39.22 ± 1.55	52.22 ± 0.65	44.39 ± 5.20	36.50 ± 2.97

* Treatment mean and standard error of values evaluated in triplicate.

rated highest in most parameters, with the phosphate omelets intermediate, and the starch and gum omelets lowest.

Results of these experiments indicate that water-holding capacity and textural acceptability of the precooked, frozen omelets improved with the addition of xanthan gum, the use of cryogenic freezing, or the application of moist heat. When dry heat (baking) was used to cook omelets, a better product was achieved with the addition of xanthan gum, combined with cryogenic freezing. However, when moist heat (steaming) was used, an omelet without any additives when cryogenically frozen was only slightly less desirable than the baked, xanthan gum – containing omelets.

As illustrated by Fig. 3, cryogenic freezing improves the water-holding capacity and structural integrity of the xanthan gum and starch omelets, with and without CRSM, when compared to conventional freezing at −23°C. Fig. 4 (Experiment E) demonstrates the effects of cryogenics on air cell size of omelet ultrastructure. Rate of freezing dramatically influenced omelet structure. Omelets frozen in liquid nitrogen had a more compact structure than those frozen at higher temperatures (−23°C). The addition of starch, xanthan gum, or phosphates did not appear to affect ultrastructure. Although the exact relationship between ultrastructure and water-holding capacity of omelets has not been established by this study, it appears that the more compact the ultrastructure, the greater the water-holding capacity and textural acceptability of the omelets.

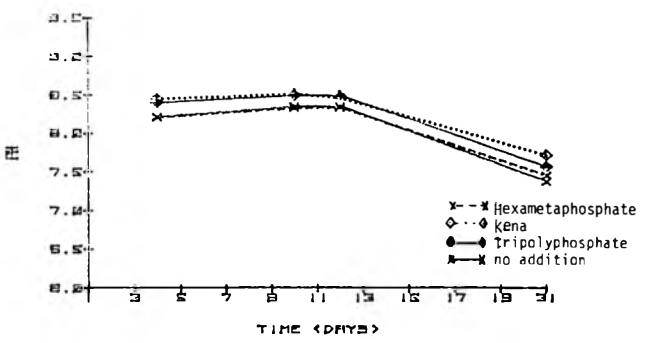


Fig. 1—Alteration of unbaked omelet pH by tripolyphosphate, kena and hexametaphosphate.

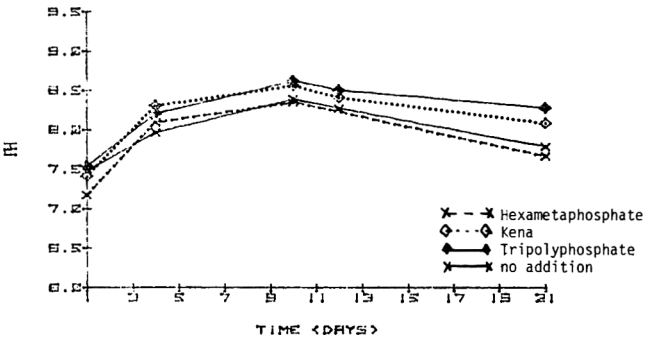


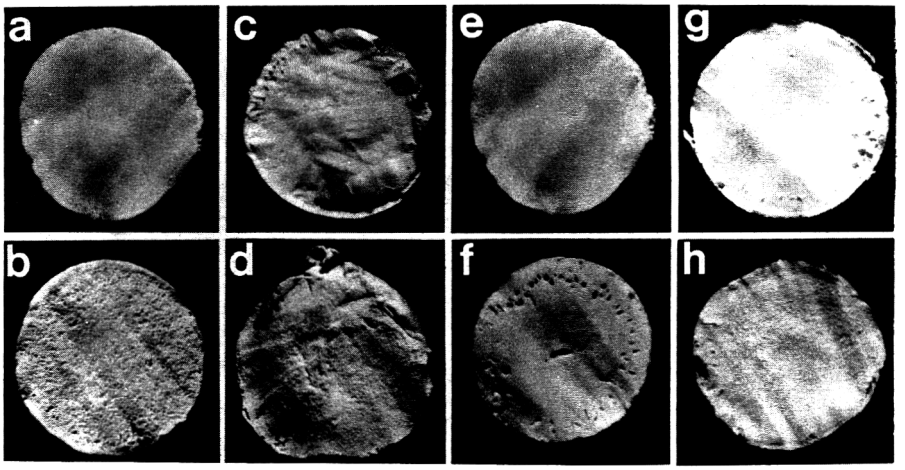
Fig. 2—Alteration of baked omelet pH by tripolyphosphate, kena and hexametaphosphate.

*Table 8—Sensory evaluation of steamed omelets, frozen with liquid N_2 and stored one week at −23°C still air**

	Sensory Parameter				
Addition	Color	Texture	Tenderness**	Flavor	Overall Desirability
Pregelatinized tapioca starch	7.22[a]	5.22[a]	6.33[a]	5.00[a]	4.78[a]
Xanthan Gum	7.56[a]	5.44[a]	7.44[b]	6.44[a]	5.44[a,b]
Tripolyphosphate	7.67[a]	5.67[a]	3.56[c]	7.22[a]	5.33[b,c]
Hexametaphosphate	7.22[a]	5.67[a]	4.33[c]	6.44[a]	6.11[a,b,c]
Not frozen, no addition	6.89[a]	5.44[a]	6.22[a]	6.67[a]	6.11[a,b,c]
Frozen, no addition	7.00[a]	5.89[a]	5.56[a]	7.00[a]	7.11[c]

* Values given are treatment mean responses of nine panelists. a, b and c differ significantly ($P \leq 0.05$)
**Optimum tenderness = 5, too tender = 9, too tough = 1.

Fig. 3—Structural integrity of frozen, reheated omelets. (a) Pregelatinized tapioca starch + CRSM, liquid CO_2 frozen; (b) Pregelatinized tapioca starch + CRSM, −23°C forced air frozen; (c) Pregelatinized tapioca starch, liquid CO_2 frozen; (d) Pregelatinized tapioca starch, −23°C forced air frozen; (e) Xanthan gum + CRSM, liquid CO_2 frozen; (f) Xanthan gum + CRSM, −23°C forced air frozen; (g) Xanthan gum, liquid CO_2 frozen; (h) Xanthan gum, −23°C forced air frozen.



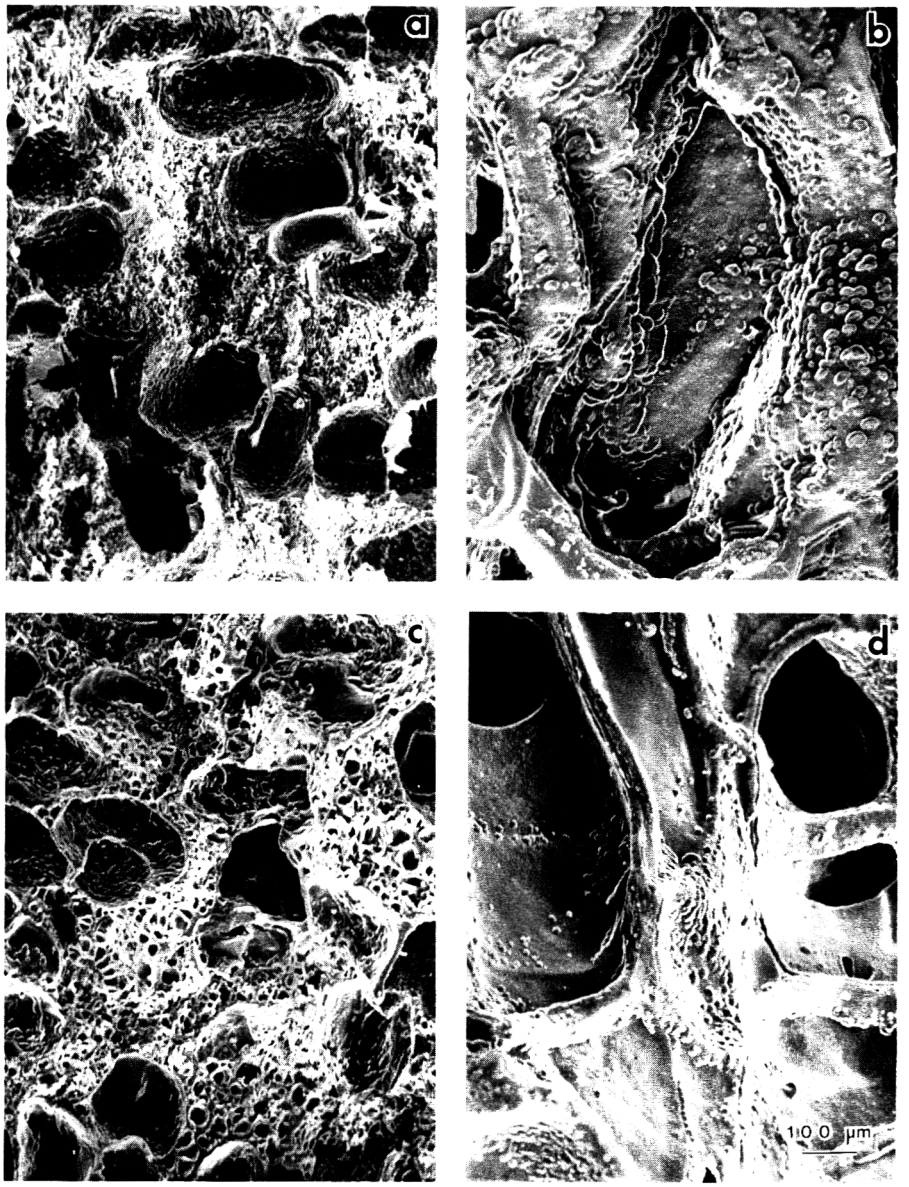


Fig. 4—Scanning electron micrographs of omelets made with and without pregelatinized tapioca starch, and frozen at different rates. (a) without starch, frozen in liquid N_2; (b) without starch, frozen at −23°C; (c) with starch, frozen in liquid N_2; (d) with starch, frozen at −23°C. Marker = 100 µm.

REFERENCES

Dintzis, F.R., Babcock, G.E., and Tobin, R. 1970. Studies on dilute solutions and dispersions of the polysaccharide from Xanthomonas campestris NLRL b-1450. Carbohyd. Res. 13: 257.

Dowell, L.G., Moline, S.W., and Rinfret, A.P. 1962. A low-temperature X-ray diffraction study of ice structures formed in aqueous gelatin gels. Biochim. Biophys. Acta 59: 158.

Ellinger, E.H. 1972. "Phosphates as Food Ingredients." CRC Press, Cleveland, OH.

Ferry, J. 1948. Protein gels. Adv. Protein Chem. 4: 1.

Golding, S.H. 1963. The effect of rate of heating, final internal temperature, and sugar level on protein gel formation. M.S. thesis, Cornell Univ., Ithaca, NY.

Greenwood, C.T. 1976. Starch. In "Advances in Cereal Science and Technology," Vol. 1. Amer. Assoc. Cer. Chem., St. Paul, MN.

Osman, E. 1972. Starch and other polysaccharides. In "Food Theory and Application," p. 151. Wiley & Sons, Inc., New York.

Rees, D.A. 1972. Shapely polysaccharides. Biochem. J. 126: 257.

Shemwell, G.A. and Stadelman, W.J. 1976. Some factors affecting weepage of baked spinach souffle. Poultry Sci. 55: 1467.

Taborsky, G. 1970. Effect of freezing and thawing on the conformation of phosvitin. J. Biol. Chem. 245: 1054.

Western Dairy Products. 1962. Comminuted meat products containing calcium-reduced skim milk. U.S. Patent 3,050,400.

Ms received 7/13/81; revised 10/26/81; accepted 10/31/81.

This research was made possible by a scholarship and grant from the American Egg Board.

Prediction and Characterization of Normal and Vat-Failed Cottage Cheese

M. H. WU and D. Y. C. FUNG

ABSTRACT

An acidification-heat-coagulation test has been developed for predicting cottage cheese vat-failure potential of milk. Milk is first acidified to pH 5.06 at 10°C and then heated at a slow rate (1°C increment per min). Poor quality acidified milk (> 10^4 CFU/ml) forms small curds at 37°C and below. Good quality acidified milk (< 10^4 CFU/ml) will form small curds at higher temperatures. By this procedure cottage cheese vat-failure potential of milk containing different levels of psychrotrophs can be predicted. Normal and vat-failed cottage cheese curds are characterized by % of grit in cottage cheese and amount of curd fines in whey.

INTRODUCTION

MILK QUALITY and manufacturing procedures are two major factors which determine cottage cheese quality. Psychrotrophic bacteria are a predominant factor in damaging milk quality for the manufacture of cottage cheese (Elliott et al., 1974). Cottage cheese plants often store raw milk at refrigeration temperatures for 3 days or more before pasteurization. This long storage may result in poor quality or vat-failed cottage cheese due to biochemical and microbiological changes. Angevine (1976) indicated that milk supply, antibiotics, rancid milk, starter cultures, and total solids content of milk were factors which caused problems in cottage cheese making. Mohamed and Bassette (1979) reported that some of the factors which may result in cottage cheese vat-failure are (1) large numbers of psychrotrophic bacteria, (2) extended storage of raw milk, (3) fluctuation in temperature of milk during transportation, storage, and separation, and (4) low total solids milk. The combination of proteolysis by psychrotrophs and high milk separation temperature may be the main factor contributing to cottage cheese vat-failure.

In cottage cheese vat-failure, normal cheese curds do not form. Mohamed and Bassette (1979) defined typical cottage cheese vat-failure as (1) porous and spongy coagulum before cutting, and (2) extensive disintegration of curds during cooking and agitation.

The objective of this study was to develop a quick and reliable method to test milk to predict cottage cheese vat-failure and to characterize cottage cheese vat-failure by using the grit test (Kosikowski, 1963) and the curd fines test (Raab et al., 1964).

MATERIALS & METHODS

Milk treatment

Raw milk with known protein concentrations, was obtained from selected cows on the Kansas State University Dairy Farm, and then standardized to 3.1 ± 0.05% protein. After pasteurization (63°C for 30 min), milk was divided into three portions of 6L each; two portions were inoculated individually with *Pseudomonas fluorescens* MC 60 and incubated at 8°C; the third portion was left uninoculated as a positive control for successful cottage cheese manufacturing. After 24 hr incubation, milk samples were separated individually at 49°C in a farm-type cream separator (McCormick-Deering KS60 FSA), and then the skimmilk was made into cottage cheese. To study the effect of natural flora on cottage cheese vat-failure, raw milk samples (without inoculation) were incubated at 4°C for 3, 5, and 7 days before separation and pasteurization, and then made into cottage cheese.

Authors Wu and Fung are affiliated with the Dept. of Animal Sciences & Industry, Kansas State Univ., Manhattan, KS 66506.

Source and treatment of microorganism

Pseudomonas fluorescens MC 60, a highly proteolytic strain, was obtained from North Carolina State University through the courtesy of Dr. D.M. Adams. Flasks with the test microorganisms in nutrient broth (Difco) were placed in a water bath shaker at 28°C for 14 hr before inoculating into milk. The amount of inoculum added to the milk varied according to the desired level of microorganism in milk (ranging from 10^3–10^6/ml) after 24 hr incubation at 8°C.

Method developed to predict cottage cheese vat failure

1. One hundred forty ml of milk were measured into a 250 ml beaker, and acidified to pH 5.06 at 10°C by adding about 0.75 ml of a mixture of phosphoric acid and lactic acid (Vitex 750, Diamond-Shamrock, 1:1 dilution), dropwise. The milk was held below 8°C at the beginning of acidification and reached 10°C at pH 5.06.
2. Milk was heated slowly (1°C per 1 min) in a reciprocating water-bath shaker and temperature was measured with a digital thermometer.
3. A 152 mm chemical scoop was dipped in the milk and periodically withdrawn as the temperature increased. The appearance of tiny flocculus curds on the scoop indicated the end point. The temperature to induce coagulation of chilled and acidified milk was termed "coagulation Temperature" (C.T.).

Cottage cheese manufacturing

Cottage cheese was made by either the culture method (Emmons and Tucky, 1967; Kosikowski, 1963) or by the direct-acid-set method (Vitex/American) with 5L of milk for each vat. The starter culture used was supplied by Chr. Hansen's Laboratory. A small transparent plexiglass vat (internal dimension: 23.5x23.5x24.5 cm) was designed for this particular purpose. Specially designed vertical and horizontal knives with wires 0.25" apart were used to cut the curd.

One hundred sixty eight vats of cottage cheese were made from milk inoculated with *Pseudomonas fluorescens* MC 60 and 60 from milk with normal flora.

Curd fines measurement

We used a modification of the curd fines measurement described by Raab et al. (1964). Instead of taking 1 pt of whey as they described, the total amount of whey was collected by passing the cheese-whey mixture through a strainer (size of mesh, 1.98 mm^2). The whey was stirred to obtain a homogenous suspension and two 30 ml aliquots were transferred to 30 ml conical-bottom graduated centrifuge tubes. After centrifuging for 5 min at 178x*g*, the volume of curd fines in each tube was read directly, the two replicates averaged, and reported as ml of curd fines per 30 ml of whey.

Grit percentage measurement

One pound of curd was introduced into a beaker containing 2L of cold water and then gently stirred for 1 min with a 20-cm blade spatula. The mixture was poured into the topmost of a battery of four nested copper sieves with 1/2", 1/4", 1/8", and 1/16" openings (U.S. Standard sieve sizes, 12.7, 6.35, 3.35, and 1.70 mm) and

shaken on a rotary sieve shaker (Tyler, Model RX-24) for 30 sec. Each sieve was wiped free of water droplets and weighed. Percent of curd on each sieve was calculated. The percentage of curd particles on the smallest sieve was reported as percent grit.

Analytical methods

Skim milk samples were analyzed for total protein content by Udy dye-binding method (Udy, 1956), fat content by the Milk-O-Tester (AOAC, 1975) and proteolysis by the Hull test (Hull, 1947). The method developed by Oliveria and Parmalee (1976) was used for psychrotrophic counts.

Data were analyzed using Spearman's nonlinear correlation coefficient (Snedecor and Cochran, 1972), Gauss' method (Draper and Smith, 1966), and the Fishere Z transformation (Morrison, 1976).

RESULTS & DISCUSSIONS

A TYPICAL SUCCESSFUL COTTAGE CHEESE should have uniform curd particles (Angevine et al., 1958), with 5% or less grit in uncreamed curds (Kosikowski, 1963), and with 0.4 ml or less of curd fines per 30 ml of whey (Raab et al., 1964). Mohamed and Bassette (1979) considered a cheese vat-failed when "normal" cheese curds were not obtained. We considered cottage cheese as normal when the grit was less than 4.20% and curd fines volume less than 0.60 ml in 30 ml of whey for direct-acid-set method, and less than 4.70% grit and less than 0.65 ml curd fines for culture method (Table 1). Our data indicated that vat-failed cottage cheese had grit percentage of 4.60% or more and curd fines volume of 0.65 ml or more in 30 ml of whey for direct-acid-set method, and grit percentage of 5.20% or more and curd fines volume of 0.90 ml or more for culture method. There was also an intermediate group of cottage cheese with grit percentages and curd fines volumes in between these two extremes (Table 1). Our grit percentage for normal cottage cheese is lower than that reported by Kosikowski (1963) and curd fines volume higher than that reported by Raab et al. (1964) probably because they made cottage cheese in a large vat with mechanical aids. We made ours manually in a small vat.

The temperature at which the chilled and acidified milk coagulates (Coagulation Temperature, C.T.) predicts vat-failure. Table 1 indicated that cheese vat-failed if the C.T. was below 33°C. When C.T. was above 37°C all milk samples made normal cottage cheese. When C.T. was between 33 and 37°C the milk had a high probability of vat-failure.

Milk inoculated with *Pseudomonas fluorescens* MC 60 and with final psychrotrophic count of 1×10^4 CFU/ml or less provided normal curd by direct-acid-set and culture methods. When the final count of inoculated milk was 3.0×10^4 CFU/ml or more vat-failure occurred. Since we used a highly proteolytic strain of *Pseudomonas fluorescens* (Adams et al., 1975), it is not surprising that 3.0×10^4 CFU/ml would induce vat-failure. Mohamed and Bassette (1979) who used a less proteolytic strain reported no vat failure at 2.4×10^5 CFU/ml.

The mean grit percentages of normal cottage cheese (3.97% for direct-acid-set and 4.49% for culture) were lower than those with potential vat-failure (4.30% for direct-acid-set and 5.00% for culture) and overt vat-failure (8.87% for direct-acid-set and 10.10% for culture) (Table 2). The mean volume of curd fines in whey from normal cottage cheese also was lower, (0.58–0.62 ml/per 30 ml) than cheese with potential (0.62–0.71 ml/30 ml) or overt (1.43–1.73 ml/30 ml) vat-failure.

When the C.T. of chilled and acidified milk decreased (i.e. poor quality milk), a concomitant increase in grit percentage and curd fines volume was observed (Tables 3, 4, 5 and 6). The correlation coefficients between milk C.T. and grit percentage were −0.90 for the direct-acid-set method and −0.92 for the culture method, for milk inoculated with *P. fluorescens* MC 60. The correlation coefficient between milk C.T. and curd fines volume was −0.90 for both methods (Table 7). When *P. fluorescens* MC 60 inoculated milk had more than 3.0×10^4 CFU/ml the C.T. was lower (<33°C) than for milk with final lower levels of *P. fluorescens* MC 60 (Table 1, 3 and 4). The C.T. of milk inoculated with *P. fluorescens* MC 60 in milk after incubation and the number of *P. fluorescens* MC 60 in milk were related inversely ($r = -0.95$, Table 7), which indicated that milk samples with higher bacteria count had lower C.T. (Table 3–6). The correlation coefficient between *P. fluorescens* MC 60 count and grit percentage and curd fines was 0.91–0.92 for both direct-acid-set and culture methods (Table 8).

The C.T. of milk with normal flora also was related to possible vat-failure (Table 5-6). Cottage cheese made from 3-day-old milk (7.3×10^6 CFU/ml) showed normal cheese curds by both direct-acid-set and culture methods. How-

Table 1—Effect of Pseudomonas fluorescens MC60 on milk coagulation temperature and the status of cottage cheese curd

Manufacturing method	No. of Observations	*P. fluorescens* MC60 count/ml	Milk Coagulation temperature, °C	Grit %	Curd fines ml	Status of cheese curd
Direct-acid-set method	35	$>3.0 \times 10^4$	<33	>4.60	>0.65	failure
	8	$1.0–3.0 \times 10^4$	33–37	4.60–4.20	0.60–0.65	potential of failure
	42	$<1.0 \times 10^4$	>37	<4.20	<0.60	normal
Culture method	33	$>3.0 \times 10^4$	<33	>5.20	>0.90	failure
	10	$1.0–3.0 \times 10^4$	33–37	5.20–4.70	0.65–0.90	potential of failure
	40	$<1.0 \times 10^4$	>37	<4.70	<0.65	normal

Table 2—Comparisons of grit percentage and curd fines volume in fat-failed, potential of vat-failed, and normal cottage cheese[a]

	Vat-failed cottage cheese		Potential of vat-filled cottage cheese		Normal cottage cheese	
	Direct-acid-set method n=35	Culture method n=33	Direct-acid-set method n=8	Culture method n=10	Direct-acid-set method n=42	Culture method n=40
Grit, %	8.87	10.10	4.30	5.00	3.97	4.49
Curd fines, ml	1.43	1.73	0.62	0.71	0.58	0.62

[a] n = Number of observations.

Table 3—Observations on cottage cheese made from pasteurized milk by the direct-acid-set method in which milk was inoculated 24 hr with Pseudomonas fluorescens MC60 before separation[a]

Pseudomonas fluorescens MC 60 count Mean, per ml	Hull test % T		Milk coagulation temperature, °C		Grit %		Curd fines ml		Status of cheese curd
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
2.3×10^6 n = 14	59.7	1.43	28.2	0.52	12.51	2.13	2.10	0.23	failure
4.6×10^5 n = 11	60.0	2.54	29.8	0.46	7.57	1.24	1.22	0.30	failure
4.3×10^4 n = 17	60.2	1.57	33.0	1.93	4.84	0.52	0.68	0.07	failure and potential of failure
6.5×10^3 n = 15	61.1	1.11	41.2	2.53	4.03	0.11	0.59	0.02	normal
1.4×10^2 n = 28	65.0	0.79	43.5	1.37	3.96	0.05	0.58	0.01	normal

[a] n = Number of observations.

Table 4—Observations on cottage cheese made from pasteurized milk by the culture method in which milk was inoculated 24 hr with Pseudomonas fluorescens MC60 before separation[a]

Pseudomonas fluorescens MC 60 count Mean, per ml	Hull test % T		Milk coagulation temperature, °C		Grit %		Curd fines ml		Status of cheese curd
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
3.7×10^6 n = 12	58.7	1.51	27.8	0.62	14.88	1.93	2.39	0.19	failure
4.7×10^5 n = 12	60.0	1.24	29.6	0.54	8.74	1.78	1.63	0.29	failure
4.1×10^4 n = 16	60.3	0.91	32.6	1.36	5.35	0.30	0.89	0.15	failure and potential of failure
7.1×10^3 n = 16	60.9	1.06	40.2	2.97	4.56	0.15	0.63	0.01	normal
1.5×10^2 n = 27	65.2	0.75	42.9	1.66	4.49	.02	0.61	0.06	normal

[a] n = Number of observations.

Table 5—Observations on cottage cheese made from milk by the direct-acid-set method in which raw milk was stored at 4°C for 3, 5, and 7 days before separation and pasteurization

Days of raw milk storage at 4°C	Hull test % T		Milk coagulation temperature, °C		Grit %		Curd fines ml		Status of cheese curd
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
3 n=10	62.3	0.98	39.1	1.49	3.98	0.15	0.60	0.01	normal
5 n=10	60.6	1.06	30.8	0.83	6.02	1.12	0.88	0.26	vat-failed
7 n=10	58.3	1.08	27.3	0.88	15.81	2.66	2.49	0.32	vat-failed

n = Number of observations.

Table 6—Observations on cottage cheese made from milk by the culture method in which raw milk was stored at 4°C for 3, 5, and 7 days before separation and pasteurization[a]

Days of raw milk storage at 4°C	Hull test % T		Milk coagulation temperature, °C		Grit %		Curd fines ml		Status of cheese curd
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	
3 n=10	62.3	0.99	39.1	1.46	4.54	0.14	0.63	0.02	normal
5 n=10	60.7	0.94	30.8	0.77	6.34	1.15	1.17	0.23	vat-failed
7 n=10	58.4	1.18	27.2	0.89	16.29	2.03	2.57	0.25	vat-failed

[a] n = Number of observations.

ever, 5 day and 7 day-old milk (2.8×10^7 and 1.3×10^8 CFU/ml for 5 and 7 days, respectively) consistently displayed vat-failure (Table 5–6).

All correlation coefficients relating C.T. of natural flora milk to grit percentage or curd fines volume, by the direct-acid-set or culture method ranged from 0.94 to 0.96 (Table 7).

The correlation coefficient between milk C.T. and bacterial count was high, ($r = -0.95$) for milk inoculated with *P. fluorescens*, but that for milk with natural flora was lower, ($r = -0.88$). The difference probably was because natural milk flora was less proteolytic than our test culture. Olsen et al. (1955) reported that the type of contaminating microorganism was more critical than the number in determining milk keeping quality.

Hull test values decreased (indicating increased proteolysis) as the number of *P. florescens* MC 60 in milk increased (Table 3 and 4). In normal milk, Hull test values also decreased with storage time (Table 5 and 6). However, the correlation coefficients between Hull test and grit percentage and curd fines volume were relatively low ($r = -0.77$ to -0.82; Table 9). Those between C.T. and the same parameters were -0.90 to -0.96 (Table 7)., indicating that the C.T. is a more sensitive predictor of vat-failure than the Hull test.

The following equations were derived from empirical data using Gauss Method (Draper and Smith, 1966) and showed the relationship between milk C.T. and grit percentage and curd fines volumes.

1. Relationship between C.T. (X) and ml curd fines/30 ml(Y)

$$Y = 0.568 + 2257e^{-0.00923X^2} \text{ (Direct-acid-set)}$$
$$Y = 0.596 + 138e^{-0.00565X^2} \text{ (Culture)}$$

2. Relationship between C.T.(X) and grit percentage (Y)

$$Y = 3.983 + 19589e^{-0.00974x^2} \text{ (Direct-acid-set)}$$
$$Y = 4.419 + 4433e^{-0.00789X^2} \text{ (Culture)}$$

Based on the equations above, curves were drawn and shown in Fig. 1–4. Those curves are all significant at $\alpha = 0.01$. Those equations can be used by the cottage cheese industry to predict the probability of vat-failure from milk C.T.

To utilize this information the experimental conditions reported in this study must be followed carefully.

—Continued on next page

Table 7—Correlation coefficients between coagulation temperaure of milk inoculated with Pseudomonas fluorescens MC 60 and of milk supporting the growth of natural flora, and grit percentage, curd fines volume, and concentration of psychrotrophs[a]

	Coagulation temperature of milk			
	P. fluorescens MC 60 (1×10^2–7×10^6/ml)		Natural flora (5.7×10^6–3.3×10^8/ml) (3, 5, and 7 days' incubation)	
	Direct-acid-set method n = 85	Culture method n = 83	Direct-acid-set method n = 30	Culture method n = 30
Grit percentage	−0.90	−0.92	−0.94	−0.96
Curd fines volume	−0.90	−0.90	−0.95	−0.95
Bacterial count	−0.95[c]	−0.95[b]	−0.88[c]	−0.87[b]

[a] n = number of observations.
[a,b,c] Significant difference at $\alpha = 0.05$ in values with same letter.

Table 8—Correlation coefficients between psychrotrophic count, and grit percentage and curd fines volume[a]

	P. fluorescens MC 60 (1×10^2–7×10^6/ml)		Natural flora 5.7×10^6–3.3×10^8/ml	
	Direct-acid-set method n = 85	Culture method n = 83	Direct-acid-set method n = 30	Culture method n = 30
Grit percentage	0.92[e]	0.92[b]	0.81[e]	0.80[b]
Curd fines volume	0.91[c]	0.92[d]	0.79[c]	0.81[d]

[a] n = number of observations.
[a,b,c,d,e] Significant difference at $\alpha = 0.05$ in values with same letter.

Table 9—Correlation coefficients between Hull test value of milk inoculated with Pseudomonas fluorescens MC 60 and of milk supporting the growth of normal flora, and grit percentage and curd fines volume[a]

	Hull test value			
	P. fluorescens MC 60 (1×10^2–7×10^6/ml)		Natural flora (5.7×10^6–3.3×10^8/ml) (3, 5, and 7 days' incubation)	
	Direct-acid-set method n = 85	Culture method n = 83	Direct-acid-set method n = 30	Culture method n = 30
Grit percentage	−0.77	−0.80	−0.81	−0.81
Curd fines volume	−0.77	−0.81	−0.81	−0.82

[a] n = number of observations.

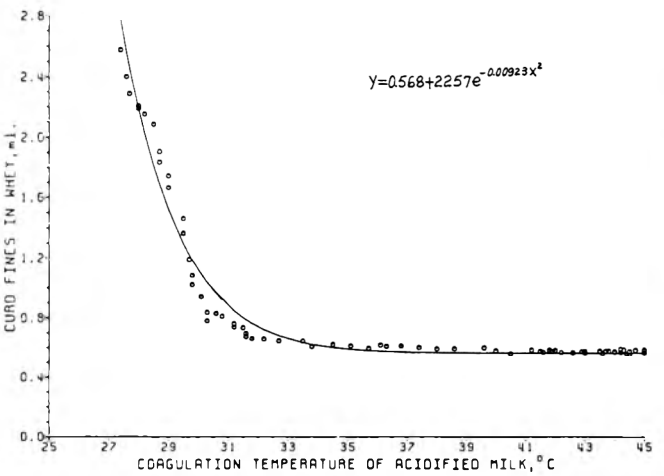


Fig. 1—Effect of milk coagulation temperature on curd fines in cottage cheese whey (direct-acid-set method).

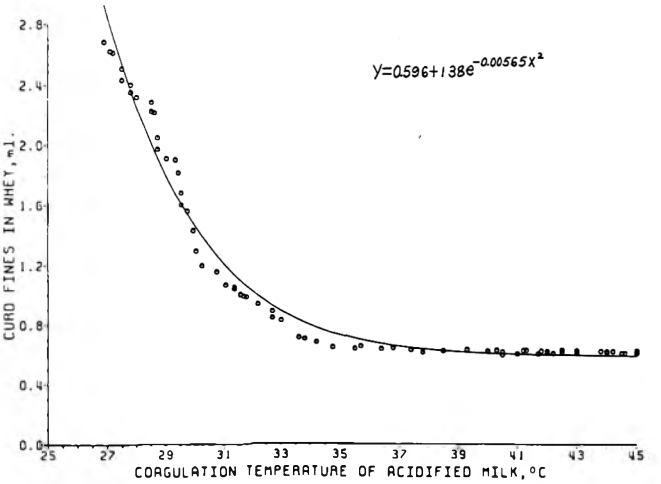


Fig. 2—Effect of milk coagulation temperature on curd fines in cottage cheese whey (culture method).

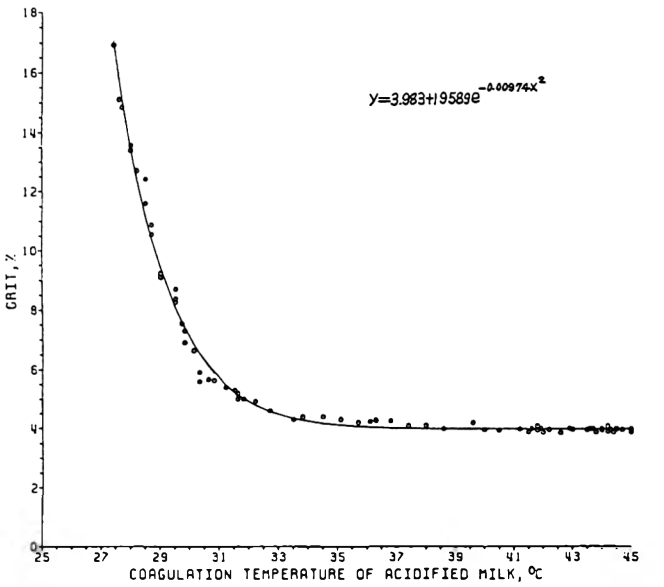


Fig. 3—Effect of milk coagulation temperature on grit of cottage cheese (direct-acid-set method).

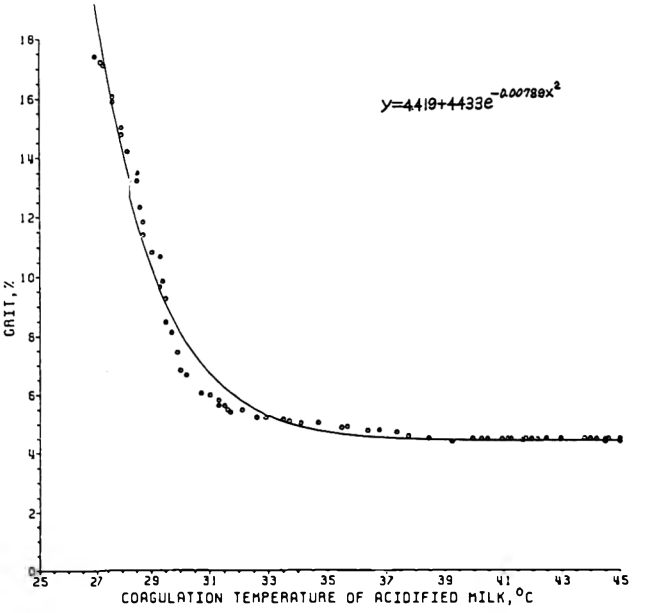


Fig. 4-Effect of milk coagulation temperature on grit of cottage cheese (culture method).

In conclusion, we have developed a laboratory method for predicting success in cottage cheese manufacturing and have characterized vat-failed cottage cheese in terms of grit percentage and curd fines volume. Now, this "coagulation temperature" procedure needs to be field-tested.

REFERENCES

Adams, D.M., Barach, J.T., and Speck, M.L. 1975. Heat resistant proteases produced in milk by psychrotrophic bacteria of dairy origin. J. Dairy Sci. 58: 828.

Angevine, N.C., Harmon, L.H., Olson, H.C., Tuckey, S.L., and Irvin, D.M. 1958. Score-card and guide for cottage cheese. J. Dairy Sci. 41: 214.

Angevine, N.C. 1976. Cures for some cottage cheese problems. Cultured Dairy Products J. 11(3): 14.

AOAC. 1975. "Official Methods of Analysis," 12th ed., p. 260. Association of Official Analytical Chemists, 12th ed. Washington, DC.

Draper, N.R. and Smith, H.S. 1966. "Applied Regression Analysis," p. 269. John Wiley & Sons, Inc., New York.

Elliott, J.A., Emmons, D.B., and Yates, A.R. 1974. The influence of the bacterial quality of milk on the properties of dairy products. A review. Can. Inst. Food Tech. J. 7: 32.

Emmons, D.B. and Tuckey, S.L. 1967. "Cottage Cheese and Other Cultured Milk Products," p. 18. Chas. Pfizer & Co., Inc., New York, N.Y.

Hull, M.E. 1947. Colorimetric determination of the partial hydrolysis of the proteins in milk. J. Dairy Sci. 30: 881.

Kosikowski, F.V. 1963. Some distribution patterns of cottage cheese particles and conditions conditions contributing to curd shattering. J. Dairy Sci. 46: 391.

Mohamed, F.O. and Bassette, R. 1979. Quality and yield of cottage cheese influenced by psychrotrophic microorganism milk. J. Dairy Sci. 62: 222.

Morrison, D.F. 1976. "Multivariate Statistical Methods," p. 104. McGraw-Hill Book Co., New York, N.Y.

Oliveria, J.S. and Parmelee, C.E. 1976. Rapid enumeration of psychrotrophic bacteria in raw and pasteurized milk. J. Milk & Food Technol. 29: 269.

Olson, J.C. Jr., Parker, R.B., and Mueller, W.S. 1955. The nature, significance and control of psychrophilic bacteria in dairy products. J. Milk & Food Technol. 18: 200.

Raab, J.A., Liska, B.J., and Parmelee, C.E. 1964. A simple method for estimating curd fines in cottage cheese whey. J. Dairy Sci. 47: 92.

Snedecor, G.W. and Cochran, W.G. 1972. "Statistical Methods," p. 194. Iowa State University Press, Ames, IA.

Udy, D.C. 1956. A rapid method for estimating total protein in milk. Nature 178: 314.

Vitex/American-Inline Cottage Cheese Acidification System Instruction. Diamond-Shamrock Corp., St. Louis, MO.

Ms received 6/22/81; revised 9/18/81; accepted 9/21/81.

Contribution No. 81-470-J, Kansas Agricultural Experiment Station, Manhattan, KS 66506.

Stabilization of Frozen Goat Milk Concentrates by Enzymatic Lactose Hydrolysis

E.J. GUY

ABSTRACT

Frozen 3:1 and 4:1 whole goat's milk concentrates were stored at −14°C for up to 10 months with minimal changes in physical stability and no loss of flavor quality when reconstituted. The processing sequence required HTST pasteurization, lactose hydrolysis by lactase enzyme (36% hydrolysis for 3:1 concentrates and 52% hydrolysis for 4:1 concentrates), repasteurization, condensing in vacuo, post heating at 71°C for 30 min, addition of Tenox 6 (0.01g/100g fat), canning, sealing, and freezing. Post heating of the concentrates was necessary for their stability but slightly lowered their hedonic flavor ratings. These stored concentrates resist freeze-thaw cycling up to 3 times without any physical destabilization or further change in flavor scores.

INTRODUCTION

GOAT MILK is consumed primarily by infants or children who are allergic to cow's milk. Because the milk is usually distributed in small amounts, it incurs high transportation and handling costs. Consequently, fresh goat milk is frequently sold at a price three to four times higher than the price of cow's milk.

Considerable amounts of goat milk sold commercially are of poor flavor quality. The presence of so-called "goaty" flavor in the milk has been a barrier to more wide-spread use by consumers in the United States. Our investigations with goat milk showed it to be of good flavor quality if properly handled and obtained fresh.

The availability of goat milk is highly seasonal; much more is produced during the summer months than in the late fall and winter. To increase its distribution and make goat milk available throughout the year, it would be desirable to process the surplus and distribute it throughout the country. It was thought that frozen concentrates would permit distributon for sale in freezer cases of health food stores, drugstores, or supermarkets. Processing and distribution costs could be offset in part by savings from the reduced bulk and the potential demand for good quality goat's milk concentrates.

The objective of the present work was to investigate the factors of processing, concentrating, freezing, and storing of goat's milk which would produce a concentrate of good storage stability and of acceptable flavor when reconstituted.

No information is available on frozen condensed goat's milk, but numerous publications are available on frozen condensed cow's milk. Several problems are associated with frozen concentrated cow's milk. It lacks stability at home freezer temperatures (−12 to −14°C) due to lactose crystallization which causes an increase in viscosity and protein destabilization. Oxidized flavors may also develop in the stored product. Physical stability can be improved by storing at lower temperatures (−23 to −29°C) (Bell and Mucha, 1952), by post heating the concentrate to 68°C for 25 min (Bratz and Winder, 1959), partially hydrolyzing the lactose (Guy et al., 1974; Turmerman et al., 1954), and by addition of polyphosphates (Doan and Warren, 1947). Oxidized flavor is minimized by judicious use of heat treatment (Bell, 1939; Bell and Mucha, 1951) and use of Tenox 6 (Johnson, 1970). Johnson stated studies on the effect of various freezing rates and handling of milk concentrates have produced conflicting results probably because the influence of lactose nucleation and crystallization on protein stability were not recognized. He recommends cooling milk concentrates in an ice water bath for 30 min at 0°C prior to freezing at −12°C in 2–3 hr.

Author Guy is with the UDSA Eastern Regional Research Center, Agricultural Research Service, 600 E. Mermaid Lane, Philadelphia, PA 19118.

MATERIALS & METHODS

Milk

Fresh pooled goat's milk was obtained from a single source of mixed breed herd. All milk scored good to excellent in initial taste and exhibited minimal goaty flavor.

Miscellaneous materials

Food grade "Maxilact" lactase (Enzyme Development Co.) of 40,000 ONPG μ/g (lot #101) (actual 18,000 ONPG μ/g) was used. Eastman Tenox 6 antioxidant preparation and JT Baker sodium hexametaphosphate (P_2O_5 66.8–68%) were also used.

Processing

Fresh raw goat milk was pasteurized at 76°C for 15 sec (HTST); one lot was pasteurized at 63°C for 30 min. All lots except a portion of lot 1 were homogenized at 105.5/35.2 kg/cm^2. Milks were treated with 0.01–0.0175% lactase at 30°C for 2 hr to hydrolyze 36% or 52% of the lactose present. Hydrolyzed lactose milks (LH) were promptly HTST pasteurized to stop the enzymatic reaction. 3:1 or 4:1 concentrates were prepared by condensing in a Wiegand falling film evaporator. For one test evaluating sodium hexametaphosphate, 4g/L were added to the concentrate before post heating. The concentrates were post heated in open 1-gal stainless steel containers at 63, 66 or 71°C for 30 min. To obtain 26% LH concentrates, 50/50 blends of 52% LH and an untreated control were made up. Tenox 6 (0.01 g/100g fat) in a 10% suspension of 95% ethanol was added to the concentrates after post heating. The concentrates were sealed in enameled 8 oz cans at 45–50°C, the cans promptly cooled for 30 min in ice water and then frozen by holding in spaced positions at −14°C.

Milk Composition

Total protein (total N X 6.38) was determined by the micro-Kjeldahl procedure (AOAC, 1970b), ash by the standard method for milk (AOAC, 1970a), and total solids and fat by the Mojonnier procedures (Milk Industry Foundation, 1959a, b). Lactose was determined colorimetrically in untreated milk (Folin and Wu, 1919), by reduction of copper salts in alkaline Fehling's solution using USP lactose hydrate for the standard curve.

Extent of hydrolysis

The Tauber Kleiner method (Tauber and Kleiner, 1932) based upon the reduction of copper salts in acid solution was used to determine monosaccharides in the presence of lactose. A standard curve was made with a 50/50 mixture of glucose and galactose.

Viscosity

Viscosities were determined on freshly thawed concentrates at 23°C using the Brookfield Synchroelectric Viscosimeter. Viscosities

of samples below 100 centipoise were obtained using the mean of duplicate readings each at 12 and 30 rpm with the UL adapter and #1 spindle. Viscosities above 100 centipoises were obtained employing higher numbered spindles. Comparative viscosities were obtained within a sample series using the same spindle and rpm as much as possible.

Lactose crystallization

The fraction of lactose crystallized was determined on weighed portions of frozen milk concentrate according to the technique of Tumerman et al. (1954) using the Sharp and Doob (1941) procedure. Samples weighing 10g were placed in 200 ml beakers, held frozen at −14°C and analyzed within 8 hr for extent of lactose crystallization.

Sedimentation

Solubility indices were determined on 11% total solids milks reconstituted from their concentrates (ADMI 1947) and are expressed as milliliters sediment per 50 ml reconstituted milk.

Organoleptic evaluation

Samples of freshly thawed concentrate were reconstituted with tap water, held overnight at 2°C, warmed to room temperature, and evaluated on a 9-point hedonic preference scale (Peryam and Pilgrim, 1957) in a panel room equipped with subdued lighting and individual booths by a group of 8–12 laboratory personnel. About half of the tasters were experienced dairy product judges. Goatiness and off flavors were judged on a 5-point scale, with 0 as none, 1 questionable, 2 slight, 3 moderate, and 4 excessive. (Note: Goatiness can be described as typical goat-like taste and smell of goat's milk which is generally objectionable. Some describe it as mutton-like or buck-like.) Samples were withdrawn from storage for evaluation at intervals of 1, 1.5, or 2 months over a 10-month period. Upon completion of each panel, the 9-point hedonic data were analyzed for significance by analysis of variance and Duncan's multiple range test.

Freeze-thawing

To simulate abuse in transit, storage, or home use, samples of concentrate were thawed in unopened cans 2–2½ hr at room temperature to the point of being ice-free but yet cold. They were then refrozen at −14°C. Thawings were conducted at weekly intervals during the first month of storage.

Table 1—Composition of goat's milk

Lot no.	Percent				
	Total solids	fat	Lactose	Protein	Ash
1	14.76	5.31	4.12	3.98	.79
2	12.00	3.50	4.26	3.18	.71
3	10.91	3.00	4.20	2.66	.64
4	13.00	4.28	4.32	–	–

RESULTS

THE TOTAL SOLIDS, fat, protein, and ash content of goat's milks vary considerably, although the lactose content is more constant (Table 1). A 3:1 concentrate of Lot No. 1 had 42.5% total solids, higher than the ususal range of 33–36%.

To determine the effect of the temperature of post heating on the physical stability of a 3:1 concentrate during storage, samples of the same lot (lot 2) were post heated at 63, 66, or 71°C and compared to an unheated control. The rates of lactose crystallization, viscosity increase, and protein sedimentation during 4.5 months of storage were progressively decreased as the temperature of post heating increased to 71°C (Table 2). Because physical stability in terms of these three parameters was best at 71°C, this temperature was used for post heating in all subsequent processing runs.

The intensity of goaty flavor in all post heated samples was increased over that of the unheated controls (Table 2). However, there were no significant differences in goaty flavor intensity attributable to post heating temperatures or storage times.

As little as 26% lactose hydrolysis of goat's milk improved the physical stability of its frozen 3:1 concentrates (42.5% total solids) (Table 3). With lactase-treated concentrates, post heating was not necessary to prevent lactose or sugar crystallization and extensive sedimentation during the storage period, but was necessary to prevent viscosity increases in the stored material. Post heating delayed, but did not prevent, lactose crystallization and protein sedimentation of the stored control. The viscositites of the concentrates of the unheated control and hydrolyzed lactose concentrates were high and comparable but the consistencies of the high viscosity samples were different; those of the hydrolyzed concentrates were smooth whereas that of the control was lumpy and coagulated. Extensive fat separation was also observed upon reconstitution of the unheated samples after 6 or more months of frozen storage, probably because these samples were not homogenized.

Lactose hydrolysis confers stability on a 4:1 frozen concentrate (Table 4). While 26% lactose hydrolysis was not sufficient to stabilize the concentrate completely because lactose crystallized out after 4 months of storage, 52% hydrolysis prevented crystallization for up to 10 months. Viscosity slowly increased in both sample sets over the storage period; viscosities were higher in the sample containing 26% hydrolyzed lactose. No unhydrolyzed 4:1 control concentrate was prepared because post heated 3:1 control concentrates showed sharp viscosity increases after only 3 months of storage (Table 3). Although higher for the 26% lactose hydrolysis, the sedimentation in both sample sets did not vary much over the storage period.

Although post heating of the goat milk concentrates promoted physical stability, it also appeared to promote goaty flavor development (Table 2). To investigate this

Table 2—Effect of post heating temperature of a 3:1 goat's milk concentrate (Lot 2) on storage stability at −14°C (Samples pasteurized at 63°C 30 min, homogenized at 105.5/35.2 kg/cm², post heated 30 min)

Months storage	Post heating for 30 min in °C															
	% Lactose crystallized				Centipoise viscosity at 23°C				Milliliters sedimentation				Goatiness score			
	Unheated	63	66	71	Unheated	63	66	71	Unheated	63	66	71	Unheated	63	66	71
0	0	–	–	–	10.8	10.8	12	12.5	0.1	–	–	–	1.4	1.7	2.6	2.7
1.5	54	0	0	0	14.2	11.7	11.9	12.8	0.1	0.2	0.1	0.1	1.5	1.7	2.1	1.8
3.0	81.5	27.5	20	0	87.4	13.9	14.6	13.0	3.5	0.7	0.5	0.5	1.8	2.4	2.7	2.5
4.5	90.0	75.5	73.5	64	1375[a]	44	39	30	4.5	0.9	0.8	0.6	1.5	2.4	2.7	1.6

[a] coagulated.

Table 3—Effect of partial lactose hydrolysis (LH) and post heating at 71°C for 30 min on storage stability at −14°C of 3:1 goat milk concentrates compared to an unhydrolyzed control concentrate (c) (lot 1) (unheated samples not homogenized; heated samples homogenized 105.5/35.2 kg/cm²)

	% Lactose crystallized				Viscosity 23°C (centipoise)						Milliliters sedimentation					
	Unheated		Post heated		Unheated			Post heated			Unheated			Post heated		
Months storage	C	26% LH and 52% LH	C	26% LH and 52% LH	C	26% LH	52% LH	C	26% LH	52% LH	C	26% LH	52% LH	C	26% LH	52% LH
0	0	–	–	–	27	30	30	93	–	83	0.1	–	–	–	–	–
1.5	–	–	–	–	136	98	192	101	105	110	–	–	–	–	–	–
3.0	70.4	0	3.5	0	21[a]	24.8[a]	26.8[a]	395	130	115	5.0	0.1	0.1	0.1	0.1	0.1
4.5	83.5	0	4.0	0	24[a]	20[a]	50[a]	865	172	150	8.0	0.4	0.5	2.2	0.1	0.1
6.0	–	0	73.5	0	–	27[a]	45[a]	16[a]	220	165	–	0.4	0.5	4.5	0.2	0.2
7.5	–	0	–	0	–	21.5[a]	54.5[a]	28[a]	231	178	–	0.3	0.4	6.0	0.3	0.3

[a] ×10³

observation further, samples from each stage of processing of an untreated control and a lactase treated 3:1 concentrate were evaluated for overall acceptability and for goaty and oxidized and bitter off-flavors (Table 5). Nubian goats milk, obtained fresh from one supplier, was used in these studies because its mild flavor makes it suitable to evaluate the effects of heat on flavor development. Because of taste panel limitations, it was not possible to taste all samples on the same day.

The post heating of goat milk concentrates significantly lowered the initial hedonic rating three out of four times. Although not significantly different, post heating also increased goaty and oxidized and bitter off-flavors. The post heating temperature of 71°C was evidently not severe enough to produce any noticeable increase in cooked flavor. The concentration step had no significant effect on panel acceptability of the milks and the results suggest that lactase treatment did not change acceptability. It is important to note that in spite of the decrease in hedonic ratings brought about by post heating, the scores still fell within the acceptable range of the rating scale used.

Because it was known that the development of oxidized off-flavors in frozen cow's milk concentrates could be minimized by addition of antioxidants (Johnson, 1970), Tenox 6, a common antioxidant, was tested for its effect on the flavor stability of 3:1 and 4:1 goat milk concentrates during storage.

Tenox 6, added at a level of 0.01g/100g fat, maintained the hedonic flavor ratings of stable 3:1 condensed 26% lactose hydrolyzed goat milk concentrates after 4 months storage, while those of the untreated controls declined somewhat and leveled off (Fig. 1). The Tenox 6 treated concentrates also had lower levels of oxidized and bitter flavors than those of the untreated concentrates. Overall goatiness of the concentrates remained relatively constant with time.

The 4:1 lactase treated concentrates with Tenox 6 also had higher hedonic ratings when reconstituted and contained less oxidized and bitter flavors than those of their controls after 4 months storage and were similar in goatiness.

Since the differences in hedonic ratings between the control and antioxidant treated samples were not as marked in most instances as with the 3:1 concentrates, the data are not shown. The degree of lactose hydrolysis (26 or 52%) did not affect acceptability. Also flavor scores for reconstituted unhydrolyzed 3:1 concentrates stored up to 4 months paralleled those of both the hydrolyzed 3:1 and 4:1 concentrates that did not contain added antioxidants during the early stages of storage. The unhydrolyzed samples thickened and coagulated as storage time progressed so they were not tasted.

Table 4—Effect of partial lactose hydrolysis (LH) on storage stability at −14°C of 4:1 (48% total solids) goat milk concentrates (Lot 4) (Samples homogenized 105.5/35.2 kg/cm², post heated at 71°C 30 min)

	% Lactose crystallized		Viscosity 23°C (centipoise)		Milliliters sedimentation	
Months storage	26% LH	52% LH	26% LH	52% LH	26% LH	52% LH
0	0	0	274	215	–	–
2	0	0	295	225	0.2	<0.1
4	0	0	320	280	0.6	<0.1
6	9.5	0	480	358	0.4	<0.1
8	32.6	0	710	365	0.5	<0.1
10	33.0	0	2200	480	0.6	0.1

Abuse of frozen products once they have been shipped by the manufacturer is becoming increasingly common. Therefore, the effects of freeze-thaw cycling on the physical stability of 3:1 (35% total solids) frozen goat milk concentrates were determined. Abusing 3:1 control concentrates by thawing and refreezing up to three times resulted in accelerated destabilization after total elapsed storage times of 1.5 and 3 months (Table 6). As the samples were repeatedly thawed and refrozen, viscosity, lactose crystallization, and sedimentation all increased. After 3 months of storage, freeze-thawing even once completely destabilized the stored concentrate.

When 3:1 concentrates with 26% hydrolyzed lactose were abused by freezing and thawing (FT) three times, viscosity and "apparent" lactose crystallization increased slightly after only 2 months of storage and rapidly after 4 months (Fig. 2). However, 36% and 52% lactose hydrolysis, respectively, stabilized their 3:1 and 4:1 concentrates against three freeze-thaw abuse cycles. These samples stored up to 10 months showed only small viscosity increases and no lactose crystallization and maintained sedimentation values of 0.1 cc or less. Viscosities of 36% lactose hydrolyzed 3:1 concentrates increased from 10.9 and 15.2 centipoises; FT cencentrate viscosity increased to 16.8 centipoises. Viscosities of 52% lactose hydrolyzed 4:1 concentrates increased from 225 to 395 centipoises; those of the FT concentrates increased to 590 centipoises.

FT of both 3:1 and 4:1 stored frozen concentrates had no statistically significant effect on the overall panel acceptability of their reconstituted milks (Table 7), but in seven out of eight trials, hedonic scores of these samples were at least equal to or slightly higher than their controls. However, the effects of FT on oxidized and bitter as well as goaty scores were not consistently different.

Polyphosphates are frequently added to concentrated milks to prevent thickening and coagulation on storage.

—Continued on next page

Table 5—Effect of processing steps in hedonic rating and goaty and oxidized off-flavor development during 3:1 concentrate processing

Sample	Hedonic rating[a] 9-Point scale Fresh	Hedonic rating[a] 9-Point scale day old	Goatiness 5-Point scale Fresh	Goatiness 5-Point scale day old	Oxidized and bitter 5-Point scale Fresh	Oxidized and bitter 5-Point scale day old
Raw	6.55[bc]	–	0.55	–	0.11	–
		Control				
Pasteurized 77°C 15 sec Homogenized 105.5/35.2 kg/cm^2	6.88[c]	6.50[bc]	0.44	0.80	0.33	0
Condensed 3:1 Reconstituted to 11.8% total solids	6.55[bc]	–	0.67	–	0.33	–
Condensed 3:1 Post heated 71°C 30 min Reconstituted	5.44[b]	6.40[bc]	1.22	0.80	0.55	0.20
		Lactose treated (LH) –33% hydrolyzed lactose				
Pasteurized, Homogenized LH	–	7.20[c]	–	0.50	–	0.20
LH condensed 3:1 Reconstituted	–	7.20[c]	–	0.50	–	0.20
LH condensed 3:1 Post heated Reconstituted	5.44[b]	5.80[b]	1.33	1.10	0.22	0.60

[a] 5.0 Neither like nor dislike; 6.0 Like slightly; 7.0 Like moderately.
[b,c] Means in the same line bearing different superscript letters differ, $p < 0.05$.

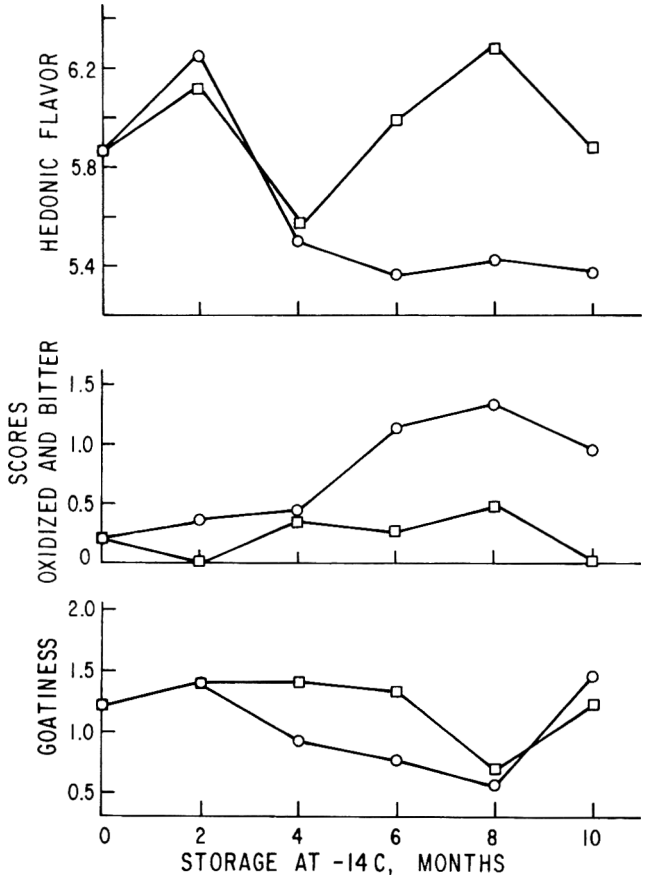


Fig. 1—Effect of Tenox 6 on the flavor scores of milks reconstituted from 3:1 (36% solids) hydrolyzed lactose (LH) goat milk concentrates: 0——0 26% LH untreated; □——□ 26% LH with Tenox 6 (0.01 g/100g fat) (Lot 4).

Table 6—Effect of repeated thawing of 3:1 (35% total solids) concentrates (Lot 3) 2 hr at 23°C, then refreezing and storing at −14°C on physical stability

Age in weeks when thawed	Viscosity at 23°C (centipoise)	% Lactose crystallized	Milliliters sedimentation
	Stored 1.5 months		
(Not thawed)	12	0	0.1
1	412	69.5	0.1
1, 2	750	91.5	0.2
1, 2, 4	1,400	101.0	1.0
	Stored 3 months		
(Not thawed)	24	7.1	7.7
1	725	90.5	10.0

Addition of 0.4% sodium hexametaphosphate to milks not treated with lactase resulted in concentrates of increased physical stability (although they contained high levels of crystallized lactose) but of poor flavor quality on extended storage (Table 8). Because the addition of sodium hexametaphosphate to goat milk concentrates lowered hedonic ratings and increased oxidized and bitter flavors, its use is not recommended even though physical stability was enhanced.

DISCUSSION

AVAILABILITY of fresh goat milk to the consumer is highly seasonal and often limited. Therefore, an important step in market expansion and development of new markets for goat milk is to preserve the milk for distribution year round. Freezing has always been considered to be a desirable way of preserving cow's milk because minimal flavor changes are caused by the freezing process.

In the work reported here, it has been demonstrated that 3:1 and 4:1 concentrates of whole goat's milk may be stored frozen for up to 10 months with minimal changes in

physical stability provided the lactose is sufficiently hydrolyzed and the concentrates are post heated before canning. Although it was observed that stored lactase treated 3:1 concentrates (not post heated and not homogenized) showed marked viscosity increases over the storage period (Table 3), no significant sedimentation and "apparent" lactose crystallization took place. These results were in contrast to those reported for cow's milk (Guy, 1974) where heavy coagulation occurred in concentrates containing 89% hydrolyzed lactose. The cow's milk samples had been homogenized but not post heated.

The values of crystallized lactose reported in samples containing partially hydrolyzed lactose should be considered only as "apparent" values. The analytical procedure used to measure crystalline lactose is a polarimetric one (Sharp and Doob, 1941); the glucose and galactose present in the samples as a result of lactose hydrolysis could also crystallize out during storage and contribute to the changes in optical rotation measured.

Bitter and oxidized flavor scores were added together because they constituted the main criticisms of the milk which could adversely affect flavor. Also average scores of oxidized and bitter flavor may be slightly low because several members of the taste panel had difficulty detecting them or describing them due to their inexperience and presence of other flavors. Because the total number of panelists was limited, in part due to adverse bias against the product, rigid panel selection was not practical. Several members, however, were experienced dairy product judges. For these reasons and the limitation of time and samples for repeat testing, no attempt was made to analyze statistically these scores. However, because Tenox 6 antioxidant consistently lowered oxidized and bitter scores in concentrates stored beyond 4 months, it was believed the average scores were a meaningful indication of differences. With the exception of one or two panelists, goaty flavor was consistently detected and scored by the panel. Although these scores were statistically analyzed, because of the range of scores obtained and limited panel members, no significant differences in scores were obtained for any one testing.

—Continued on next page

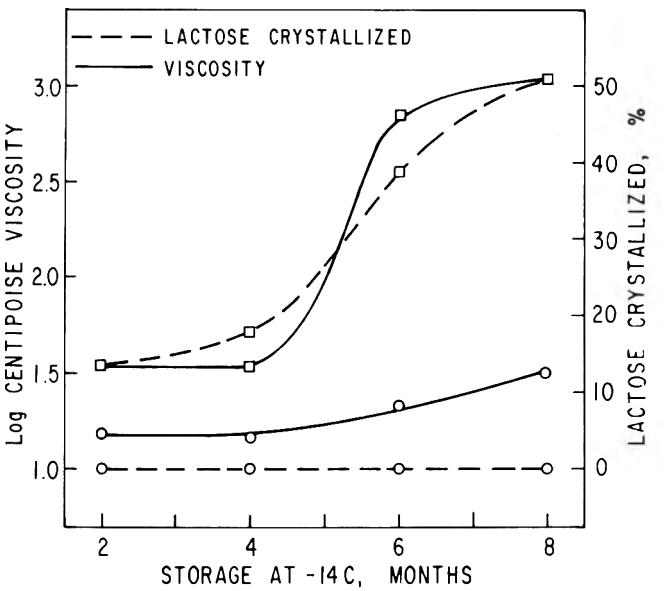


Fig. 2—Effect of storage time on the viscosity and lactose crystallization of goat milk concentrates subjected to freeze-thaw abuse (3X): O——O 3:1, (36% total solids), 26% hydrolyzed lactose (LH); □——□ freeze-thawed 26% LH.

Table 7—Effect of freeze-thawing (FT) on the panel acceptability of goat milk reconstituted from frozen lactose hydrolyzed (LH) 3:1 and 4:1 concentrates stored at −14° C

Concentrate	Months storage	9-Point hedonic score
3:1 26% LH		
Control	6	5.37
FT-3X	6	5.75
Control	8	5.42
FT-3X	8	5.42
3:1 36% LH[a]		
Control	7.5	4.62
FT-3X	7.5	5.37
Control	9	4.70
FT-3X	9	5.30
4:1 26% LH		
Control (T-6)[b]	6	5.55
FT-1X	6	5.90
Control	8	6.28
FT-1X	8	6.28
4:1 52% LH		
Control	8	5.73
FT-3X	8	5.87
Control (T-6)	10	6.75
FT-2X	10	6.20

[a] Lot 3; remainder lot 4
[b] T-6 = Tenox 6, 0.01 g/100 g fat

Table 8—Effect of 0.4% sodium hexametaphosphate (NaHP) on the storage characteristics of 3:1 concentrated goat's milk (Lot 4)

Months storage	Viscosity at 23°C (centipoise)		% Lactose crystallized		Milliliters sedimentation		9 Point hedonic score		Oxidized and bitter		Goatiness	
	Control	NaHP	Control	NaHP	Control	NaHP	Control	NaHP	Control	NaHP	Control	NaHP
0	18.8	22.6	0	0	–		6.25	5.86	0.11	0.55	1.56	1.77
2	145	28	57	0	0.4	0.2	5.42	5.85	0.12	0.62	1.6	1.6
4	318	200	51.5	71	2.5	<0.1	5.33	4.58	0.41	0.75	1.5	1.6
6	1720[a]	257	89	81	10	<0.1	5.85	4.42	0.85	0.85	1.1	1.4
8	2100[a]	435	95	79.5	11.5	0.1	4.25	4.12	0.66	1.86	0.44	1.00
10	–	688	–	86	–	0.1	–	3.50	–	1.89	–	0.75

[a] Coagulated.

An intermediate level of Tenox 6, (0.005g/100g fat), was also tested in some concentrates. Hedonic flavor ratings were found to be intermediate between those received by reconstituted samples containing 0.01% antioxidant and their controls. It might be noted that the effect of antioxidant is important in samples stored beyond 4 months' time; omission of it had no effect on hedonic ratings of concentrates stored for short periods of time. The overall data agree in showing that the concentrates containing Tenox 6 (0.01g/100g fat) stored beyond 4 month's time consistently received higher flavor ratings than the untreated controls.

Sweetness of 26 and 52% hydrolyzed lactose reconstituted concentrates was oftentimes detected by panelists but did not significantly alter the hedonic panel scores from those of the unhydrolyzed controls. This is in agreement with the findings that 30–60% lactose hydrolysis of cow's milk does not significantly affect the hedonic panel scores. Above 60% hydrolysis, cow's milk received a lower panel rating (Guy et al., 1974).

CONCLUSIONS

THE OVERALL RESULTS of the study show the processing conditions which stabilize cow's milk concentrates during frozen storage also are useful for goat's milk. In addition, repeated thawing and refreezing may even increase the flavor scores of frozen goat's milk concentrates when part of the lactose present has been hydrolyzed.

Commercial adoption of the processing sequence [HTST pasteurization, partial lactose hydrolysis with lactose enzyme (36% hydrolysis for 3:1 concentrates and 52% hydrolysis for 4:1 concentrates), repasteurization, condensing in vacuo, post heating the concentrate at 71°C for 30 min, addition of Tenox 6 (0.01g/100g fat), canning, sealing, and freezing] can help ensure a year round supply of acceptable whole goat's milk to the consumer, thereby improving economic benefits to the dairy goat farmer. Reduction of lactose content of goat's milk by treatment with lactase enzyme could provide an additional benefit to those consumers who are ot only allergic to cow's milk protein but are also lactose intolerant.

REFERENCES

American Dry Milk Institute. 1947. The grading of dry whole milk and sanitary and quality standards, p. 24, Bull. 913.
AOAC. 1970. "Official Methods of Analysis," 11th ed.: (a) p. 266; (b) p. 858. Assoc. of Official Analytical Chemists, Washington, DC.
Bell, R.W. 1939. Effects of cold storage temperature, heat treatment homogenization pressures on the properties of frozen concentrated milk. J. Dairy Sci. 22: 89.
Bell, R.W. and Mucha, T.J. 1951. Heating as a means of preventing the oxidized flavor in milk during frozen storage. J. Dairy Sci. 34: 432.
Bell, R.W. and Mucha, T.J. 1952. Stability of milk and its concentrates in frozen storage at various temperatures. J. Dairy Sci. 35: 1.
Bratz, D.R. and Winder, W.C. 1959. A method to improve the storage life of frozen concentrated milk. Abstract. J. Dairy Sci. 42: 910.
Doan, F.J. and Warren, F.G. 1947. Observations on the insolubility of the protein phase of frozen concentrated milk. J. Dairy Sci. 30: 837.
Folin, O. and Wu, A. 1919. A system of blood analysis. J. Biol. Chem. 38: 81.
Guy, E.J., Tamsma, A., Konston, A., and Holsinger, V.H. 1974. Lactase-treated milk provides base to develop products for lactose-intolerant populations. Food Prod. Dev. 8(8): 80.
Johnson, C. 1970. Some factors affecting storage stability of frozen milk concentrate. Ph.D. thesis, U. of Wisconsin, Madison.
Milk Industry Foundation. 1959. "Laboratory Manual, Methods of Analysis of Milk and Its Products," 3rd ed.: (a) p. 264; (b) p. 283. Washington, DC.
Peryam, P.R. and Pilgrim, F.J. 1957. Hedonic scale method for measuring food preferences. Food Technol. 11(9): Insert, p. 9.
Sharp, P.F. and Doob, H. 1941. Quantitative determination of alpha and beta lactose in dried milk and dried whey. J. Dairy Sci. 24: 589.
Tauber, H. and Kleiner, I.S. 1932. A method for determination of monosaccharides in the presence of disaccharides and its application to blood analysis. J. Biol. Chem. 99: 249.
Tumerman, L., Fram, H., and Cornely, K.W. 1954. The effect of lactose crystallization on protein stability in frozen concentrated milk. J. Dairy Sci. 37: 830.
Ms received 7/3/81; revised 9/11/81; accepted 9/15/81.

The author expresses his sincere thanks to P.W. Smith and S. Quillens for assistance in sample processing and to F.B. Talley for taste panel services.

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Storage Stability of Orange Juice Concentrate Packaged Aseptically

JOSEPH KANNER, JACOB FISHBEIN, PAULETTE SHALOM, STELA HAREL AND ITAMAR BEN-GERA

ABSTRACT

Orange juice concentrates were packaged aseptically by a "Dole" aseptic canning machine using 6 oz metal cans. The final juice products (11°, 34°, 44°, 58° Brix) were stored between −18° and 36°C and tested periodically for nonenzymatic browning, ascorbic acid destruction, furfural and sensory changes. Nonenzymatic browning, the main deterioration phenomena in these products, was satisfactorily retarded at 12°C or lower. Ascorbic acid destruction rate constant was dependent on temperatures between 5 and 25°C, and was affected by degree of juice concentration. Furfural accumulation in juice was higher than that in 58° Brix concentrate. Orange juice concentrate of 58° Brix did not show flavor changes after storage at 5°C or 12°C for 17 or 10 months, respectively, when evaluated after reconstitution to 11° Brix.

INTRODUCTION

THE ASEPTIC PROCESSING of food products is a technique which is now applied in several food industries. The types of products processed by this method include: milk products, puddings, banana purée, and orange, apple and guava juices. Bulk aseptic storage of tomato products is no longer new (Green, 1978; Anon., 1978).

The aseptic bulk storage system for acid fluid products (tomatoes, apples and grapes) were developed by utilization of a relatively new and advanced technology for aseptic canning of foods. The process involves pulping concentration, pre-heating, de-aeration, heating to achieve microbiological and enzymatic stabilization, and cooling prior to storage. Storage of the sterilized product can be done in drums or in very large silos or tanks (20–100 tons) which have been previously sterilized by steam and/or chemicals (Lawler, 1974; Scott, 1974; Rother, 1977).

The bulk storage system reduced the problems associated with seasonal processing, and with warehouse and rehandling costs. Transferring the product to portable units, such as railcars or truck tankers which are capable of maintaining aseptic conditions for shipment purposes, reduced the cost of packaging and transportation (Rother, 1977; Anon., 1978).

A saving of 30% energy was obtained by aseptic-packaging and refrigerated storage of fruit-based products when compared with frozen storage (Robe, 1981).

The literature on citrus products, especially concentrates, processed aseptically is very limited. Lawler (1974) and Scott (1974) described a process for sterile-cold-filling of citrus juice in glass containers. Both these workers reported a better flavor in the aseptic than in the "hot pack" processed product.

For several years, aseptically processed single-strength orange juice has been marketed succesfully in TetraPak and PurePak packages and in recent years also as a concentrate (Anon., 1975; Anon., 1978). Johnson and Toledo (1975) reported that orange concentrate aseptically packaged in glass containers could be stored at 15°C for not more than 2 months without significant flavor changes.

The purpose of this research was to identify conditions that would allow the use of aseptic packaging technology as an alternative to frozen storage and bulk transportation of citrus products.

Authors Kanner, Fishbein, Shalom, Harel and Ben-Gera are affiliated with the Dept. of Food Technology, Agricultural Research Organization, The Volcani Center, Bet-Dagan, Israel. Dr. Kanner's current address is: c/o Dr. J.E. Kinsella, Cornell University, Dept. of Food Science, Stocking Hall, Ithaca, NY 14853.

MATERIALS & METHODS

SINGLE-STRENGTH ORANGE (var. Valencia) JUICE and concentrates of 34°, 45° and 58° Brix were produced from the same batch, in a line which comprised the following units in the order of the processed material flow: an FMC juice extractor industrial plate evaporator (A.P.V., three effects were used at temperatures of 78, 65 and 50°C with flash cooling to 15°C); product preheater; vacuum de-aerator; pasteurizer; product cooler; and aseptic filling and seaming machine (Dole, model 1302, James Dole Corporation, U.S.A.). All heat exchangers were of the plate type. Aseptic conditions during filling and seaming were achieved in the Dole machine by superheated steam.

The following condition existed along the material flow line: juice and concentrates were fed at 20°C; preheated and piped at 60°C to deaerator; de-aerated at 40 mm mercury (absolute pressure); pasteurized at 92–94°C for 30 sec; cooled and filled at 35°C; sealed under positive steam pressure and water cooled to 20°C. All products were packed in 6-oz cans, which were tin plated and coated inside with epoxy-phenolic resin. Cans were stored at −18, 5, 12, 17, 25, and 36°C, and tested over a period of 18 months. Essence recovery and add-back systems were not employed. One hundred twenty cans were prepared for each storage temperature and six of these cans were opened after each storage period.

Ascorbic acid was determined by titration with 2,6-dichlorophenol indophenol (AOAC, 1970). If the color of the juice was too dark the concentrates were diluted to 5° Brix. Furfural was determined using an improved method of Dinsmore and Nagy (1973), based on the well-known aniline acetic acid reaction with furfural. The color of juice and concentrates was determined directly by a Gardner Tristimulus Colorimeter, model KL 10. The instrument was calibrated against a white plate, L = 91.6, a = −1.8, b = + 1.8.

The flavor of samples adjusted to 11° Brix was evaluated at various times during storage by at least 25 test panelists from among department personnel; the same assessors participated in all the tests. At each session, the tasters compared two sets of the three samples by the triangle test (Kramer and Twigg, 1970). The samples were presented as reconstituted orange juice, without the addition of cut-back flavors and coded by two-digit random numbers. The panel was not trained. Instructions to the tasters were to find the different samples. the samples were tested in a room with daylight, (the caramel off-flavor appear before significant browning could be detected). The reference sample, stored at −18°C, was aseptically canned and of the same concentration as the test sample. All the results are presented on the basis of 11° Brix except those results on nonenzymatic browning. The cloud of reconstituted juice was stable during the test. The stability of the cloud on reconstituted juice was tested by "settling" in a conus tube fore 3 hr at room temperature. We don't evaluate the stability of the cloud for a longer period of time.

RESULTS & DISCUSSION

Nonenzymatic browning

Color deterioration of orange concentrates during storage at high temperatures was investigated. Browning ex-

pressed as an increase in the tristimulus attribute L showed significant changes in 58° Brix concentrate stored at 25°C for at least 200 days (Fig. 1). The 58° Brix concentrate was stable at 5° and 12°C for 18 and 12 months, respectively. Products of lower concentration, such as 45° Brix, could be stored at those temperatures for 24 and 18 months, respectively, without significant color deterioration (Kanner et al., 1978). Table 1 shows the effect of juice concentration on nonenzymatic browning at 17°C. It is well known that increasing the concentration of foods significantly enhances browning reactions (Labuza et al., 1970).

Ascorbic acid degradation

Ascorbic acid destruction in citrus products has been reported under aerobic or anaerobic conditions (Boyd and Peterson, 1945; Kefford et al., 1959, Nagy and Smoot, 1977, Nagy, 1980). Most of the experiments were done with juices, and a few on concentrates. (Curl, 1947; DuBois and Kew, 1951; Bisset and Berry, 1975). It was found that in concentrates the loss in ascorbic acid was very limited at −18°, 5° and 12°C but increased markedly at 25° and 36°C. Fig. 2 describes the results obtained from the degradation of a 58° Brix concentrate. Results show that degradation of ascorbic acid follows first-order reaction kinetics at temperature of 25°C and below. At 36°C the degradation of ascorbic acid did not describe a first order reaction.

Data (Fig. 2) on 58° Brix concentrate and other concentrates (Kanner et al., 1978) are in good agreement with the results of Nagy and Smoot (1977) on ascorbic acid degradation in stored canned single-strength orange juice, and differ from those of others (Brenner et al., 1948; Huelin, 1953), who found a first-order reaction of ascorbic acid degradation until 40°C or higher temperatures but for a few minutes only (Saguy et al., 1978). Apparently, results, (Fig. 2) differ from the others because of the long storage time. During this period many breakdown products develop from juice constituents, which seem to affect and accelerate the degradation of ascorbic acid (Clegg, 1964; 1966). The destruction of ascorbic acid was found to be affected by the degree of juice concentration (Fig. 3). Similar results were found by Curl (1947) and Saguy et al. (1978). The rate constants degradation of ascorbic acid for the first 100 days are 1.27 and 3.71 mg/wk/100g of 58° Brix concentrate stored at 17 and 25°C, respectively. The same results calculated to 11° Brix are 0.24 and 0.70 mg/wk respectively, which is about 2.6-fold higher than those in stored orange juice (Fig. 3) and 3- to 4-fold higher than those reported by Nagy and Smoot (1977).

Johnson and Toledo (1975) found a high level of degradation (64%) of ascorbic acid in orange concentrate (55° Brix) filled aseptically in glass containers, after 150 days at 15°C. With a similar product, stored for the same period of time and temperature, results of the present study showed ascorbic acid degradation of less than 10%.

Table 1—Nonenzymatic browning and furfural accumulation affected by juice concentrationstored at 17°C for 200 days[a]

Juice conc (°Brix)	Nonenzymatic browning[b] (−Δ L)	Furfural[c] (ppb)
11	0.3	590
34	3.8	305
44	4.3	260
58	5.2	196

[a] Values are the mean of three replicatlons. The change in L was measured on the concentrates taken out of the can after mixing. −ΔL = L (zero time) − L (200 days).
[b] Significant differences at a level of 0.05 = 1.2
[c] Significant differences at a level of 0.05 = 27 ppb

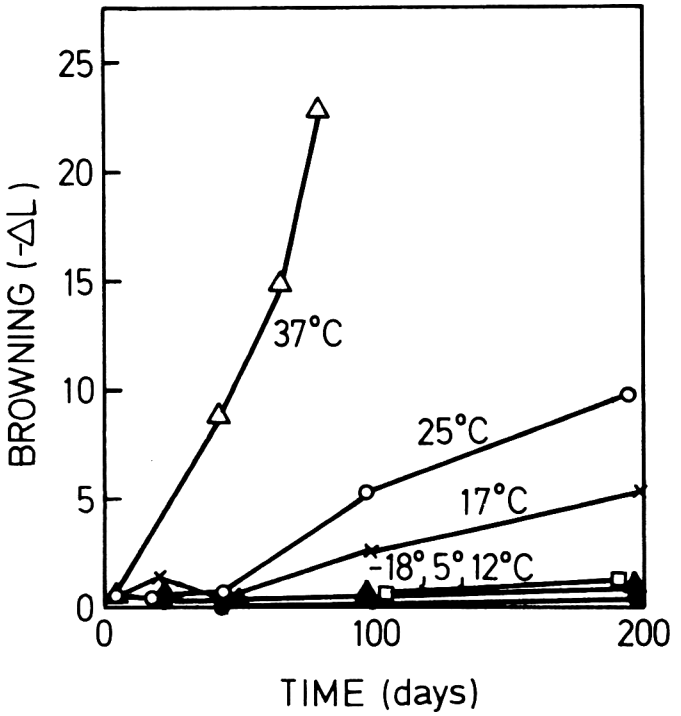


Fig. 1—Nonenzymatic browning of orange juice concentrate 58° Brix as affected by storage temperature: −18°C (●) 5°C (▲); 12°C (□); 17°C (X); 25°C (○); 37°C (△). (Values are the mean of three replications; significant differences at a level of 0.05 = 1.2).

Furfural accumulation

Blair (1964) was one of the first researchers to show that furfural accumulates during storage of citrus products. Recent reports pointed out that furfural does not directly contribute to the flavor changes but its accumulation parallels that of other compounds that alter flavor (Maraulja et al., 1973; Nagy and Randall, 1973; Nagy and

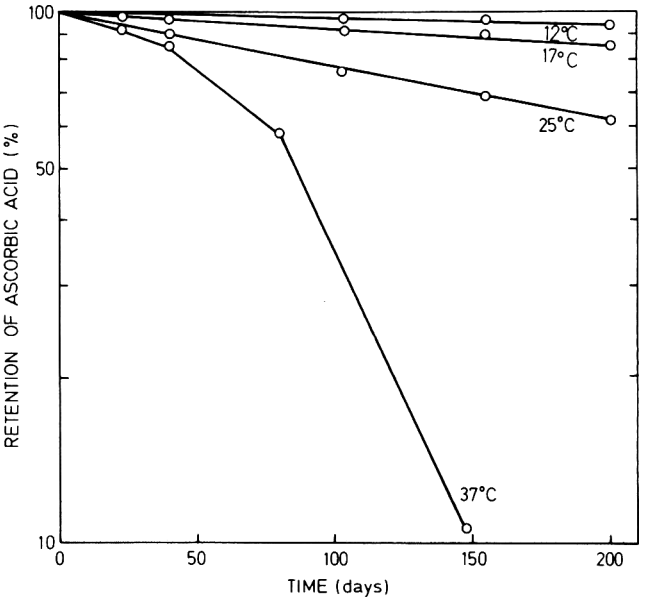


Fig. 2—Retention of ascorbic acid in orange concentrate (58°C Brix). (Values are the mean of three replications; significant differences at a level of 0.05 = 5.8%).

Smoot, 1977). There appears to be a critical temperature above which furfural accumulation is extremely rapid (Fig. 4).

In orange juice of 11° Brix, the accumulation of furfural was greater than in juice concentrate of 58° Brix (Table 1). As furfural is a breakdown product of ascorbic acid (Tatum et al., 1969) and the destruction of ascorbic acid depends on temperature and concentration, the accumulation in the 58° Brix concentrate should have been greater than in the juice. Another reason for expecting a higher accumulation of furfural in the 58° Brix concentrate was a decrease in pH which was shown by Huelin et al. (1953) to increase furfural production in a model system. Furfural is known as a reactive compound (Dunlop and Peters, 1953); in the presence of acids it tends to over condensation with aldehydes, ketones and amino acids (Rizzi, 1974).

Results of the present study indicate that further reaction of furfural with other compounds seems to have occurred at increasingly high rates with increasing product (compounds) concentration, and for this reason the accumulation of furfural was less in concentrates than in juice (Table 1); and Kanner et al., 1981).

In juice, it appears that furfural can serve as a quality deterioration index, as has been recommended by several researchers (Maraulja et al., 1973; Nagy and Randall, 1973; Nagy and Smoot, 1977); however, for concentrates this index needs further study.

Sensory quality

The concurrent changes in the flavor quality of orange concentrates filled aseptically and stored at high temperature were investigated. In contrast with Johnson and Toledo's (1975) findings, results (Fig. 5) show no statistically significant differences between 58° Brix concentrate stored at −18°C and those stored at 5°, 12° and 17°C for 17, 10, and 8 months, respectively. After this period, off-flavor was developed which was associated mainly with a caramel-like taste. Deaeration of the concentrates could account for the difference in the results of this study and that of Johnson and Toledo (1975).

Our results show that the aseptic process and storage at a refrigerated temperature could be recommended to replace the present freezing process of citrus concentrates as a means of reducing energy consumption (Robe, 1981) during production, storage, and bulk transportation.

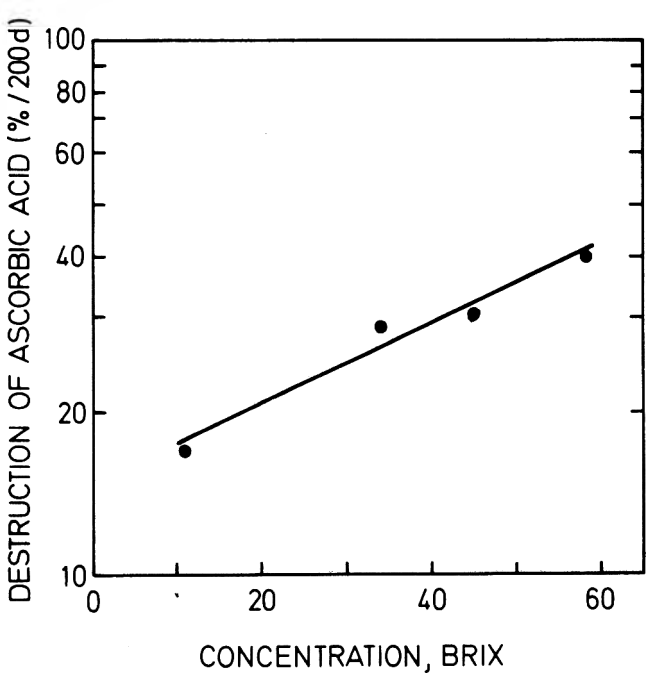


Fig. 3—Destruction of ascorbic acid in orange juices as affected by degree of concentration after storage of 200 days at 25°C.

REFERENCES

Anon. 1975. Orange concentrate in aseptic package DC builds business for Swedish dairy. Food Eng. 47: 21.

Anon. 1978. Zur kartonabfulung von fruchtsaften und nektaren. Flussinges Obst. 45: 446.

AOAC. 1975. "Official Methods of Analysis," 12th ed. Amer. Assoc. of Analytical Chemists, Washington, DC.

Belshaw, F., 1978. Interest in aseptic systems grows as energy problems approach. Food Product Develop. 12: 36.

Bisset, U.W. and Berry, R.E. 1975. Ascorbic acid retention in orange juice as related to container type. J. Food Sci. 40: 178.

Blair, J.S. 1964. Unpublished work at Fla. Citrus Exp. Sta.

Boyd, J.M. and Peterson, G.T., 1945. Quality of canned orange juice. Ind. Eng. Chem. 37: 370.

—Continued on page 436

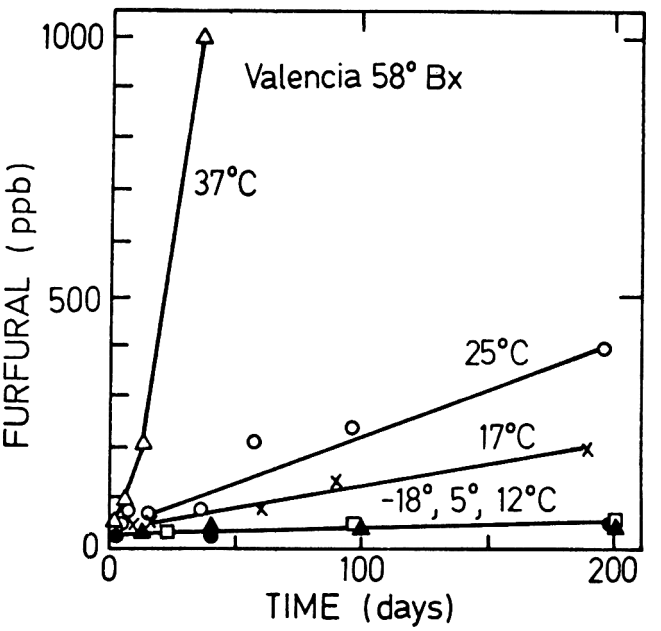


Fig. 4—Furfural accumulation in orange juice concentrate (58°C Brix) as affected by storage temperature. (Value are the mean of three replications; significant difference at a level of 0.05 = 27 ppb).

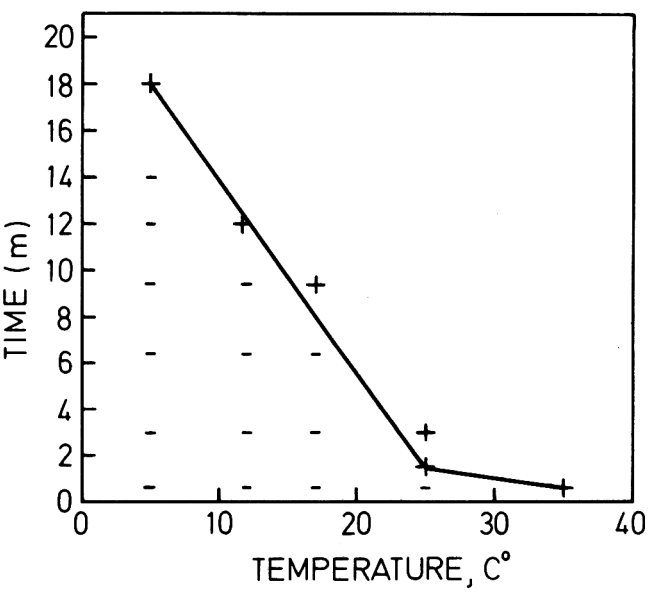


Fig. 5—Concurrent changes in the flavor quality of orange juice concentrate (58° Brix) as affected by temperature. No (−); Yes (+) statistically significant difference at a level of 0.05 between samples and the same orange juice concentrate stored at −18°C. (Time m = months).

Controlled Atmosphere and Ethylene Effects on Quality of California Canning Apricots and Clingstone Peaches

JEFFREY K. BRECHT, ADEL A. KADER, CHRISTI M. HEINTZ, and ROBERT C. NORONA

ABSTRACT

Storage at 2% O_2 plus 5% CO_2 at 1.1°C maintained higher flesh firmness and lower pH and retarded decay more effectively than air storage of immature (M1) and over-mature (M3) Patterson and Tilton apricot fruits. CA storage of fruits picked at the optimum maturity stage (M2) produced little benefit over air storage, however. Treatment with 100 ppm ethylene for 48 hours accelerated softening and color change at 20°C compared to ripening in air and may potentially be used to prepare immature apricot fruits for canning in the shortest possible time. Large differences in storageability and canned quality following storage were found among the five clingstone peach cultivars tested. Loadel and Carolyn peaches, if in sound condition at harvest, can be stored for up to 4 wk under 2% O_2 + 5% CO_2 at 1.1°C. Andross, Klamt and Halford peaches should be stored for shorter storage periods only. Fruits ripened at 20°C with ethylene (100 ppm for 48 hr) were similar to those ripened without it in appearance, texture, and flavor.

INTRODUCTION

CALIFORNIA APRICOTS and clingstone peaches are harvested for canning on the basis of their skin and flesh color, respectively, and are normally processed within one day of harvest. Currently, when canning capacity is exceeded by daily harvested tonnage, processors may store the fruit a few days (apricots) or up to 2 wk (cling peaches) at 0–2°C in air. As more restrictions on water use and waste disposal are imposed, longer storage may become necessary to regulate processed quantity per day. In addition, increased use of mechanical harvesting may result in a wide range of maturities at harvest, including minimally mature fruit which require ripening before canning.

Previous work (Salunkhe et al., 1966; Wankier et al., 1970; Claypool and Pangborn, 1972) has shown that canning quality of apricots can be affected by cultivar, maturity, and length and type of storage. Claypool and Pangborn (1972) tested Blenheim and Tilton apricots and judged Tilton to have limited storage potential compared to Blenheim. In this study we compare Tilton to Patterson, a recently introduced cultivar in California.

Anderson et al. (1969) recommended a controlled atmosphere (CA) of 1% O_2 plus 5% CO_2 for storage of fresh market peaches for longer than three weeks duration. Claypool and Davis (1959) found no beneficial effect of 1 or 2.5% O_2 and observed that flavor ratings of canned peaches decreased as the CO_2 concentration increased above 5%. Most previous studies on the effect of maturity or ripeness on canning quality (Salunkhe et al., 1966; Boggess et al., 1974) have been limited to air storage without added ethylene.

The objectives of this study were (1) to explore the possibility of maintaining good quality in storage through use of controlled atmospheres (CA), and (2) to evaluate the possible use of ethylene (C_2H_4) treatments to ripen green fruits before canning in order to achieve faster and more uniform ripening.

Authors Brecht, Kader and Heintz are with the Dept. of Pomology, Univ. of California, Davis, CA 95616. Author Norona is with the Tri/Valley Growers, Modesto, CA 95353.

MATERIALS & METHODS

Source of fruit and sorting by maturity

Apricots (Patterson and Tilton) and clingstone peaches (Loadel, Andross, Klamt, Carolyn, and Halford) were obtained from Tri-Valley Growers (Modesto, CA). The fruits were hand harvested and sorted in the orchard into three maturities, designated M1, M2 and M3. These were defined as follows: (M1) hard fruit with overall green color, (M2) firm fruit with yellow to yellow-orange ground color on the cheeks and little or no green on the suture or ends, (M3) moderately soft fruit with an overall yellow-orange ground color. The initial color and firmness measurements corresponding to these maturity stages are shown in Table 1 (apricots) and Table 3 (cling peaches).

Handling

Fruits were transported to Davis within 24 hr of harvest and stored at 0°C in air if overnight holding was required. Peaches were dipped in 20°C fungicidal suspension of benomyl and 2,6-dichloro-4-nitroaniline, a treatment which Wells (1972) has shown to be very effective in controling decay caused by *Monilinia* and *Rhizopus* fungi. All fruits were sorted for freedom from bruising and decay, and for uniformity of size range and maturity stage among replicates before being placed in 9.5-liter jars fitted with rubber stoppers with inlet and outlet tubes. Three replicates of each treatment were prepared for both the storage and ripening experiments.

Storage experiment

The effects of CA on storage life at 1.1°C, ripening of M1 fruit at 20°C after storage, and canning quality were compared to air storage using three maturities of each cultivar. Individual lots of fruits were exposed to one of four different atmospheres: air control, 2% O_2 in N_2, 5% CO_2 in air, and 2% O_2 plus 5% CO_2 in N_2. The gases were mixed in a flow-through system using needle valve type flowmeters and the total flow rates delivered to each jar adjusted so that CO_2 accumulation from respiration remained below 0.2%. Gas mixtures were monitored by gas chromatography. The relative humidity in the jars was maintained at 95–100% by bubbling the gases through water before entering the sample chamber. Storage times were 1, 2, and 3 wk for apricots and 2 and 4 wk for cling peaches at 1.1°C.

Ripening experiment

Ripening responses at 20°C in air, and in air containing 100 ppm ethylene for the initial 48 hr of ripening, were compared using M1 and M2 fruits. Samples to be ripened were held at 20°C until color and hand-held firmness were judged indicative of optimum stage for immediate canning, i.e., M3. Gas mixing, flow rate adjustment, monitoring of mixes and attainment of desired relative humidity were as mentioned above.

Evaluation and canning of samples

Samples of ten fresh fruits per replicate were evaluated initially and after the various treatments. Initial and post-treatment samples were also sent to Tri-Valley Growers for canning. A syrup consisting of one-third sucrose, one-third high fructose corn syrup, and one-third corn syrup was used. Fruits were cooked in continuous rotary cookers at 100°C for 14 min. Evaluations of canned fruits from the ripening experiment and from the CA storage treatments were performed after about 10 months storage at 20°C.

Color

The skin color of fresh and canned fruits was measured with a Gardner XL-23 Color Difference Meter. The instrument was cali-

brated with a standard white reference plate (X = 81.7, Y = 84.1, Z = 97.9). One reading was taken of each cheek on a fruit.

Firmness

With flesh exposed by peeling a small area of skin on each cheek, a UC Firmness Tester with a 0.79-cm tip was used to measure the fruit flesh firmness.

Chemical analysis

Fruit samples were homogenized in a Waring Blendor, centrifuged for 20 min at 13,200 x *g* and the supernatant juice decanted. Soluble solids content (SSC) of juice and syrup was measured with an Abbe refractometer. The pH of the juice was measured directly with a Corning pH Meter 130. Total titratable acidity (TTA) was determined by diluting a 6.00 g aliquot of juice with 50 ml deionized water and titrating to pH 8.2 with 0.1N NaOH. Results were expressed as percent malic acid. All samples of canned fruit were rinsed for one minute in deionized water and drained for two minutes to remove the canning syrup before chemical analyses were performed.

Discarded fruits

Percent discard is the average of three replicates and includes fruit which were unsuitable for canning due to either physiological or pathological deterioration.

Sensory evaluation by a taste panel

A taste panel of 11 individuals scored canned fruit samples for firmness, sweetness, sourness, and flavor intensity by marking an unscaled 10-cm line labeled at opposite ends with 'none' or 'extreme.' The judges were trained prior to testing by being given fruit samples representative of the ranges of the various attributes as well as definitions of the terms. These were as follows: "firmness," the force required to penetrate the fruit with the back molars; "sweetness," the taste associated with sucrose; "sourness," the taste associated with acids; "flavor intensity," the relative intensity of the flavor associated with a fresh apricot or cling peach.

Judges were given three pieces of each sample placed under red light to minimize color bias and labeled with random three-digit numbers. A maximum of ten samples were tasted per day. Judges were instructed to expectorate samples and to rinse with water between samples.

Statistical analysis

Data were treated by analysis of variance (ANOVAR) with least significant difference (LSD) between treatment means determined at the 5% level.

RESULTS & DISCUSSION

Apricots

The 'a' color value, which indicates the relative greeness or redness of a sample, gave the best correlation with firmness readings and changed to a greater degree during ripening than either 'Rd' or 'b' values, which measure relative whiteness/blackness and yellowness/blueness, respectively. Thus, 'a' values seem a better measure of ripeness and are included in the data presented here while 'Rd' and 'b' values are not.

The 2% O_2 plus 5% CO_2 CA storage treatment was the most consistently effective in retaining the quality attributes of fresh apricots and was therefore chosen for further evaluation of canned samples. For this reason data are presented for this CA treatment only.

Effect of controlled atmosphere. CA storage was more effective than air storage in retarding the loss of greeness by fresh M1 and M2 fruits (Table 1). CA storage had no effect on the SSC of fresh apricots, but did result in greater firmness, lower pH, and higher TTA, as well as lower decay than air storage.

When objective evaluations of the canned fruits were performed these differences between air- and CA-stored

Table 1—Compositional and quality attributes of fresh apricots as related to storage treatment at 1.1°C

Variable	Treatment	Days	Patterson M1[a]	Patterson M2	Patterson M3	Tilton M1[a]	Tilton M2	Tilton M3
Color ('a' value)		0	7.3	17.6	26.9	−4.9	17.3	26.2
	Air	7	6.3	24.4	27.1	−2.1	18.0	24.8
		14	21.7	25.5	27.3	16.4	19.4	25.3
		21	14.0	25.6	28.1	8.6	21.5	24.7
	CA	7	7.3	21.5	28.8	−4.2	18.1	26.0
		14	16.5	23.8	27.2	11.1	19.9	26.6
		21	11.4	24.0	28.6	10.6	18.3	25.6
LSD @ 5%			2.1	1.5	1.2	2.2	1.5	NS
Firmness (kg)		0	5.6	3.6	0.9	7.9	4.0	1.3
	Air	7	4.1	1.5	0.4	5.7	2.3	0.7
		14	0.6	1.5	0.7	1.0	2.0	0.7
		21	1.5	1.5	0.7	1.4	2.1	0.8
	CA	7	4.7	2.7	1.0	6.4	3.3	0.8
		14	1.8	2.0	1.0	2.5	2.5	0.9
		21	3.2	2.3	0.9	2.7	2.7	1.1
LSD @ 5%			0.6	0.4	0.2	0.6	0.6	0.2
SSC (%)		0	10.4	12.6	16.7	8.8	12.0	14.6
	Air	7	10.7	14.0	16.3	4.2	12.4	9.8
		14	10.6	14.2	15.7	8.8	12.5	15.3
		21	10.4	13.8	15.4	8.9	12.3	14.8
	CA	7	10.2	13.6	15.8	7.0	12.5	14.9
		14	10.6	14.3	15.9	8.7	12.5	15.3
		21	10.5	13.5	15.3	9.0	12.4	15.3
LSD @ 5%			NS	0.6	0.8	0.9	NS	0.9
pH		0	3.74	3.88	4.28	3.89	4.17	4.37
	Air	7	3.69	3.98	4.26	4.01	4.20	4.28
		14	3.96	4.09	4.36	4.04	4.21	4.35
		21	3.79	4.22	4.43	4.02	4.34	4.50
	CA	7	3.79	3.99	4.14	3.93	4.11	4.29
		14	3.85	4.02	4.18	4.04	4.17	4.31
		21	3.73	4.06	4.24	3.85	4.21	4.38
LSD @ 5%			0.04	0.07	0.11	0.06	0.07	0.10
Titratable Acidity (%)		0	1.34	1.09	0.60	1.45	0.84	0.54
	Air	7	1.46	0.89	0.62	0.81	0.49	0.45
		14	1.20	0.78	0.56	1.05	0.65	0.46
		21	1.37	0.69	0.55	1.05	0.67	0.45
	CA	7	1.50	1.00	0.72	1.07	0.62	0.50
		14	1.39	0.90	0.69	1.10	0.66	0.48
		21	1.43	0.87	0.65	1.11	0.78	0.55
LSD @ 5%			0.08	0.08	0.06	0.20	0.06	0.09
Discard (%)		0	–	–	–	–	–	–
	Air	7	0	0	0.8	0	0	0
		14	0	0	5.3	20.0	0	6.7
		21	3.3	0	16.9	4.2	0	14.2
	CA	7	0	0	0.4	0	0	0
		14	0	0	0.9	0	0	1.0
		21	0.8	0	3.4	4.2	0	6.7
LSD @ 5%			NS	NS	6.0	NS	NS	3.8

[a] Ripened for 0, 4, and 2 or 0, 5, and 4 days at 20°C after removal from air storage or CA storage, respectively.

fruits remained generally similar. CA-stored fruits were lower in 'a' values at M1 and M2 stages with no significant differences among M3 fruits (data not shown). The pH of CA-stored fruits was slightly lower and the acidity higher than air-stored fruits. The taste panel judges found CA-stored M1 fruits firmer than air-stored fruits, but the firmness differences between air- and CA-stored M2 and M3 fresh fruits were essentially lost after canning. The taste panel detected little or no differences in sweetness, sourness, or flavor intensity between the two treatments, but off-flavors were more frequently noted in air than in CA fruits. These data are in agreement with the results of Claypool and Pangborn (1972) indicating that CA retards development of off-flavors.

The potential applicability of CA to storage of canning apricots appears to depend on the condition of the fruit to be stored. For fruit of the M2 maturity stage, CA has little beneficial effect over air storage, but fruits picked at the M1 and M3 stages may benefit from CA storage. CA storage results in a firmer M1 canned product with no perceptible detrimental effect on flavor. In addition, whereas decay may be a problem in air-stored M1 and M3 fruits, it is effectively retarded by CA storage.

A potentially important effect of CA storage concerns the retention of low pH values compared to air storage. This is especially apparent after longer storage of the higher maturity fruits. The high pH values recorded in canned M3 apricots from the 3-wk storage treatment (4.3) are approaching the upper limit for safe canning (4.5). CA storage may allow the use of lower cooking times and/or temperatures than would otherwise be necessary to safeguard against possible contamination, thereby resulting in better textural and nutritive quality.

Table 2—Compositional and quality attributes of fresh apricots ripened in air or air plus 100 ppm ethylene for 48 hr at 20°C

		Patterson		Tilton	
Variable	Treatment	M1	M2	M1	M2
Ripening time (days)	Air	8	6	8	6
	C_2H_4	7	5	7	5
LSD @ 5%		NS	NS	NS	NS
Color ('a' value)	Initial	5.0	22.0	−0.2	8.8
	Air	16.0	28.6	14.5	18.4
	C_2H_4	20.3	28.0	16.5	20.4
LSD @ 5%		2.5	1.2	2.9	1.1
Firmness (kg)	Initial	4.9	3.0	5.4	3.6
	Air	2.9	1.0	1.0	1.3
	C_2H_4	1.0	0.5	0.5	0.5
LSD @ 5%		0.2	0.3	0.2	0.05
SSC (%)	Initial	8.8	11.6	10.6	11.5
	Air	9.6	12.8	9.9	11.1
	C_2H_4	9.3	12.6	9.8	11.3
LSD @ 5%		NS	0.5	0.6	NS
pH	Initial	3.72	3.95	4.03	4.14
	Air	3.78	4.21	4.20	4.28
	C_2H_4	3.94	4.12	4.31	4.24
LSD @ 5%		0.14	0.06	0.05	0.05
Titratable acidity (%)	Initial	0.82	0.58	0.66	0.57
	Air	0.66	0.47	0.58	0.52
	C_2H_4	0.73	0.45	0.55	0.53
LSD @ 5%		0.10	0.03	0.05	0.02

Effect of ethylene treatments. Fresh apricots were significantly softer and less green after treatment with ethylene even though ripened for one day less than air-ripened fruits (Table 2). There were no differences in SSC, TTA, or percent discard between the two ripening treatments; differences in pH were small.

After canning, the ethylene-treated fruits were still higher in 'a' value, but no significant differences were observed in SSC or TTA. While C_2H_4-treated fruits had a higher (by about 0.2 units) pH than air control M1 fruits, no differences in pH were noted in the M2 fruits. The taste panel found ethylene-treated M1 fruits softer than those from air while the firmness differences seen in fresh M2 fruits were no longer detectable. In addition, canned M1 fruits were judged less sour when ripened with ethylene. Consequently, the ethylene-treated M1 fruits were also judged to have less flavor intensity than air-ripened fruit. Panel members found no differences among M2 fruits canned after the two ripening treatments (data not shown).

The results of the ripening experiment indicate that ethylene treatment (100 ppm for 48 hr at 20° C) accelerates the ripening process compared to air storage. Even though the ethylene-treated fruit were held for one day less at 20°C than the air-ripened fruit, they were softer and showed less green color. It seems possible that manipulation of holding time, temperature, and/or ethylene concentration can be used to develop ripening schedules which could be applied to achieve faster and more uniform ripening of canning apricots.

Clingstone peaches

The 'a' color value, which indicates the relative greeness or redness of a sample, gave the best correlation with firmness readings and has been shown to correlate well with flavor and visual quality by Leonard et al. (1961). It thus seems to be a better measure of ripeness than either 'Rd' or 'b' values, and therefore, only 'a' values are presented here.

The 2% O_2 plus 5% CO_2 storage treatment was the most effective in retarding color change and development of decay in fresh peaches and was therefore chosen for further evaluation of canned samples. Data are presented for this CA treatment and for Carolyn and Halford peaches only. The relative responses of the other three cultivars will be discussed.

Effect of controlled atmosphere. There were small or no significant differences between air- and CA-stored fruit in terms of color, firmness, SSC, pH and TTA for each storage duration (Table 3). However, CA storage was more effective than air storage, in some cases, in lowering the incidence of decay observed after 4 wk at 1.1°C.

Canned M3 Halford peaches from CA storage showed higher 'a' values than air-stored fruits. Canned M1 fruits which were held in CA had a slightly higher pH than air-stored fruits. Otherwise, the objective evaluation revealed no statistically significant differences between the two storage treatments. The taste panel judges also detected no significant differences between fruits from the two storage treatments except that CA-stored M3 peaches were scored firmer than air-stored fruits.

An expert panel from Tri-Valley Growers subjectively rated canned Carolyn peaches good to very good in flavor and appearance with M1 fruits from CA storage judged to be of significantly better overall appearance than air-stored fruits (data not shown). Canned Loadel peaches were rated good while Halford and Klamt peaches scored low and Andross peaches were intermediate in appearance and flavor quality attributes.

An important result of this study is the observed variation in responses to storage among the cultivars tested. It seems

that the most important factor in the successful storage of cling peaches may be the cultivar that is chosen. Large differences were found with respect to off-flavors, flesh browning, and bruising. Both Wankier et al. (1970) and Watada et al. (1979) cited development of off-flavors as a major factor limiting CA storage of fresh market peaches. We observed no significant increase in off-flavors in CA-stored canning cling peaches compared to air. Among the five cultivars, Halford and Klamt were badly discolored and exhibited off-flavors described as "fishy" and "caramelized"

Table 3—Compositional and quality attributes of fresh peaches as related to storage treatment at 1.1°C

Variable	Treatment	Days	Carolyn M1[a]	Carolyn M2	Carolyn M3	Halford M1[a]	Halford M2	Halford M3
Color ('a' value)		0	4.8	18.7	24.8	1.5	10.5	21.4
	Air	14	8.9	12.3	20.4	8.0	10.0	18.3
		28	7.6	13.4	18.6	9.4	10.8	18.1
	CA	14	10.4	13.8	19.1	8.4	8.6	16.8
		28	8.9	11.4	17.9	7.9	8.8	16.2
LSD @ 5%			2.0	0.9	1.9	2.2	1.8	1.6
Firmness (kg)		0	4.8	4.2	3.4	4.0	3.2	3.5
	Air	14	2.7	4.3	3.4	2.2	3.3	3.2
		28	3.8	4.1	2.8	3.2	3.1	2.7
	CA	14	2.5	4.1	3.6	2.4	3.2	3.4
		28	3.3	4.1	2.8	3.0	3.3	3.0
LSD @ 5%			0.4	NS	NS	0.3	NS	0.5
SSC (%)		0	9.6	11.1	10.7	9.3	9.9	14.1
	Air	14	8.9	9.9	10.3	9.1	9.8	12.5
		28	8.6	10.4	9.8	9.6	9.3	12.1
	CA	14	8.5	9.9	10.3	9.3	9.7	12.4
		28	8.9	9.9	10.2	8.9	9.6	12.2
LSD @ 5%			NS	0.3	0.8	NS	NS	0.7
pH		0	3.76	3.88	3.99	3.91	4.01	4.03
	Air	14	3.99	3.93	4.24	4.21	4.16	4.28
		28	4.03	4.14	4.44	4.25	4.29	4.58
	CA	14	3.98	3.99	4.27	4.19	4.20	4.31
		28	3.97	4.19	4.65	4.32	4.41	4.53
LSD @ 5%			0.02	0.08	0.15	0.07	0.06	0.07
Titratable acidity (%)		0	0.82	0.58	0.50	0.66	0.57	0.50
	Air	14	0.67	0.57	0.42	0.60	0.51	0.42
		28	0.63	0.44	0.29	0.51	0.45	0.30
	CA	14	0.62	0.51	0.41	0.59	0.52	0.42
		28	0.65	0.44	0.26	0.52	0.45	0.29
LSD @ 5%			0.05	0.07	0.05	0.04	0.05	0.02
Discard (%)		0	—	—	—	—	—	—
	Air	14	0	0	0	0	0	0
		28	6.7	1.4	18.1	22.7	1.4	0
	CA	14	0	0	0	0	0	0
		28	6.7	0	4.2	8.0	2.8	0
LSD @ 5%			NS	NS	NS	10.1	NS	NS

[a] Ripened for 3 or 4 and 3 days at 20°C after removal from air storage or CA storage (14 and 28 days), respectively.

by the judges. Andross, Loadel, and Carolyn fruits ranged from good to excellent in both appearance and flavor after four weeks storage followed by canning, regardles of the storage atmosphere.

There was also a wide range of susceptibility to decay among the cultivars which was most serious in M1 fruits ripened after storage. *Alternaria* was most common on these fruits, while *Monilinia* was more common on fruits affected during storage or the ripening experiment. While Andross, Carolyn, and Halford peaches were relatively unaffected by decay, Klamt peaches were heavily infected, and Loadel peaches were subject to such heavy decay that none of the M1 fruits transferred to 20°C after 4 wk storage were salvageable after a few days at room temperature.

When M1 fruits were ripened immediately after harvest, decay was not a significant problem. Therefore, the much greater amounts of decay observed in M1 fruits ripened after storage compared to immediate ripening indicates that when long-term storage of cling peaches is necessary, green fruits should probably be ripened before storage rather than after. Although CA storage proved to offer only small benefit over air storage in terms of canning quality, it would tend to reduce decay compared to air storage if initial sorting and ripening of green fruits is not feasible.

It is known that susceptibility of fruits to chilling injury is lessened with increasing maturity (Fidler, 1968; Lyons, 1973) and accelerated decay incidence is a common symptom of chilling injury in other commodities upon transfer to room temperature (Lyons, 1973; Ryall and Pentzer, 1974). Therefore, the decay observed in this study may have been a symptom of chilling injury to the M1 peaches.

—Continued on next page

Table 4—Compositional and quality attributes of fresh peaches ripened in air or air plus 100 ppm ethlyene for 48 hr at 20°C

Variable	Treatment	Carolyn M1	Carolyn M2	Halford M1	Halford M2
Ripening time (days)	Air	8	6	8	5
	C_2H_4	8	5	8	5
LSD @ 5%		NS	NS	NS	NS
Color ('a' value)	Initial	4.8	18.7	1.5	10.5
	Air	12.2	19.3	10.1	15.0
	C_2H_4	11.1	22.2	10.6	13.5
LSD @ 5%		3.8	1.3	2.3	NS
Firmness (kg)	Initial	4.8	4.2	3.9	3.2
	Air	2.9	2.8	2.9	2.6
	C_2H_4	2.7	3.0	2.9	2.7
LSD @ 5%		0.4	0.4	0.1	0.2
SSC (%)	Initial	9.6	11.1	9.3	9.9
	Air	8.7	9.8	9.0	10.1
	C_2H_4	8.2	9.4	8.4	9.9
LSD @ 5%		1.0	0.4	0.3	NS
pH	Initial	3.76	3.88	3.91	4.01
	Air	3.93	4.08	4.13	4.16
	C_2H_4	3.92	4.10	4.09	4.08
LSD @ 5%		0.05	0.06	0.05	0.08
Titratable acidity (%)	Initial	1.17	0.75	1.07	0.97
	Air	1.25	0.68	0.94	0.77
	C_2H_4	1.07	0.62	0.85	0.70
LSD @ 5%		0.06	0.06	0.12	0.05

Effect of ethylene treatment. Only small differences were found in rate or uniformity of ripening between the ethylene- and air-ripening treatments. However, ethylene-ripened fruits were somewhat lower in SSC, pH, and significantly lower in acidity than air-ripened fruit (Table 4). Decay was not a problem in this experiment in contrast to the M1 fruit ripened after storage (Table 3).

Objective evaluation of the canned Halford peaches showed no significant differences between air- and ethylene-ripened fruits. The taste panel, however, judged M1 fruits both firmer and more sour when treated with ethylene, while ethylene-treated M2 fruits were scored as softer and less sour than air-ripened fruit.

The Tri-Valley Growers panel found no significant differences between fruits from the two ripening treatments but rated M1 and M2 Carolyn fruits good and very good, respectively, in flavor and rated both maturities excellent in overall appearance.

When ripening of cling peaches is desired before canning, there is apparently little to be gained from ethylene treatment. Fruits ripened in air, and in air after a 2-day ethylene treatment at 20°C, were essentially equal in appearance (including decay) and flavor quality. Although the ethylene treatment used in this study did not accelerate the ripening process, use of a longer duration and/or a higher ethylene concentration may potentially exhibit this effect. Otherwise, it would seem that simply holding the fruits in air at 20°C will suffice to allow ripening which produces peaches of good flavor and appearance when canned.

REFERENCES

Anderson, R.E., Parsons, C.S., and Smith, W.L. Jr. 1969. Controlled atmosphere storage of Eastern-grown peaches and nectarines. U.S. Dept. Agr. Mktg. Res. Rept. 836.

Boggess, T.S. Jr., Heaton, E.K., Shewfelt, A.L., and Parvin, D.W. Jr. 1974. Effects of ripeness and postharvest treatments on the firmness, acidity, and canning characteristics of Babygold #6 peaches. J. Milk Food Technol. 37: 164.

Claypool, L.L. and Davis, L.D. 1959. The effect of cold and modified atmosphere storage on the canning quality of cling peaches. Food Technol. 13: 208.

Claypool, L.L. and Pangborn, R.M. 1972. Influence of controlled atmosphere storage on quality of canned apricots. J. Amer. Soc. Hort. Sci. 97: 636.

Fidler, J.C. 1968. Low-temperature injury to fruits and vegetables. In "Low Temperature Biology of Foodstuffs." Recent Adv. Food Sci. 4: 271.

Leonard, S.J., Luh, B.S., Chichester, C.P., and Simone, M. 1961. Relationship of fresh clingstone peach color to color and grade after canning. Food Technol. 15: 492.

Lyons, J.M. 1973. Chilling injury in plants. Annu. Rev. Plant Physiol. 24: 445.

Ryall, A.L. and Pentzer, W.T. 1974. "Handling, Transportation, and Storage of Fruits and Vegetables." Vol. 2. "Fruits and Tree Nuts." Avi Publishing Co., Westport, CT.

Salunkhe, D.K., Schvaneveldt, N.S., Walker, D.R., Anderson, J.L., Wilcox, E.B., and Norton, R.A. 1966. Evaluation of variety, maturity, and storage on the processing quality of Utah-grown fruits. Special Report 20, Agric. Exp. Sta., Utah State Univ., Logan, UT.

Wankier, B.N., Salunkhe, D.K., and Campbell, W.F. 1970. Effects of controlled atmosphere storage on biochemical changes in apricot and peach fruit. J. Amer. Soc. Hort. Sci. 95: 604.

Watada, A.E., Anderson, R.E., and Aulenbach, B.B. 1979. Sensory, compositional, and volatile attributes of controlled atmosphere stored peaches. J. Amer. Soc. Hort. Sci. 104: 626.

Wells, J.M. 1972. Heated wax emulsions with Benomyl and 2,6-dichloro-4-nitroaniline for control of postharvest decay of peaches and nectarines. Phytopathology 62: 129.

Ms received 7/31/81; revised 9/21/81; accepted 9/24/81.

STORAGE STABILITY OF ASEPTICALLY PACKAGED OJ CONC . . . From page 431

Brenner, S., Wodicka, V.O., and Dunlop, S.G. 1948. Effect of high Temperature storage on the retention of nutrients in canned foods. Food Technol. 2: 207.

Clegg, K.M. 1966. Citric acid and the browning of solutions containing ascorbic acid. J. Sci. Food Agric. 17: 546.

Clegg, K.M. 1964. Nonenzymatic browning of lemon juice. J. Sci. Food Agric. 15: 878.

Curl, A.L. 1947. Concentrated orange juice storage studies. The effect of degree of concentration and temperature of storage. canner 105: 14.

Dinsmore, H.L. and Nagy, S. 1973. Improved colorimetric determination for furfural in citrus juices. J. AOAC 57: 772.

Du Bois, C.W. and Kow, T.J. 1951. Storage temperature effects on frozen citrus concentrates. Refrig. Engin. 59: 772.

Dunlop, A.P. and Peters, F.N. 1953. "The Furans," p. 638. Reinhold, New York, NY.

Green, J. 1978. A closer look at aseptic canning. Food Eng. Int'l. 3: 21.

Huelin, F.E. 1953. Studies on the anaerobic decomposition of ascorbic acid. Food Res. 18: 663.

Johnson, R.L. and Toledo, R.T. 1975. Storage stability of 55° Brix orange juice concentrate aseptically packaged in plastic and glass containers. J. Food Sci. 40: 433.

Kanner, J., Fishbein, J., Shalom, P., Harel, S., Juven, B. and Ben-Gera, I., 1978. Aseptic filling of citrus products in metal cans: Orange concentrate. Volcani Center Publications, Preliminary Report 772, p. 23.

Kanner, J., Harel, S., Fishbein, Y., and Shalom P. 1981. Furfural accumulation in stored orange juice concentrates. J. Agric. Food Chem. 29: (in press).

Kefford, J.F., McKenzie, H.A., and Thompson, P.C.O. 1959. Effects of oxygen on quality and ascorbic acid retention in canned and frozen orange juice. J. Sci. Food Agric. 19: 51.

Kramer, A. and Twigg, B.A., 1970. "Quality Control for the Food Industry," 3rd ed. Avi Publishing Co., Westport, CT.

Labuza, T.P., Tennenbaum, S.R., and Karel, M. 1970. Water content and stability of low-moisture and intermediate-moisture foods. Food Technol. 24: 543.

Lawler, F.K. 1974. Two citrus advances: Aseptic juice in glass; Year round packaging. J. Food Technol. 18: 81.

Maraulja, M.D., Blair, J.S., Olsen, R.W., and Wenzel, F.W. 1973. Furfural as an indicator of flavor deterioration in canned citrus juices. Proc. Fla. Sta. Hort. Soc. 86: 270.

Nagy, S. 1980. Vitamin C contents of citrus fruit and their products: a review, J. Agric. Food Chem. 28: 8.

Nagy, S. and Randall, V. 1973. Use of furfural content as an index of storage temperature abuse in commercially processed orange juice. J. Agr. Food Chem. 21: 272.

Nagy, S. and Smoot, J.M. 1977. Temperature and storage effects on percent retention and percent U.S. recommended dietary allowance of vitamin C in canned single-strength orange juice. J. Agric. Food Chem. 25: 135.

Rizzi, P.G. 1974. Formation of N-Alkyl-2 acylpyrroles and aliphatic aldimines in model nonenzymatic browning reactions. J. Agr. Food Chem. 22: 279.

Robe, K. 1981. Aseptic-pack fruits retain color, flavor, save 30 processing energy. Food Processing 42: 86.

Rother, H. 1977. Der Tankcontainer, eine möglichketi zur rationalisierung in der fruchtsaftindustrie. Flussiges Obst. 44: 363.

Saguy, I., Kopelman, I.J., and Mizrahi, S. 1978. Simulation of ascorbic acid stability during heat processing and concentration of grapefruit juice. J. Food Process. Eng. 2: 213.

Scott, H.C. 1974. Commercially sterile cold filling of citrus juices in glass containers. Food Production/Management 97: 8.

Tatum, H.J., Shaw, P.E., and Berry, R.E. 1969. Degradation products from ascorbic acid. J. Agric. Food Chem. 17: 38.

Ms received 3/17/81; revised 10/9/81; accepted 10/12/81.

This study was conducted under contract with the Israel Can Company Ltd., Petah-Tikva, and in cooperation with Pri-Ze/Pardes factories. Thanks are expressed to Dr. I. Rotman, Mr. M. Saguy and Mr. N. Barazovski. Presented in part at the 13th Symposium of International Federation of Fruit Juice Producers, Bled, Jugoslavia, April 23—25, 1980.

Estimation of Fungal Contamination in Tomato Products by a Chemical Assay for Chitin

R. H. BISHOP, C. L. DUNCAN, G. M. EVANCHO, and H. YOUNG

ABSTRACT

A chemical method for the quantitative measurement of fungal chitin was used to estimate the level of mold contamination in tomato products. The alkaline degradation of chitin results in a polyglucosamine which is measured colorimetrically and the results expressed as μg of fungal glucosamine. Addition of fungal mycelium to tomato products gave recoveries of 97 ± 3% of the expected level of glucosamine. Analysis of various types of tomato products showed a significant correlation ($P < 0.01$) between fungal glucosamine content and the Howard mold count.

INTRODUCTION

THE HOWARD MOLD COUNT (HMC) is a microscopic method for the determination of fungal contamination in tomato products. It is subjective and often imprecise. When "milled" products are examined, the inaccuracy is further compounded because comminution breaks up the mycelium yielding higher HMC's for the same initial level of contamination. A chemical method for the determination of mold should be more accurate and reliable.

Chitin is a major constituent of fungal cell walls but little or no chitin-like materials occur in higher plants, such as tomatoes. Therefore, the chitin content of tomato products should be a good measure of mold contamination.

Previous methods for the chemical assay for chitin utilizing acid, with or without subsequent enzymatic hydrolysis, were lengthy and subject to interference from plant materials (Ride and Drysdale, 1971). Ride and Drysdale (1972) described a method in which fungal chitin is deacetylated by alkaline hydrolysis to produce a group of compounds called chitosan. The chitosan is treated further with nitrous acid to yield an aldehyde which is measured colorimetrically (Tsuji et al., 1969). The advantages of this method are that vigorous alkaline treatment reduces the possibility of artifacts from plant materials, specific enzymes are unnecessary, and the time for the assay is reduced (Ride and Drysdale, 1972). Jarvis (1977) used the alkaline hydrolysis method to assess the level of fungal contamination of tomato juices and purees. Results with spiked samples showed more than 95% recovery of the added fungal glucosamine. Comparison with the Howard Mold Count showed a high correlation coefficient for nonhomogenized juices and purees, but a low correlation for homogenized juices.

Likewise, Donald and Mirocha (1977) used chitin analysis to measure the degree of fungal invasion of stored corn and soybean seed. The method had an advantage over standard plating methods in that results could be obtained in hours rather than days and the results reflected the total amount of mycelium present (viable and nonviable).

Because of a lack of information in the literature, further work on the method is required before chitin content of a tomato product can be used to establish acceptable or unacceptable levels of mold. Information also is lacking on whether the chitin content of molds differs and to what extent the chitin content is affected by substrate, age and growth conditions. And, as the exoskeleton of mature insects also contains chitin, work was also needed to assess the affect of insect contamination.

This study was conducted to evaluate a chemical method for the assessment of fungal contamination in tomato products based on the estimation of chitin, a constituent of the fungal cell wall.

All authors are affiliated with the Campbell Institute for Research & Technology, Campbell Place, Camden, NJ 08101.

MATERIALS & METHODS

Fungi

Cultures of the fungi *Alternaria tenuis, Colletotrichum phomoides, Fusarium oxysporium,* and *Geotrichum candidum,* previously isolated from tomatoes, were used in the study.

Glucosamine content of fungi

All fungal cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of a semi-synthetic liquid medium. The medium consisted of glucose, 10g; yeast extract, 1g; KH_2PO_4, 1g; $MgSO_4$, 0.5g; tomato juice, 400 ml; distilled water 600 mL The medium was autoclaved for 15 min at 121°C. Each flask was inoculated with three agar disks (5 mm diam) of the appropriate fungus which had been grown on Potato Dextrose Agar for 5 days at 22°C. Broth cultures were incubated at 22°C for up to 21 days, either statically or on a rotary shaker (160 rpm). Mycelium was harvested, washed with distilled water and homogenized in an Omnimixer for 1 min (1g wet wt/10 ml H_2O). The dry weight of the fungal suspensions was determined by drying duplicate 1 ml samples of blended mycelium in tared aluminum weighing cups to constant weight at 100°C. Portions of the suspensions of disrupted mycelia of pure cultures were added to tomato product samples to determine the rate of recovery.

Tomato studies

Tomato puree, paste, and ketchup samples were purchased from local markets or were obtained from regular factory production.

Howard mold count

The standard method (44.001) described in the *official Methods of Analysis* AOAC (1980) was used throughout the study. Pastes and purees were diluted to 8.3% soluble solids while ketchup was diluted 1:1 before testing.

Detection of insect fragments

Drosophila fly eggs (1, 2, and 4) and maggots (1 and 2) were added to two gram samples of tomato paste with moderate (24%) and low (5%) Howard mold counts. These egg and maggot levels equal or exceed the proposed, more stringent defect action levels (DAL) of one fly egg or two maggots/100g of tomato paste. The above contamination levels were considered to be the worst conditions which could be encountered in a 2 gram sample of acceptable tomato paste. In addition, fragments of mature insects (Family *Cucujidae*) were added to a sample of tomato paste naturally contaminated with fragments of tomato pinworm (*Gnorimoschema lycopersicella*) and thoroughly blended. This level (600 fragments/100g paste) was diluted with quantities of insect-free paste to provide levels of 6 and 60 fragments/100g. Each sample was analyzed by the standard AOAC method (44.096) to ensure proper distribution of the insect fragments. *—Continued on next page*

Fungal chitin assay

Fungal chitin, expressed as glucosamine, was determined by a modified procedure adopted from methods of Ride and Drysdale (1971, 1972), Tsuji et al. (1969), and Jarvis (1977). The method is based on the alkaline deacetylation of chitin to chitosan, the glucosamine residues of which are deaminated with nitrous acid to an aldehyde which is measured colorimetrically.

Portions of fungal mycelial suspensions in water or tomato samples (2g) and unadulterated tomato samples (2g) were diluted with 9 ml of acetone in a 15 ml graduated centrifuge tube. The samples were centrifuged at 1500 x *g* for 2 min, resuspended in 9 ml acetone and recentrifuged. The dried pellet was suspended in 4 ml concentrated KOH (120g KOH/100 ml) and placed in a 130°C oven for 1 hr or autoclaved for 15 min at 121°C. After cooling, 8 ml of ice cold 75% ethanol were added and the mixture allowed to stand in an ice-bath for 15 min. A celite suspension (0.9 ml), prepared by mixing 1g of celite "545" in 20 ml of 75% ethanol and allowing to stand for 2 min, was layered on top of the ethanol suspension and centrifuged at 1500 x *g* for 10 min at 2°C. The pellet was resuspended in 8 ml of ice cold 40% ethanol, stirred thoroughly, and recentrifuged at 1500 x *g* for 10 min at 2°C. The pellet was washed and centrifuged twice with cold distilled water.

The residue was adjusted to 1.5 ml with water, and 1.5 ml each of 5% $KHSO_4$ and 5% $NaNO_2$ were added. The mixture was stirred for 15 min and centrifuged at 1500 x *g* for 2 min at 2°C. A 1.5 ml portion of the supernatant was mixed with 0.5 ml of 12% NH_4SO_4-NH_2, and the mixture was shaken for 5 min, and 0.5 ml of 0.5% 3-methyl-2-benzothiozolone hydrazone (MBTH) added and mixed. The mixture was heated in a boiling water bath for 3 min, cooled, and 0.5 ml of 0.5% $FeCl_3$ added. After standing at room temperature for 30 min, the absorbance was read at 650 nm. Results, expressed as μg glucosamine/sample, were determined from the standard curve prepared with known concentrations of glucosamine HCl. The standard solutions prepared in 1.5 ml water were treated with 1.5 ml each of 5% $KHSO_4$ and 5% $NaNO_2$ and the procedure contined as described above. The standard curve was linear over the range 0–50 μg. Standard solutions of glucosamine-HCl (20, 30, 40 μg in 1.5 ml water) and water blanks were run concurrently with each assay.

RESULTS & DISCUSSION

THE CHITIN CONTENT (expressed as μg of fungal glucosamine per mg dry wt) of four fungi commonly isolated from diseased tomatoes varied with the fungus, the cultural conditions (aeration and availability of nutrients), and the age of the culture (Table 1). Mycelium grown in shake cultures reached a maximum chitin level in 3–4 days and then remained constant through 10 days of incubation. However, the glucosamine level reached in shake cultures never was higher than that reached by mature stationary cultures. Static cultures required at least 7 days to reach the chitin level achieved in the 3-day shake cultures, after which the level continued to increase slightly through 14 days. The differences in chitin content of the cultures observed at early stages of growth apparently reflect the influence of growth rate. The mean glucosamine level of the static cultures of the four fungi was 29 μg/mg dry wt of mycelium. This figure compares favorably with a mean of 32 μg for 10 similar fungi reported by Jarvis (1977). Mold contamination of tomatoes in nature obviously will consist of a variety of fungi at different stages of growth. The results of this study indicate that a mean value of 29–30 μg of glucosamine/mg dry wt of this diverse fungal population may be a statistically valid figure.

Addition of known amounts of fungal mycelium (mg dry wt) or glucosamine to tomato products prior to hydrolysis gave recoveries of 94–103% of the expected level (Table 2). The type of tomato sample, type of mold or the amount of mold mycelium added did not affect the recovery.

Insects contain chitin in their exoskeleton and contamination of tomato products with insects possibly would increase the glucosamine level of the contaminated products. The most common form of insect contamination found in tomato paste is Drosophila fly eggs and maggots. The proposed new FDA guideline for tomato paste lists the DAL as 1 fly egg/100g, or 0.5 fly egg and 1 maggot/100g, or 2 maggots/100g. To simulate the worst condition which might occur in acceptable paste, up to four fly eggs or two maggots were added to the 2g samples employed in the chitin procedure. The glucosamine level of a tomato paste sample with a moderate but acceptable HMC of 24% did not increase after the addition of the fly eggs or maggots. However, the addition of one or two maggots to a 2g sample of relatively mold-free paste did produce a noticable increase in the low initial glucosamine level, but this increase never elevated the glucosamine value to a level which suggested an unacceptable HMC. There also is an FDA guideline for other insect fragments in tomato products. The present unofficial standard for tomato paste is 25 insect fragments/100g. Results in Table 3 show that a paste sample containing 60 insect fragments/100g had a glucosamine content similar to the clean control sample. An exaggerated level of 600 insect fragments/100g (20 times the acceptable level) raised the glucosamine level only slightly. The small number of insect fragments normally present in acceptable tomato products would appear to have no significant effect on the total glucosamine level. Since insect fragments are reported as numbers/100g of sample it is unlikely there would be a significant number in the 2g sample used in the chitin assay.

To determine whether sugars in tomato products affect the results by adding to the glucosamine values, samples of the different tomato products were analyzed unhydrolyzed. It was felt that this value would provide a zero correction factor or baseline to correct for the presence of naturally occurring sugars. Little glucosamine (10–20 μg/g solids) was detected in most unhydrolyzed samples.

Mean values from duplicate chitin analyses of 20 tomato paste samples were plotted (Fig. 1) to obtain the relationship between the HMC and fungal glucosamine. The linear

Table 1—Glucosamine content of fungal mycelium grown in a glucose-yeast extract broth containing tomato juice

Fungus	Culture[a]	μg Fungal glucosamine/mg dry wt				
		Days				
		3	4	7	10	14
Alternaria tenuis	Static	5.7[b]	–	20.5	21.7	29.5
	Shake	22.7	23	25	23	–
Colletotrichum phomoides	Static	9.2	–	27.7	36	40
	Shake	30.7	–	30.3	28	–
Geotrichum candidum	Static	11.2	–	21	28	–
	Shake	28.7	–	28	26	–
Fusarium oxysporium	Static	–	20	38	40	43

[a] Cultures grown in 50 ml broth in a 250 ml Erlenmeyer flask at 22°C.
[b] Results reported are the mean of duplicate samples.

regression equation relating HMC and glucosamine is: μg glucosamine = 3.73 HMC + 65.99. The curve indicates that a glucosamine level of approximately 219 μg/g solids for paste samples would be equivalent to the HMC defect action level of 41%. With one exception, samples with a HMC less than the defect action level of 41% also had glucosamine levels less than the calculated value of 219 μg. The correlation coefficient of 0.902 indicates a significant correlation between HMC and the fungal glucosamine level.

Fig. 2 shows the plotted results of the chitin analysis of 25 ketchup samples. Again, the regression equation relating HMC and glucosamine level (Glucosamine = 2.92 HMC + 71.92) shows that all samples with HMC values of less than the 31% defect action level also had glucosamine levels lower than the expected value of 162.5 μg. However, two samples with greater than a 31% HMC did have glucosamine levels less than 162.5 μg. The fact that the samples represent several brands of ketchup, each receiving different degrees of milling, may explain the higher than expected HMC's.

The relationship of the glucosamine level of 13 tomato puree samples to the HMC is shown in Fig. 3. All samples with a HMC of less than the 41% defect action level also had a glucosamine level below the projected value of 257 μg/g solids. Samples with unacceptable HMC levels (>41%) contained glucosamine in the range 275–425 μg. All unacceptable samples would have been rejected using either method.

Results proved to be highly reproducible; duplicate samples never varied by more than 5% and generally were within 2%. This was true not only of duplicate samples run simultaneously, but also for identical samples run at different times.

Table 2—Recovery of glucosamine from spiked tomato products

Sample	Added	μg Glucosamine/g solids Expected	Observed[a]	% Recovery
Puree	None	—	20	—
	0.5 mg dry wt, mold[b]	40	38	95
	1.0 mg dry wt, mold	60	58.6	97.6
	1.5 mg dry wt, mold	80	81.2	101.5
	Glucosamine (30 μg.)	50	47	94
Paste	None	—	22	—
	0.5 mg dry wt, mold[c]	36.5	34.2	93.6
	1.0 mg dry wt, mold	51	52.8	103.5
	1.5 mg dry wt, mold	65.6	63.5	96.8
	Glucosamine (30 μg.)	52	50.5	97.2
Ketchup	None	—	28	—
	0.5 mg dry wt, mold[b]	48	45.5	94.8
	1.0 mg dry wt, mold	68	66.8	98.2
	1.5 mg dry wt, mold	88	84.2	95.7
	Glucosamine (25 μg.)	53	54	101.8

[a] Results are the mean of duplicate analyses.
[b] 1.0 mg dry wt of F. oxysporium = 40 μg glucosamine.
[c] 1.0 mg dry wt of C. phomoides = 29 μg glucosamine.

Table 3—Glucosamine level of tomato paste spiked with insect fragments

Sample	Insect fragments per 100g[a]	Howard mold count	μg Glucosamine/g
1	<1	26%	153
2	6	26%	160
3	60	26%	145
4	600	26%	180

[a] Fragments of adult insects added to clean paste and thoroughly blended.

The procedure requires approximately four hours to complete, but 20 samples can easily be analyzed simultaneously. Although the time factor makes the procedure unsuitable for on-line quality control, it is well suited as a confirmatory method to evaluate the quality of products with questionable Howard mold counts. The results of this study show that the chemical assay for fungal chitin could be used as a valuable adjunct to the HMC in assessing the quality of tomato products. *—Continued on page 444*

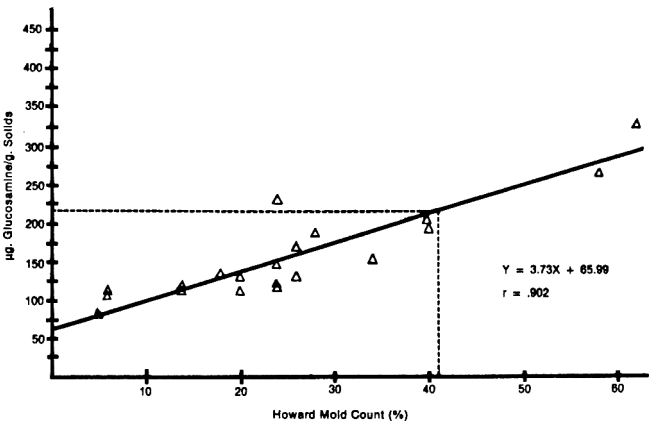


Fig. 1—Correlation of Howard mold counts with fungal glucosamine levels of 20 tomato paste samples.

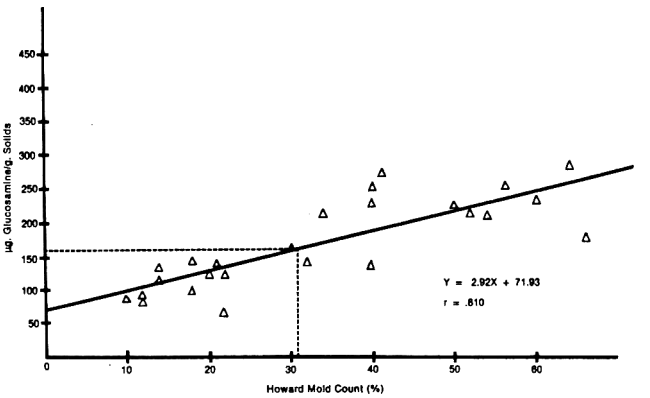


Fig. 2—Correlation of Howard mold counts with fungal glucosamine levels of 25 ketchup samples.

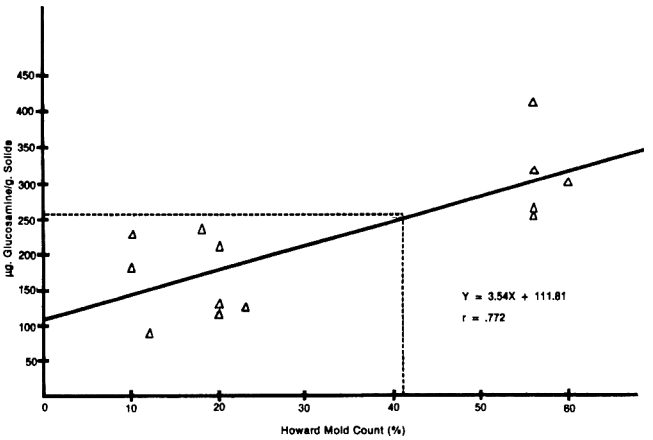


Fig. 3—Correlation of Howard mold counts with fungal glucosamine levels of 13 puree samples.

Effect of Temperature on Firmness of Raw Fruits and Vegetables

MALCOLM C. BOURNE

ABSTRACT

The firmness of a number of fruits and vegetables was measured by deformation, extrusion and puncture tests over the temperature range 0–45°C. Most commodities showed decreasing firmness with increasing temperature but there were several exceptions to this general rule. For the majority of the commodities tested the firmness-temperature relationship was approximately linear. The firmness-temperature coefficient is defined as [(firmness at T_2 – firmness at T_1)/(firmness at $T_1 \cdot (T_2 - T_1)$] x 100 (percent change in firmness per degree temperature change) where T_1 = lowest temperature and T_2 = highest temperature at which firmness is measured. The firmness-temperature coefficient ranged from –1.65 for apricot to +0.12 for carrot using the puncture principle, from –0.97 for Baby Gold peach to +7.7 for large Canoga strawberries tested between 30–45°C using the deformation principle, and from –0.04 for Golden Delicious apple stored 7 months to –1.34 for NK199 sweet corn using the extrusion principle.

INTRODUCTION

FIRMNESS is an important quality factor in many fruits and vegetables. For example, apples and pears are often harvested at a given firmness level; the maturity of green peas is usually determined by an objective firmness measurement which is used to establish a quality grade and price.

It has long been recognized that the temperature of the commodity at the time of testing can affect the numerical value of the objective firmness measurement. Fruits that have been held in cold storage for several hours usually give a higher firmness reading than when tested at field temperature. A number of researchers have published data on the effect of temperature on objective firmness measurements of raw fruits and vegetables. Some of these data are summarized in Table 1. Much of these data were published 40–60 yr ago using nonstandardized puncture testers. Data published during the last 40 years usually used well established tests such as the Magness Taylor tester (puncture) or a compression-extrusion test such as the Tenderometer, Shear Press or Ottawa Pea Tenderometer.

We thought it was time to expand the list of commodities and their condition for which firmness-temperature data were available. It is now recognized that the three principles that are most widely used for measuring the firmness of fresh horticultural crops are puncture, deformation and extrusion (Bourne, 1976). Therefore, we generated data using all three methods of firmness measurements and modern instrumentation.

EXPERIMENTAL

A NUMBER OF commodities were harvested from the Experiment Station farms at the usual harvest time. Unless noted otherwise, firmness measurements were made within 1 or 2 days of harvest. Snap beans, peas and sweet corn were always tested on the day of harvest. Apples were stored at 0°C and 90% RH except for the MacIntosh cultivar which was stored at 2°C and 90% RH.

Author Bourne is with the New York State Agricultural Experiment Station and Institute of Food Science, Cornell Univ., Geneva, NY 14456.

Each commodity was immersed in a flaked ice-water slush for a minimum of 15–45 min (the time depending on the size of the commodity) to bring it to equilibrium with the temperature bath. After draining, the firmness was measured promptly before any appreciable change in temperature could occur. The commodity was then immersed in a water bath held at 15 ± 1°C until temperature equilibrium was reached and the firmness measured again. This procedure was repeated using 30 ± 1°C and 45 ± 1°C water baths. A temperature of 45°C was the highest temperature used because preliminary experiments indicated that some thermal damage to the tissue may have occurred at 60°C.

Several kinds of firmness measurements were performed. Shear Press measurements were performed in a Food Technology Corporation Texture Test System using the standard shear-compression cell. All other tests were performed in the Instron (Model TTCM) by mounting the appropriate fixture in the working space (Bourne et al., 1966). Puncture tests were performed at a crosshead speed of 20 cm/min (Bourne, 1965). Back extrusion tests were performed at 30 cm/min in a cell 10.2 cm ID x 12 cm high with an annulus width of 4 mm and a clearance of 6 mm between the base of the extrusion cell and the compression plunger (Bourne and Moyer, 1968). Potato firmness was measured under a 7.7 mm diameter plunger at a speed of 0.2 cm/min with the potato embedded in packed sand (Bourne and Mondy, 1967). Deformation tests on other commodities were performed at a speed of 0.5–2 cm/min using a 14.8 cm diam compression plate. In all deformation tests the deformation was measured between 5% and 105% of the stated deformation force in order to eliminate errors that arise from the initial uneven contact of the compression plate with the commodity (Bourne, 1967). Each puncture and deformation test was replicated a minimum of 25 times. Back extrusion and Shear Press tests were replicated five times. The mean values were used for calculating the firmness-temperature coefficient.

The firmness-temperature (FT) coefficient is defined as the percent change in firmness per degree temperature increase over the temperature range studied and it is obtained from the formula

$$\frac{\text{firmness at } T_2 - \text{firmness at } T_1}{\text{firmness at } T_1 \cdot (T_2 - T_1)} \cdot 100\% \text{ per degree}$$

where T_1 = lowest temperature and T_2 = highest temperature at which the firmness was measured. For most of the work reported here T_1 was 0°C and T_2 was 45°C.

RESULTS & DISCUSSION

THE RESULTS are summarized in Table 2. Most of the commodities have a negative temperature coefficient for puncture test and compression-extrusion tests indicating that the firmness decreases as the temperature increases. The numerical value of these firmness-temperature coefficients lie in approximately the same range as the literature values listed in Table 1.

Deformation should be considered as a softness measurement rather than a firmness measurement because, from the nature of the test, a higher figure indicates decreased firmness. For this reason, the positive sign on the FT coefficients in Table 2 for the deformation tests indicate that the deformation increases with increasing temperature i.e. firmness decreases as temperature increases.

With a few exceptions, which are noted below, the firmness decreases with increasing temperature for all commodities and for every type of firmness measurement. There is a wide range of firmness-temperature sensitivity from commodity to commodity. The stored Golden Delicious apples

tested with the Shear Press and the SHA-47 breeding line of apricots had a FT coefficient of zero while the cherry, the 30–40–126 breeding line of plum, and the Shear Press test on three cultivars of sweet corn had coefficients numerically greater than 1% per degree. Most of the data shown in Table 2 have a FT coefficient between 0.1% and 1% per degree.

One would expect to find the wide variations from one commodity to another in the FT coefficient shown in Table 2, but it is interesting to see that the coefficient can vary widely within the same commodity. The FT coefficient ranges from 0 to −0.73 for apples and from −0.07 to −0.62 for green peas. For three cultivars of sweet corn, the coefficient measured by the Shear Press is higher than the coefficient determined by back extrusion, while for most apple cultivars tested the Shear Press coefficient is lower than for the back extrusion coefficient.

The New Yorker tomato cultivar has a squat shape with a smooth contour across the blossom end that lends itself to deformation with the stem-blossom axis in the vertical position. Nova cultivar tomato fruits usually have a pronounced nipple-like protuberance at the blossom end which renders it unsuitable for testing in a vertical position. Therefore, this cultivar was tested with the stem-blossom axis in a horizontal position in order to avoid the high reading that would have been obtained by compressing the small diameter nipple at the blossom end. Previous work has shown that the deformability of tomatoes is usually a little higher when tested in the horizontal position than in the vertical position.

Snap beans, carrots, cucumbers, onions, clingstone peaches, and potatoes are exceptional in that they sometimes show a small positive FT coefficient indicating that they become firmer with increasing temperature. The reason for this anomalous behavior is not known.

Plots of some of the FT data are shown in Fig. 1. The deformation test (curves no. 1, 2) have a positive slope indicating the increase in deformation as the commodity warms and becomes less firm. The other firmness measurements have a negative slope indicating decreasing firmness with increasing temperature. All the curves in Fig. 1 except no. 5 are essentially linear which justifies the assumption of

Table 1—Effect of temperature on firmness of raw fruits and vegetables—Literature values

Commodity	Type of measurement	Temp range °C	Change per 1°C increases	Reference
Apple, Idared	puncture 7/16" diam tip	2–21	force −0.42%	Blanpied et al. (1978)
Apple, R.I. Greening	puncture 7/16" diam tip	2–21	force −0.39%	Blanpied et al. (1978)
Apple, Rome	puncture 7/16" diam tip	2–21	force −0.61%	Blanpied et al. (1978)
Apple, Baldwin	puncture 1/4" diam tip	2–33	rupture stress −0.44% rupture strain +0.58% rupture energy −0.27%	Fletcher (1975)
Blackberry, Erie	puncture 0.060 mm diam tip	13–26	force −0.92%	Hawkins and Sando (1920)
Blackberry, Erie	puncture 0.060 mm diam tip	13–27	force −0.83%	Hawkins and Sando (1920)
Blackberry, Lawton	puncture 0.060 mm diam tip	13–28	force −1.07%	Hawkins and Sando (1920)
Blackberry, Lawton	puncture 0.060 mm diam tip	13–25	force −1.52%	Hawkins and Sando (1920)
Blackberry, Wachuset	puncture 0.060 mm diam tip	16–26	force −2.30%	Hawkins and Sando (1920)
Blackberry, unknown variety	puncture 0.060 mm diam tip	13–26	force −2.01%	Hawkins and Sando (1920)
Blackberry, unknown variety	puncture 0.060 mm diam tip	12–27	force − .90%	Hawkins and Sando (1920)
Cherry, Napoleon	puncture 2 mm diam tip	0–32.2	force −0.93%	Hartman and Bullis (1929)
Cherry, Montmerency	puncture 0.068 mm diam tip	16–27	force −1.95%	Hawkins and Sando (1920)
Cherry, Montmerency	puncture 0.068 mm diam tip	13–29	force −1.12%	Hawkins and Sando (1920)
Pears, Bartlett	puncture 1/2" diam tip	0.6–36.1	force −0.38%	Hartman (1924)
Peas, Admiral size 2	FMC Tenderometer	2–49	−0.30 Tenderometer units	Martin et al. (1938)
Peas, Admiral size 4	FMC Tenderometer	2–49	−0.31 Tenderometer units	Martin et al. (1938)
Peas, Alaska size 2	FMC Tenderometer	2–49	−0.46 Tenderometer units	Martin et al. (1938)
Peas, Alaska size 4	FMC Tenderometer	2–49	−0.48 Tenderometer units	Martin et al. (1938)
Peas, Tall Alderman	FMC Tenderometer	2–45	−0.48 Tenderometer units, early season	Campbell (1942)
Peas, Tall Alderman	FMC Tenderometer	2–45	−0.40 Tenderometer units, late season	Campbell (1942)
Peas, 4 varieties	FMC Tenderometer	not stated	−0.5 Tenderometer units	Makower et al. (1953)
Peas, test 1	Ottawa Pea Tenderometer	18–32	−0.27 kg force	Voisey and Nonnecke (1972)
Peas, test 2	Ottawa Pea Tenderometer	18–32	−0.16 kg force	Voisey and Nonnecke (1972)
Raspberry, black	puncture 0.121 mm diam tip	14–25	force −2.60%	Hawkins and Sando (1920)
Raspberry, red	puncture 0.313 mm diam tip	16–27	force −2.20%	Hawkins and Sando (1920)
Raspberry, red	puncture 0.313 mm diam tip	16–28	force −2.16%	Hawkins and Sando (1920)
Strawberry, Cooney	puncture 0.636 mm diam tip	16–29	force −1.08%	Hawkins and Sando (1920)
Strawberry, Cooney	puncture 0.636 mm diam tip	13–24	force −2.54%	Hawkins and Sando (1920)
Strawberry, Cooney	puncture 0.636 mm diam tip	16–26	force −3.21%	Hawkins and Sando (1920)
Strawberry, Cooney	puncture 0.636 mm diam tip	16–23	force −3.14%	Hawkins and Sando (1920)
Strawberry, No. 29-5	puncture 0.636 mm diam tip	16–29	force −2.92%	Hawkins and Sando (1920)
Strawberry, unnamed variety	puncture 0.636 mm diam tip	13–23	force −2.53%	Hawkins and Sando (1920)
Strawberry, unnamed variety	puncture 0.636 mm diam tip	16–30	force −1.93%	Hawkins and Sando (1920)
Strawberry, Fletcher	puncture, Dunkley pitter	2–43.5	force −1.06%	Ourecky and Bourne (1968)
Strawberry, Fortune	puncture, Dunkley pitter	2–43.5	force −1.00%	Ourecky and Bourne (1968)
Strawberry, Frontenac	puncture, Dunkley pitter	2–43.5	force −1.19%	Ourecky and Bourne (1968)
Strawberry, NY 844	puncture, Dunkley pitter	2–43.5	force −0.67%	Ourecky and Bourne (1968)
Strawberry 1928	puncture 1/4" diam tip	0–21.1	force −1.36%	Rose et al. (1934)
Strawberry 1933	deformation[a]	0.21.1	force −0.29%	Rose et al. (1934)
Strawberry 1934	deformation[a]	0.21.1	force −0.32%	Rose et al. (1934)

[a] This test measured the force in grams to give a combined deformation of 7/8" in the fruit and spring scale.

Table 2—Effect of temperature on firmness of raw fruits and vegetables—This study

Commodity	Description	Type of measurement	Firmness-temp coeffi. (% change in firmness per 1°C increase
apple	Golden Delicious, 1972 crop, stored 9 months	back extrusion	−0.55
apple	Golden Delicious, 1972 crop, stored 9 months	Shear Press	−0.43
apple	Golden Delicious, 1972 crop, stored 9 months	puncture, 1/8" tip	−0.73
apple	Golden Delicious, 1977 crop, stored 7 months	Magness Taylor 7/16" tip	−0.45
apple	Golden Delicious, 1977 crop, stored 7 months	back extrusion	−0.19
apple	Golden Delicious, 1977 crop, stored 7 months	Shear Press	−0.04
apple	Idared, stored 7 months	Magness Taylor 7/16" tip	−0.32
apple	Idared, stored 7 months	Magness Taylor 7/16" tip yield point	−0.42
apple	Idared, stored 7 months	back extrusion	−0.90
apple	Idared, stored 7 months	Shear Press	−0.57
apple	Red Delicious, stored 1 week	back extrusion	−0.23
apple	Red Delicious, stored 1 week	Shear Press	−0.33
apple	Red Delicious, stored 7 months	Magness Taylor 7/16" tip	−0.20
apple	Red Delicious, stored 7 months	Magness Taylor 7/16" tip yield piont	−0.30
apple	Red Delicous, stored 7 months	back extrusion	−0.48
apple	Red Delicious, stored 7 months	Shear Press	−0.16
apple	McIntosh, stored 2 weeks	Magness Taylor 7/16" tip	−0.39
apple	McIntosh, stored 2 weeks	Magness Taylor 7/16" tip yield point	−0.42
apple	McIntosh, stored 2 weeks	back extrusion	−0.58
apple	McIntosh, stored 2 weeks	Shear Press	−0.61
apple	Rome, stored 1 week	Magness Taylor 7/16" tip	−0.08
apple	Rome, stored 1 week	Magness Taylor 7/16" tip yield point	−0.12
apple	Rome, stored 1 week	back extrusion	−0.22
apple	Rome, stored 1 week	Shear Press	−0.07
apricot	Morepark, firm ripe 0–30°C	Magness Taylor 5/16" tip	−0.66
apricot	Morepark, firm ripe 30–45°C	Magness Taylor 5/16" tip	−1.65
apricot	Morepark, soft ripe	Magness Taylor 5/16" tip	−0.72
apricot	SHA-47	deformation to 0.3 Newton	+0.15*
apricot	Vineland 60081	deformation to 0.3 Newton	−0.29*
apricot	Vineland 60081	back extrusion	−0.44
beans, snap	Early Wax, sieve size 3	puncture, 1/8" tip	−0.09
beans, snap	Early Wax, sieve size 4	puncture, 1/8" tip	−0.10
beans, snap	Early Wax, sieve size 5	puncture, 1/8" tip	−0.18
beans, snap	Slim Green, sieve size 4	puncture, 1/8" tip	+0.11
beans, snap	Slim Green, sieve size 5	puncture, 1/8" tip	+0.06
beets	Detroit Dark Red 2½–3" diam	deformation to 1 Newton	+0.28*
beets	Detroit Dark Red 2½–3" diam	puncture, 1/8" tip	−0.09
carrot	Chantenary, phloem tissue	puncture, 3/32" tip	+0.12
corn, sweet	NK199 10–35°C	Shear Press	−1.34
corn, sweet	NK199 10–35°C	back extrusion	−0.44
corn, sweet	Jubilee 10–35°C	Shear Press	−1.31
corn, sweet	Jubilee 10–35°C	back extrusion	−0.82
corn, sweet	Deep Gold 10–35°C	Shear Press	−1.08
corn, sweet	Deep Gold 10–35°C	back extrusion	−0.35
cucumber	Marketor	deformation to 0.5 Newton	−0.27*
cucumber	Marketor 0–30°C	puncture, 1/8" tip	+0.04
cucumber	Marketor 30–45°C	puncture, 1/8" tip	−0.65
cherry	Emperor Francis	Dunkley pitter	−1.11
onion	Autumn Keeper 1¾"–2" diam	deformation to 4 Newtons	−0.58*
onion	Autumn Keeper 1¾"–2" diam	puncture, Magness Taylor 5/16" tip	−0.18
peach	Baby Gold (clingstone) 9/5/80	deformation to 1 Newton	−0.97*
peach	Baby Gold (clingstone) 9/8/80	deformation to 1 Newton	−0.22*
peach	Hale Haven (freestone)	deformation to 1 Newton	+3.37*
peach	Sun Cling (clingstone)	Magness Taylor 7/16" tip	−0.74
peach	Bisco (freestone)	Magness Taylor 7/16" tip	−0.75
peas, green	Early Sweet 11, sieve size 3, AIS 11.6%	Shear Press	−0.32
peas, green	Early Sweet 11, sieve size 3, AIS 11.6%	Maturometer	−0.52
peas, green	Early Sweet 11, sieve size 3, AIS 11.6%	back extrusion	−0.62
peas, green	Early Sweet 11, sieve size 4, AIS 16.0%	Shear Press	−0.35
peas, green	Early Sweet 11, sieve size 4, AIS 16.0%	Maturometer	−0.30
peas, green	Early Sweet 11, sieve size 4, AIS 16.0%	back extrusion	−0.12

continued

Table 2—continued

Commodity	Description	Type of measurement	Firmness-temp coeffi. (% change in firmness per 1°C inc.)
peas, green	Early Sweet 11, sieve size 5, AIS 18.3%	Shear Press	−0.22
peas, green	Early Sweet 11, sieve size 5, AIS 18.3%	Maturometer	−0.27
peas, green	Early Sweet 11, sieve size 5, AIS 18.3%	back extrusion	−0.20
peas, green	Target, sieve size 3, AIS 11.0%	Shear Press	−0.37
peas, green	Target, sieve size 3, AIS 11.0%	Maturometer	−0.28
peas, green	Target, sieve size 3, AIS 11.0%	back extrusion	−0.26
peas, green	Target, sieve size 4, AIS 16.9%	Shear Press	−0.16
peas, green	Target, sieve size 4, AIS 16.9%	Maturometer	−0.15
peas, green	Target, sieve size 4, AIS 16.9%	back extrusion	−0.07
plum	Stanley, firm ripe	puncture, 1/8" tip	−0.66
plums	Stanley, firm ripe	back extrusion	−0.75
plum	Stanley, firm ripe	deformation to 1 Newton	+0.42*
plus	Severn Cross	deformation to 1 Newton	+0.11*
plum	Italian prune	deformation to 1 Newton	+0.61*
plum	30-4-126	deformation to 1 Newton	+0.12*
pear	Bartlett	Magness Taylor 5'16" tip	−0.47
pear	Bartlett	deformation to 1.5 Newton	+0.12*
potato	Katahdin, stored 1 month	Magness Taylor 5/16" tip	−0.02
potato	Katahdin, stored 7 months	Magness Taylor 5/16" tip	+0.06
potato	Katahdin, stored 1 month	deformation to 0.25 Newton	+0.28*
potato	Katahdin, stored 7 months	deformation to 0.25 Newton	+0.12*
potato	Monona, stored 1 month	Magness Taylor 5/16" tip	−0.10
potato	Monona, stored 7 months	Magness Taylor 5/16" tip	+0.06
potato	Monona, stored 1 month	deformation to 0.25 Newton	+0.28*
potato	Monona, stored 7 months	deforamtion to 0.25 Newton	+0.35*
potato	Russet Burbank, stored 1 month	Magness Taylor 5/16" tip	+0.06
potato	Russet Burbank, stored 7 months	Magness Taylor 5/16" tip	+0.04
potato	Russet Burbank, stored 1 month	deformation to 0.25 Newton	+0.14*
potato	Russet Brubank, stored 7 months	deformation to 0.25 Newton	+0.09*
tomato	New Yorker, stem end down, 1973	deformation to 1 Newton	+0.87*
tomato	New Yorker, stem end down, 1978	deformation to 1 Newton	+0.20*
tomato	Nova (plum type), sideways, 1973	deformation to 1 Newton	+0.58*
tomato	Nova (plum type), sideways, 1978	deformation to 1 Newton	+0.17*
strawberry	Honeoye, mean size 6.0 g 0–30°C	deformation to 1 Newton	+0.46*
strawberry	Honeoye, mean size 6.0 g 30–45°C	deformation to 1 Newton	+3.09*
strawberry	Canoga, mean size 6.1 g 0–30°C	deformation to 1 Newton	0 *
strawberry	Canoga, mean size 6.1 g 30–45°C	deformation to 1 Newton	+3.48*
strawberry	Canoga, mean size 12.9 g 0–30°C	deformation to 1 Newton	+0.09*
strawberry	Canoga, mean size 12.9 g 30–45°C	deformation to 1 Newton	+7.72*

*Since deformation is a softness measurement a + sign indicates the product decreases in firmness as the temperature rises.

linearity in the formula that is used to calculate the FT coefficient. The puncture test on the apricot (curve no. 5) has a steep slope indicating a high sensitivity to temperature change; it also has a definite change of slope at 30°C. This deviation from approximate linearity was infrequent. In such cases we calculated two FT coefficients, (1) from 0–30°C and (2) from 30–45°C.

SUMMARY

WITH A FEW EXCEPTIONS which showed a slight firming effect, most fruits and vegetables tested showed decreasing firmness with increasing temperature over the range 0–45°C. The FT relationship was approximately linear for most commodities. The FT coefficient is highly variable. It varies from commodity to commodity, from cultivar to cultivar within the same commodity, from test principle to another on the same commodity, on the same commodity during storage and from year to year. A survey of the data in Table 2 shows that one cannot predict in advance whether a particular commodity will have a low, medium or high FT coefficient. A small change in temperature is unlikely to be detected in firmness readings of most horizontal crops because the FT coefficient is small and the variation from unit to unit in horticultural crops is great. However, a large change in temperature would be sufficient to cause detectable differences in firmness. Other things being equal, the higher the FT coefficient, the lower the temperature change needed to detect temperature effects. For a product with a FT coefficient of 0.3% per degree a change of 10°C in temperature would change the firmness measurement by 3%, an amount that probably would not be detected because of the high coefficient of variation that is usually encountered in firmness measurements of horticultural crops. A change of 35°C in a product with a FT coefficient of 0.3% per degree would cause a change in firmness of 10.5%, an amount that would probably be easily detected. A product with a FT coefficient of 1.0% per degree would show a change in firmness of 10% with a 10°C temperature change, an amount that probably would be detected.

For those commodities that are graded on a firmness measurement, it is possible to change the apparent grade by adjusting the temperature by a sufficient amount in the right direction. The temperature change need not be large if the commodity is close to the boundary between grades. Researchers and marketers of the products should be aware of the possibility of temperature changes causing apparent

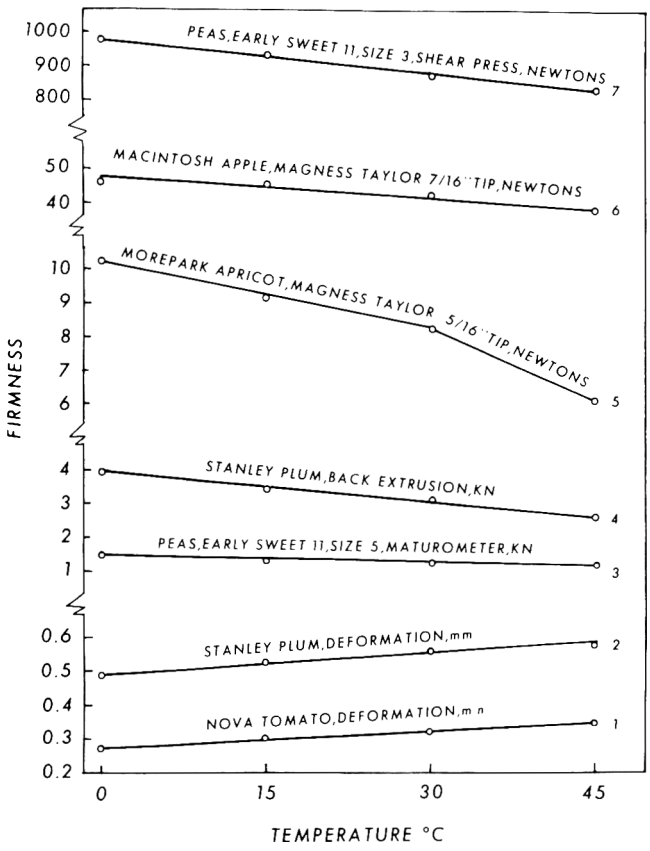


Fig. 1—Firmness of some horticultural crops as a function of the temperature at which the firmness measurement was made. The units in which each firmness measurement was made are shown alongside the commodity description.

significant differences in firmness readings on horticultural crops and should endeavor to make firmness measurements within a narrow temperature range unless preliminary studies have shown that the FT coefficient for that commodity is small.

REFERENCES

Blanpied, G.D., Bramlage, W.J., Dewey, D.H., LaBelle, R.L., Massey, L.M. Jr., Mattus, G.E., Stiles, W.C., and Watada, H.E. 1978. A standardized method for collecting apple pressure test data. New York's Food & Life Sciences Bull. 74.

Bourne, M.C. 1967. Deformation testing of foods. 1. A precise technique for performing the deformation test. J. Food Sci. 32: 601.

Bourne, M.C. 1965. Studies on punch testing of apples. Food Technol. 19: 113.

Bourne, M.C. 1976. Texture of fruits and vevetables. In "Rheology and Texture in Food Quality," Ed. J.M. deMan, P.W. Voisey, V.F. Rasper, and D.W. Stanley, p. 275. Avi Pub. Co., Westport, CT.

Bourne, M.C. and Mondy, N. 1967. Measruement of whole potato firmness with a universal testing machine. Food Technol. 21: 1387.

Bourne, M.C. and Moyer, J.C. 1968. The extrusion principle in texture measurement of fresh peas. Food Technol. 22: 1013.

Bourne, M.C., Moyer, J.C., and Hand, D.B. 1966. Measurement of food texture by a Universal Testing Machine. Food Technol. 20: 170.

Campbell, H. 1942. Temperature and Tenderometer. Western Canner & Packer. 34(Feb.): 39; 34 (May): 45.

Campbell, H. and Diehl, H.C. 1940. Quality in frozen pack peas. Western Canner and Packer. 32(10): 48 (Sept.); 32:(11):51 (Oct.).

Fletcher, S.W. 1975. Mechanical damage to the processed fruits and vegetables. In "Theory, determination and Control of Physical Properties of Food Materials," Ed. C. Rha, p. 163. D. Reidel Pub. Co., Dordrecht, Holland.

Hartman, H. 1924. Studies relating to the harvesting and storage of apples and pears. Oregon Agr. Exp. Sta. Bull. 206.

Hartman, H. and Bullis, D.E. 1929. Investigations relating to the handling of sweet cherries. Oregon Agr. Exp. Sta. Bull. 247.

Hawkins, L.A. and Sando, C.E. 1920. Effect of temperature on the resistance to wounding of certain small fruits and cherries. USDA Bull. 830.

Makower, R.U., Boggs, M.M., Burr, H.K., and Olcott, H.S. 1953. Comparison of methods of measuring the maturity factor in frozen peas. Food Technol. 7: 43.

Martin, W. McK., Lueck, R.H., and Sallee, E.D. 1938. Practical application of the Tenderometer in grading peas. Canning Age 19(3): 146; (4): 193.

Ourecky, D.K. and Bourne, M.C. 1968. Measurement of strawberry texture with an Instron machine. Proc. AM. Soc. Hort. Sci. 93: 317.

Rose, D.H., Haller, M.H., and Harding, P.L. 1934. Relation of temperature of fruit to firmness in strawberries. Proc. Am. Soc. Hort Sci. 32: 429.

Voisey, P.W. and Nonnecke, I.L. 1972. Measurement of pea tenderness. 4. Development and evaluation of the test cell. J. Texture Stud. 3: 459.

Ms received 7/17/81; revised 10/26/81; accepted 10/31/81.

CHEMICAL ASSAY FOR FUNGAL CHITIN . . . From page 439

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.

Donald, W.W. and Mirocha, C.J. 1977. Chitin as a measure of fungal growth in stored corn and soybean seed. Cereal Chem. 54: 466.

Jarvis, B. 1977. A chemical method for the estimation of mould in tomato products. J. Fd. Technol. 12: 581.

Ride, J.P. and Drysdale, R.B. 1971. A chemical method for estimating Fusarium oxysporum f. lycopersici in infected tomato plants. Physiol. Pl. Path. 1: 409.

Ride, J.P. and Drysdale, R.B. 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiol. Pl. Path. 2: 7.

Tsuji, A., Kinoshita, T., and Hoshino, M. 1969. Analytical chemical studies on amino sugars. 2. Determination of hexosamines using 2-methyl-2-benzothiazolone hydrazone hydrochloride. Chem. Pharm. Bull. 17: 1505.

Ms received 6/22/81; revised 9/16/81; accepted 9/18/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 7—10, 1981.

The authors thank Frances P. Dever for her technical assistance.

Dehydrated Blueberries by the Continuous Explosion-Puffing Process

J. F. SULLIVAN, J. C. CRAIG Jr., E. D. DEKAZOS, S. M. LEIBY, and R. P. KONSTANCE

ABSTRACT

A blueberry dehydration process which includes the unique continuous explosion-puffing system (CEPS) is described. A drying study including alternate drying pretreatments failed to increase the dehydration rate. It was found that care during processing was necessary to prevent rupture of the berries as rupture caused bleeding during drying which reduced the drying rate. Optimum operating conditions for CEPS were established for blueberries. Measurements of dried blueberry properties such as bulk density, color, rehydration, and disintegration were used to determine optimum operating conditions for pressure (103 kPa), temperature (190°C), and feed moisture (18.5%) for CEPS.

INTRODUCTION

THE FIRST HIGHBUSH cultivar was released in 1920 (Draper, 1979); since then blueberries have become a major fruit crop in the United States. Blueberries are high in iron and manganese (Hope, 1965) and their vitamin content equals or exceeds that of most fruits (USDA Handbook #8, 1963). Qualities such as disease resistance, plant vigor, high production, and suitability to machine harvest have encouraged growers to process blueberries.

The technology of blueberry processing is limited. Most of the crop is freeze-packed. Excepting a minuscule portion which is dried. In an early drying study, berries took 13 hr to dry (Friar and Mrak, 1943). Eidt apparently reduced the drying time to 4 hr by using a hot lye dip (Eidt et al., 1944). Batch explosion-puffing of high bush blueberries created rapid drying and quick rehydrating blueberries (Eisenhardt et al., 1964; 1967). Because batch processing was costly and labor intense, the continuous explosion-puffing system (CEPS) was developed (Heiland et al., 1977). Potatoes, carrots, and apples have been successfully processed in CEPS (Sullivan et al., 1977, 1980, 1981).

The blueberry crop of 1980 was about 45,000 metric tons, of which 1/3 was frozen for later use in pies, tarts, or other baked goods and desserts. Frozen storage life is limited to 6 months or less because of a textural problem, that of woodiness or grittiness which has plagued growers and processors. One of the authors (Dr. Dekazos) testing explosion-puffed dried blueberries, found that this problem did not develop when these berries were put in storage (Dekazos, 1980). Other favorable characteristics of the explosion-puffed product are excellent flavor and color, fast rehydration, ambient temperature storage, minimal storage and transportation costs, and durability.

The purpose of our experiments was to adapt the explosion-puffing process to the "Rabbiteye" blueberry species, particularly the Tifblue variety, and to optimize the continuous explosion-puffing system (CEPS) for blueberry processing.

Authors Sullivan, Craig, and Konstance are with the USDA Eastern Regional Research Center, Agricultural Research Service, 600 E. Mermaid Lane, Philadelphia, PA 19118. Author Leiby is a Student Co-op from Drexel University, Philadelphia, PA. Author Dekazos is with the USDA-SEA-AR Richard B. Russell Research Center, Athens, GA 30604.

The Rabbiteye blueberry is a species native to the Southeast and is high in flavor and palatability. The crop has not been firmly established for freezing or canning. Breeding experts believe that the Rabbiteye offers the greatest potential for future expansion of the blueberry industry (Eck and Childers, 1967). Explosion-puffing was applied to Rabbiteye blueberries to enhance the economic value of the crop by adding another and better processing possibility. The Tifblue variety, which we studied, was introduced in 1955 and is a medium large berry with high dessert quality.

EXPERIMENTAL

TIFBLUE, Rabbiteye blueberries (a commercial variety) grown in Georgia (1978 crop, moisture 82.4% (wet basis)) were used in all experiments. The blueberries were washed and for more uniform drying were size separated with a pneumatic winnower. No other preprocessing was required. In addition, unlike most fruit processing, sulfiting was not needed, as nonenzymatic browning remained minimal during dehydration. The raw and processed berries were evaluated for moisture content, bulk density, color difference, and selected minerals.

Drying studies

An effort was made to increase the drying rate of blueberries. Experiments were made in which the blueberries received the following predrying treatments: (1) two separate 0.2% lye dips at 93.3°C were made, one at 4 and one at 8 sec, respectively; (2) a mild detergent wash at 24°C; (3) a steam blanch; and (4) no treatment. After the pretreatments, each test lot was dried in the continuous belt dryer (Anon., 1963). The drying conditions for these experiments were: air velocity 2.4 m/s; direction of air flow–downward through the bed; dry bulb temperature 82°C; wet bulb temperature 33°C; belt speed 1.44 m/hr; bed depth entering dryer 5 cm; and a feed rate of 33 kg/hr to a 0.6 m-wide dryer belt.

In the study, the rate of steam required to dry blueberries from about 85% to 19% moisture in a continuous dryer was determined and these dried berries were later used for the optimization study. For the drying study, the steam condensate from the air heating coils was passed through a chilled exchanger and weighed. The drying conditions were the same with one exception; air velocity was increased from 2.4 to 3.0 m/sec. Five hundred twenty-five kg of blueberries were dried in three batches of about 175 kg each.

Optimization of the continuous explosion-puffing system (CEPS)

Optimization of CEPS was achieved by determining the effect of feed moisture, internal pressure, and temperature on the puffing of partially dried berries, reflected in the final product. These blueberries were partially dried to four experimental moistures, approximately 120 pounds at each moisture (Table 1). An experimental design of 36 conditions, including four moisture levels (12.5–24.0%), three pressure levels (103–172 kPa), and three temperature levels

Table 1–Experimental conditions for optimization tests

Moisture (%)	Pressure (kPa)	Temp (°C)
12.4	103	149.0
17.5	138	165.5
21.2	172	188.0
23.7		

(145–192°C), were used (Table 1). The design was duplicated. The equipment used was described by Heiland et al., (1977).

The partially dried blueberries were fed to CEPS at the rate of 125 kg/hr for each test. After being explosion-puffed, the berries were returned to a hot air tray dryer and dried to 4-5% moisture. Because of the limited amounts of blueberries, the drying study was not continued through final drying. The physical attributes evaluated were: bulk density, rehydration, disintegration, and color. These properties were chosen because they reflect the quality of the explosion-puffed blueberry. Fast rehydration or water pickup is a desired characteristic. The greater the puff, the more porous the product, and the more readily the water is picked up as measured by rehydration. Bulk density is decreased because of explosion-puffing. An inadequate puff is indicated by a bulk density near that of the partially dried berries. To the other extreme, overpuffing is undesirable because it results in excessive disintegration. Minimal disintegration is desired. Color values relate to eye appeal and flavor. Hunter scales (Hunter, 1942) were used for color determinations.

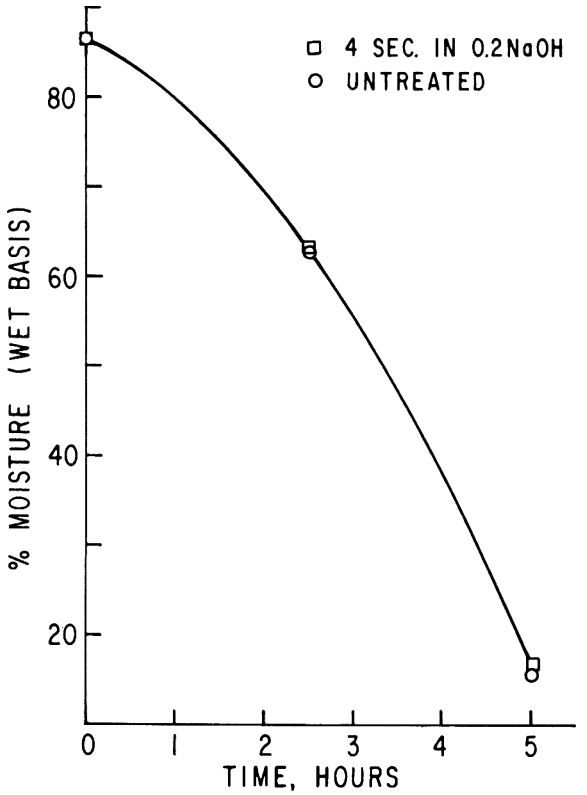


Fig. 1—Drying curves of treated vs untreated blueberries.

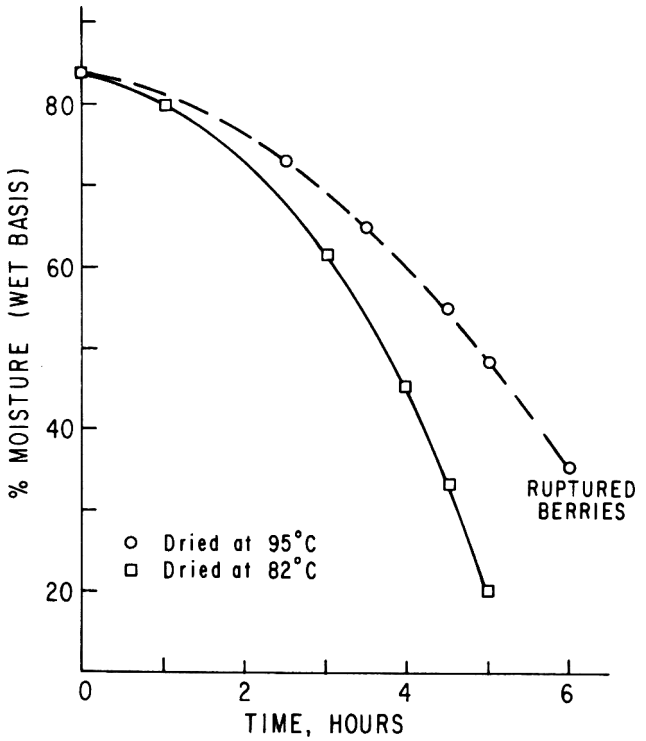


Fig. 2—Drying curves of ruptured and unruptured blueberries.

Analytical procedures

Color was determined on reconstituted blueberries at room temperature. Moisture content, bulk density, rehydration, and disintegration, were determined on the dehydrated blueberries.

Moisture

Moisture content was determined by the standard vacuum oven method. All samples were dried at 70°C under vacuum for 16 hr. Results are expressed on a wet basis.

Color measurement

A Gardner Automatic Color Difference Meter was used for all color measurements (Hunter, 1942). However, the color of the intact reconstituted berries (fresh and processed) was not measured directly because they were too dark. To insure consistency of results for comparative purposes, a calculated volume of water was added to a weighed amount of dehydrated berries so that the final moisture content of the rehydrated berries was always 87.5% (wet basis). Reconstitution was carried out by boiling the berries for 4 min, after which the liquid was filtered through cheesecloth and the colored water was collected for color measurement. To improve reflectance of the colored solution, three layers of Wahtman #1 filter paper were placed in the bottom of the cells before the colored solution was added. Once added, the solution and filter discs were allowed to soak for 10 min before measurements were made of the color of the filter discs and color solution together. Small beakers (50 ml) were used as weights to prevent the filter discs from floating. Color values for each sample were obtained in this manner and are reported as Hunter ΔE, a, b, and R_d units. The instrument was standardized each time with a standard color tile #CDR 001B (R_d = 5.9; a = +21.7; b = +6.0).

Disintegration

Disintegration was determined by manually separating disintegrated pieces in 100g sample and weighing this portion.

Rehydration

A 25g sample was boiled in water 3 min, then drained and weighed. The amount of water picked up per gram of dry solids was determined by subtracting the original sample weight (25g) from the weight of the rehydrated sample and dividing this weight by the solids in the 25g sample.

Bulk density

Bulk density was determined by filling a tared crystallizing dish of known volume with dried blueberries then weighing the blueberries and calculating the weight per volume.

RESULTS & DISCUSSION

Drying study

The initial drying of blueberries was from 85% to about 20% moisture (wet basis). Fig. 1 shows one drying curve—for fresh untreated blueberries and berries dipped in a 0.2% NaOH solution at 93°C for 4 sec. The curves are coincidental and the drying rates are the same. The other experiments, which included the mild detergent wash (to remove natural occurring wax) and the steam blanched, dried at the same rate as the untreated berries.

Temperature had a profound effect because the heat and elevated internal pressure within the berries led to easy rupture as they dried. Ruptured berries tended to bleed, which slowed drying (Fig. 2). Seasonal and area variations

yielded some heat sensitive berries that had to be dried at a lower processing temperature to avoid rupture. During the first drying run, temperature had to be reduced gradually from 93°C (a normal drying temperature) to 82°C where the incidence of rupture was reduced and the drying rate increased. Five hundred twenty-five kg of berries were dried at 82°C to a moisture of about 30% (wet basis). At a bed loading of 36.5 kg/m² and increased air velocity (3 m/sec), 175 kg of berries required slightly over 3 hr to dry to 30%. Eight hundred pounds of steam per hr were used in drying 175 kg of berries from 82.4% to 30% moisture. After equilibration, the berries were sampled for moisture. Later they were further dried from 30% to selected moistures (Table 1) for the optimization of CEPS.

Optimization study

The results (responses) of these experiments were related to the three controlled variables by simple quadratic equations. The controlled or independent variables were pressure, temperature, and moisture. The responses chosen as dependent variables were bulk density, rehydration, percent disintegration and color measurements.

A second-order regression equation in terms of the independent variables was fitted to the data of each response. These empirical equations were used as models of the explosion-puffing process. Fig. 3 shows a typical response surface.

A computer program solving constrained nonlinear optimization problems was applied to these models. Table 2 lists the models and limits imposed on the optimal solution. The limits are maximum and minimum acceptable levels for a given response. These quality limits are chosen by the experimenter, external to the optimization. A line of optima was generated as a function of the model's radius ($\sqrt{P^2 + M^2 + T^2}$). Fig. 4 displays an optimal ridge for percent disintegration. It is evident that slight improvements in percent disintegration can be realized without other harm by extrapolating to higher feed moisture. An important consideration is that explosion-puffing at higher moistures allows the more difficult drying, that is drying in the falling rate period, to be done in the faster porous state (Eisenhardt et al., 1967; Sullivan et al., 1977, 1980). Table 3 lists the optimal (recommended) operating conditions at the highest moisture that could be selected with confidence.

Final drying

The bluberries were dried from about 15–26% moisture (there is about 3% moisture pickup during explosion-puffing) to 10% moisture (dry basis). Earlier studies (Eisenhardt et al., 1967) showed that blueberries need only be dried to 10% although explosion-puffed berries can readily be dehydrated to 4% moisture or lower. They were dried in a tray drier at 77°C. Moisture absorption isotherm developed from the dried blueberry product showed a_w = 0.45 at 10%.

Comparison of raw and processed blueberries

Nutrient losses and color changes occurred during processing. Differences from the fresh berries indicated the effects of dehydration. Some of the mineral values (Dekazos, 1978) are shown (Table 4) for raw highbush berries from North Carolina and Rabbiteye (Tifblue) from Georgia, both from the 1975 season. Also listed are values for processed Tifblue variety from the 1978 season (rehydrated to fresh basis). Although a comparison with the fresh berries cannot be made directly because of the different crop years, mineral losses appear to be between 10 and 20%. There is no other apparent reason why the Na value is high in the processed berries.

Color changes were slight (on a relative basis) when the R_d, a and b values for processed samples were compared with those of fresh berries. Table 5 shows variability between the fresh and processed berries at optimum conditions. Processing changes, with pressure decreasing in CEPS and the feed moisture increasing, cause the processed berry to become lighter in color, that is, going from a dark blue to a purplish hue. The optimum value (Table 3) includes color

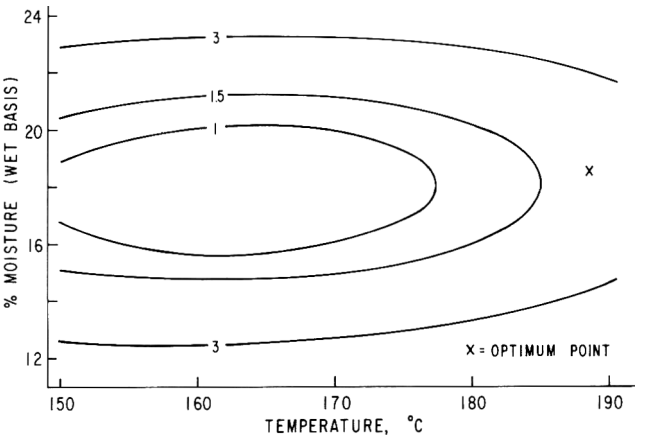


Fig. 3—Response surface of % disintegration at 103.4 kPa, % moisture vs temperature.

Table 2—Multiple correlations and coefficients of blueberry model for optimum study

Model form: $Y = b_0 + b_1 P + b_2 T + b_3 M + b_4 P^2 + b_5 T^2 + b_6 M^2 + b_7 PT + b_8 PM + b_9 TM$

Y	Bulk density	% Disintegration	Rehydration	ΔE
R^2	0.89	0.93	0.89	0.64
Upper limit	435 kg/m³	5.0	2.0g H_2O/g d solid	14.0
Lower limit	365 kg/m³	0.0	0.60 H_2O/g d solid	1.0
Intercept, b_0[a]	−711.759	115.613	7.8842	−124.210
b_1	−22.447	−4.243	0.01192	−6.07091
b_2	20.877	−0.469	−0.09770	2.7384
b_3	−27.650	−5.317	0.02123	−1.7062
b_4	0.2307	0.07473	0.000718	0.04386
b_5	−0.06658	0.001797	0.002881	−0.009358
b_6	0.44028	0.081638	−0.000165	0.01508
b_7	0.01996	−0.005510	0.0000273	0.01661
b_8	0.15474	0.18081	−0.000166	0.01883
b_9	0.02329	0.00193	0.0000661	0.002861

[a] Sufficient significant places are provided so that the reader can recalculate the response surface. No unexpected precision should be inferred.

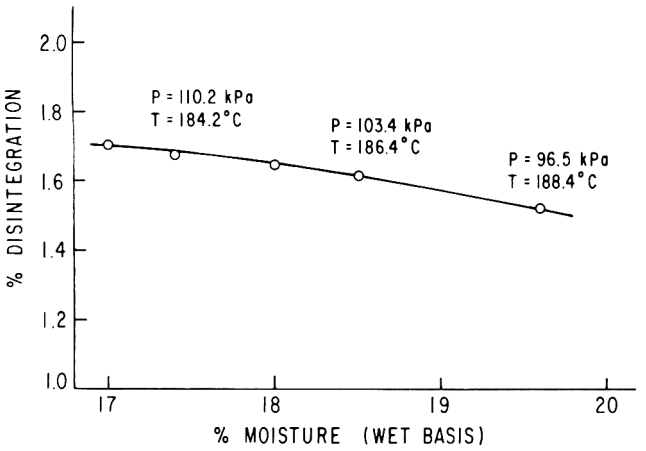


Fig. 4—Optimal ridge generated from model's radius, % disintegration vs % moisture.

Table 3—CEPS optimum point for Rabbiteye blueberries using dependent variables as constraints

Independent variables	Dependent variables	
Pressure 103 kPa	ρ (bulk density)	393 kg/m^3
Temp 190°C	% disintegration	1.80
Moisture 18.5%	Rehydration	0645g H_2O/g dry solids
	ΔE (color difference)	14

Table 4—Mineral levels in highbush and Rabbiteye blueberries (mg/100g fresh fruit)

Mineral	Raw[a] highbush (NC)	Raw[a] Rabbiteye (GA)	Dried explosion-puffed[b] Rabbiteye (GA)
Ca	5.20	5.60	5.15
P	10.70	7.95	6.81
K	84.00	84.60	67.78
Na	0.32	0.95	1.96
Fe	0.14	0.24	Trace

[a] Dekazos (1978); 1975 crops
[b] 1978 crop

Table 5—Color values of fresh and processed blueberries

Moisture	Raw 82.4% (wet basis)	Dried explosion-puffed moisture 5.75% (A_w 0.2)
ΔE	–	14
a	+28.50	+20.88
b	+ 0.09	– 7.86
R_d	3.30	10.96
b/a	+ 0.0032	– 0.376

ΔE — Color difference $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$
ΔL — is linearly related to R_d. By multiplying by a factor, R_d can be converted to L
a — Signifies redness if positive and greenness if negative.
b — Signifies yellowness if positive and blueness if negative.
R_d — Measure of the reflectance of the sample.

values (R_d, a and b) which have gone toward the purple, but these berries when reconstituted appear dark blue and when made into a pie or tart are indistinguishable in color from those in a fresh-berry pie.

CONCLUSIONS

VARIOUS PREDRYING treatments were tried but failed to affect the drying rate. Drying temperature had a pronounced effect on the drying. The drying temperature was reduced from 93° to 82°C which reduced bleeding and lessened the incidence of rupture. This indirectly increased the drying rate. CEPS was optimized for blueberries. The optimization indicated that blueberries should be dried to 18.5% moisture and processed with CEPS internal pressure and temperature at 103 kPa and 190°C. A final moisture of 10% is sufficient although lower moistures are readily obtainable.

REFERENCES

Anon. 1963. A basic new concept in pilot and research equipment. C.G. Sargent's Sons Corp, Graniteville, MA.
Dekazos, E.D. 1978. Essential mineral elements in and quality evaluation of Rabbiteye blueberry fruit. Proc. Fla. State Hort. Soc. 91: 164.
Dekazos, E.D. 1980. Private communication. Richard B. Russell Agricultural Research Center USDA, SEA, Athens, GA.
Draper, A.D. 1979. Highbush blueberry cultivars. Fruit Varieties J. 37(2).
Eck, P. and Childers, N.F. 1967. Blueberry culture, p. 11, 13, 110. Rutgers University Press, New Brunswick, NJ.
Eidt, C.C., MacArthur, M., and Hope, G.W. 1944. Dehydration of lowbush blueberries. Food in Canada 4: 22.
Eisenhardt, N.H., Eskew, R.K., and Cording, J., Jr. 1964. Explosive-puffing applied to apples and blueberries. Food Eng. 36(6): 53.
Eisenhardt, N.H., Eskew, R.K., Cording, J. Jr., Talley, F.B., and Huhtanen, C.N. 1967. Dehydrated explosion-puffed blueberries. U.S. Agr. Res. Serv., ARS 73-54.
Friar, H. and Mrak, E.M. 1943. Dehydration of huckleberries. The Fruit Pro. J. 22: 138.
Heiland, W.K., Sullivan, J.F., Konstance, R.P., Craig, J.C. Jr., Cording, J. Jr., and Aceto, N.C. 1977. A continuous explosion-puffing system. Food Technol. 31(11): 32.
Hope, G.W. 1965. A review of the suitability of the lowbush blueberry for processing. Food Technol. 19(2): 115.
Hunter, R.S. 1942. Photoelectric tristimulus coloremetry with three filters. Natl. Bur. Standards, Circ. C429. U.S. Government Printing Office, Washington, DC.
Sullivan, J.F., Konstance, R.P., Aceto, N.C., Heiland, W.K., and Craig, J.C., Jr. 1977. Continuous explosion-puffing of potatoes. J. Food Sci. 42: 1462.
Sullivan, J.F., Craig, J.C. Jr., Konstance, R.P., Egoville, M.J., and Aceto, N.C. 1980. Continuous explosion-puffing of apples. J. Food Sci. 45(6): 1550.
Sullivan, J.F., Konstance, R.P., DellaMonica, E.S., Heiland, W.K., and Craig, J.C. Jr. 1981. Carrot dehydration—Optimization process studies on the explosion-puffing process. J. Food Sci. 46(5): 1537.
USDA Handbook #8. 1963. ARS, U.S. Dept. of Agri., U.S. Government Printing Office, Washington, DC.
Ms received 4/20/81; revised 10/19/81; accepted 10/20/81.

The authors thank Ruth Campbell and Edward S. DellaMonica for their chemical analyses, and Kevin Kelly and Carlisle Ross for their technical assistance.

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Effect of Postharvest Holding Conditions on the Quality of Salt-Stock Pickles

J. P. LEE, M. A. UEBERSAX, and R. C. HERNER

ABSTRACT

Quality changes of green- and salt-stock cucumbers held at 5, 20, and 30°C up to 6 days prior to brining were evaluated. Cucumbers held at 5°C showed firm texture, low respiration rates, minimum weight loss, and good quality stock after fermentation. Increasing postharvest holding temperatures and times prior to brining resulted in loss of firmness, high respiration rates, increased weight loss, and a decrease in salt-stock quality. Salt-stock pickle defects increased dramatically under high temperature long time holding periods due to textural degradation and internal softening. Brining directly after harvest yielded the highest quality salt-stock. Cucumbers held 2 days at 20°C or 6 days at 5°C resulted in good salt-stock quality.

INTRODUCTION

THE ACREAGE OF CUCUMBERS grown for pickle manufacture is one of the largest of the national truck crops grown for processing. Michigan is the leading state in pickling cucumber production, with over 100,000 tons annually valued at over ten million dollars. At present approximately half of the total crop is utilized in the manufacture of fresh or uncured stock which is packed from the fresh state and is pasteurized in suitable spiced brines or light syrup for preservation. The remaining crop is brined at time of harvest, cured by fermentation, and stored until needed for further processing and manufacture into finished pickle products.

Cucumber pickles are not known for nutritional characteristics, but rather are consumed for their desirable crisp texture and fine flavor. Due to the rapid increased consumption of pickles throughout the world, considerable research effort has been devoted to compositional, genetic, handling, and processing factors affecting pickle quality.

Damage due to impacts during harvesting and handling is a major problem encountered with fresh cucumbers and this may lead to increased bloating in salt-stock pickles (Marshall et al., 1972; Heldman et al., 1976). The most important of losses during holding and transport of cucumbers are weight loss and softening caused by unfavorable temperature and composition of the surrounding atmosphere. Fellers and Pflug (1967) recommended a holding temperature as low as 1.1°C at 5% O_2 and 5% CO_2 controlled atmosphere. However, general recommendations for holding and transit conditions of pickling cucumber varieties have commonly been 10 ± 2°C at a relative humidity of 80–85% or higher (Lutz and Hardenburg, 1977; Ryall and Lipton, 1979). Cucumbers have a moisture content of about 95% and are very susceptible to rapid weight loss accompanied by visible shriveling. Apeland (1961) and Etchells et al. (1973) found that high temperatures and low relative humidities had a significant effect on weight loss and severe shriveling of cucumbers during holding. Prolonged holding time as well as improper holding conditions were also shown to result in undesirable softening of cucumbers (Fellers and Pflug, 1967). Eaks and Morris (1956) and Hirose (1976) indicated that at nonchilling temperatures (10–30°C) the rate of CO_2 production decreased with duration of storage, whereas at chilling temperatures (0–10°C) the rate increased with time to a plateau (at 5–10 days) that was followed by a decline. In addition, lesser amounts of CO_2 were produced at chilling temperatures.

Bloater damage and softening in commercially brined cucumbers, particularly in the larger sizes, are sources of serious economic loss to the pickle industry. The primary cause for bloater damage has been shown to be due to the production of gases, primarily CO_2, in the fermentation brines (Etchells et al., 1968). Carbon dioxide in the brine originates from the cucumber tissue and from microbial activity in the brine (Etchells et al., 1968; Fleming et al., 1973a, b). Cucumber softening is considered to be the result of action by pectic enzymes on pectic substances in the middle lamella of cucumber tissue during the active fermentation period (Bell et al., 1950, 1951). Nitrogen-purging systems developed by Costilow et al. (1977) efficiently maintain low CO_2 concentration in natural salt-stock pickle fermentations and dramatically reduce the incidence of severe bloaters; however, the problem of softening in large cucumbers is not eliminated by this process.

The Magness-Taylor fruit pressure tester (FPT) (Magness and Taylor, 1925) has commonly been used to objectively evaluate the textural quality of cucumber products. The FPT tip mounted in the Instron was also used to study the texture of raw cucumbers (Breene et al., 1974). The application of Texture Profile Analysis (TPA) using the Instron to demonstrate textural differences in cucumbers was introduced by Breene et al. (1972). They suggested that cucumber texture might be adequately assessed by measuring one or more of the three parameters: (1) brittleness, (2) hardness, and (3) total work. They also indicated that texture nearer the cucumber stem ends was firmer. Jeon et al. (1973, 1975) showed that the above three TPA parameters were well correlated with the conventional FPT firmness values in the study of raw cucumbers and salt-stock pickles.

The present work was undertaken to evaluate the effects of postharvest holding conditions on the change of green-stock and subsequent salt-stock quality.

MATERIALS & METHODS

Postharvest holding of cucumbers

Size 3B (1-3/4–2 in. diameter) cucumbers harvested at Michigan State University were received in the laboratory the same day as harvested and were carefully selected for uniformity of shape and freedom from visible disease and mechanical damage prior to use. Twenty pounds of the randomly selected cucumbers were filled into each of 26 5-gallon plastic pails. A sub-sample of about 2-lb to be used for postharvest green-stock analyses was placed on top of each pail. Two pails of cucumbers were brined immediately (controls) and the remaining pails were held open in duplicate in tempera-

Authors Lee and Uebersax are with the Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824. Author Herner is with the Dept. of Horticulture, Michigan State Univ., East Lansing, MI 48824.

ture controlled cabinets (5, 20, and 30°C) with constant RH of 90% for 1, 2, 4, and 6 days, prior to salt brining and natural fermentation. All samples were brined at approximately the same time of day following the assigned postharvest holding treatments.

The respiration rate of the fresh cucumbers held at different temperatures was determined daily by gas chromatography (as described later) throughout the holding period. Percent cucumber weight loss for each storage treatment condition was calculated from weight loss prior to brining.

Cucumbers in the sub-sample represented the green-stock of the same pail in each treatment. At the end of each holding time, the sub-sample was removed and cucumbers in the pail were brined. Five cucumbers were taken from each sub-sample and measured individually for length, width, specific gravity, and texture.

Following those measurements the cucumber samples were packed individually in plastic bags and stored at −20°C. These frozen cucumber samples were then analyzed for pH, soluble solids, and total acidity.

Brining and fermentation

The fermentation pails and the brining procedures used in this experiment were in accordance with those described and illustrated for laboratory use by Costilow and Uebersax (1978). The system employed a large number of 5-gallon plastic pails which were designed to facilitate the nitrogen gas purging during the natural fermentation process. All treatments were replicated in duplicate pails.

Cucumbers in each pail were covered with a 40° salometer brine (10.6% w/w NaCl) containing 0.05% acetic acid. Sufficient dry salt was added to the brine to equilibrate at about 25° salometer. The equilibrated brine strength was maintained until a 0.6% lactic acid concentration was attained at which time the brine strength was increased by 3° salometer weekly to a holding strength of 45° salometer. Brine strength or salt concentration was determined using a standardized hydrometer calibrated in degrees Salometer (Thomas Co., Philadelphia, PA). Brine pH and total acidity (calculated as % lactic acid) were determined periodically by methods described by Etchells et al. (1964).

Nitrogen gas purging was started immediately after brining and the brine was then purged on schedule (15 min in mornings and 15 min in evenings) to accelerate salt circulation and to reduce CO_2 accumulation in the brine. The brine fermentation and storage of cucumbers required about two months, after which salt-stock pickles were assessed for the quality by visual evaluation and instrumental textural analyses.

Green-stock cucumber analyses

Ten cucumbers from each treatment were measured for length, width, and specific gravity. Length and width (cm) of cucumbers were determined by direct measurement using a plexiglass jig. Specific gravity, defined as weight (g)/volume (ml), was calculated for each cucumber. The weight was simply measured on a top-loading balance. Volume was determined by submerging each cucumber in a 1000 ml graduated cylinder filled with 500 ml water and recording the volume displaced.

After the above measurements the cucumbers were prepared for texture evaluation as follows: two cross-sectional pieces 5/8 inch thick were cut from each cucumber tested, one from near the stem end and the other from near the blossom end. Two parallel sharp knives of fixed distance were designed to cut the pieces. Four consecutive cross-sectional slices 1/4 inch thick were cut from the central region of the cucumber. The device used to obtain cucumber slices of uniform thickness was essentially the same as that illustrated by Marshall et al. (1975). Two pieces and four slices obtained from each cucumber were used for piece crush (side crush) and slice punch (center punch) measurements, respectively.

The Instron Universal Testing Machine (Model TTBM, Instron Corp., Canton, MA) equipped with a FPT tip (7/16" diameter) was used as the force detecting device (Breene et al., 1974). The crosshead speed was set at 10 cm/min and the chart speed was 20 cm/min. The chart full scale reading was 1 kg for slice punching and 10 kg for piece crushing. Peak force (kg) indicating the force required to cause carpel suture separation of a slice punched or flesh breakage of a piece crushed, was calculated for slice punching and piece crushing. Work expressed in kg-cm was calculated for the piece crushing test. Areas under peak force curves were determined on an Instron Integrator (Model D1-53, Instron Corp., Canton, MA).

Percent deformation expressing the cripsness and firmness of the cucumber was also calculated for the piece crushing test as follows:

$$\% \text{ Deformation} = \frac{\text{Crosshead traveling distance needed to cause a sharp crack (rupture) of cucumber flesh (cm)}}{\text{Diameter of cucumber piece (cm)}} \times 100$$

Piece crush deformation was calculated to express tissue compression to rupture point as a percent of cucumber diameter. This measure was indicative of the degree of cucumber firmness such that a firm cucumber had a smaller percent deformation due to its crispness. Conversely, a soft cucumber needed a longer distance to cause a breakage or rupture due to its limpness. Increased percent deformation therefore indicated a reduction in firmness. Fig. 1 shows the typical force distance curves for textural evaluation of green-stock cucumbers.

The tested pieces and slices from individual cucumbers were collected, packed in polyethylene bags, and held at −20°C for analyses of pH, soluble solids and total acidity. Weighed frozen cucumber samples ranging from 50–150g were placed in a Waring Blendor, and blended 1:2 with distilled water for three minutes. pH was measured with a Beckman pH meter by inserting the glass electrode directly into the blended slurry. A small portion of the slurry was filtered through a #2 Whatman filter paper and soluble solids content of the filtrate was determined as degrees Brix (°B) using a Bausch and Lomb refractometer. Degrees Brix for the slurry was then multiplied by 3 to correct for dilution.

Total acidity was measured by titration of 10g slurry with 0.1N NaOH to a pH 8.1 endpoint using a Beckman glass electrode pH meter. Percent acid expressed as malic was calculated as follows:

$$\%\text{TA} = \frac{(\text{ml of NaOH})(\text{N of NaOH})(0.06703 \text{ g/meq malic acid})}{\dfrac{(10\text{g slurry} \times 1/3 \text{ dilution factor})}{\text{fresh sample weight}}} \times 100$$

Respiration rate (carbon dioxide production) of the cucumbers was monitored in a continuous air flow-through system. Fresh

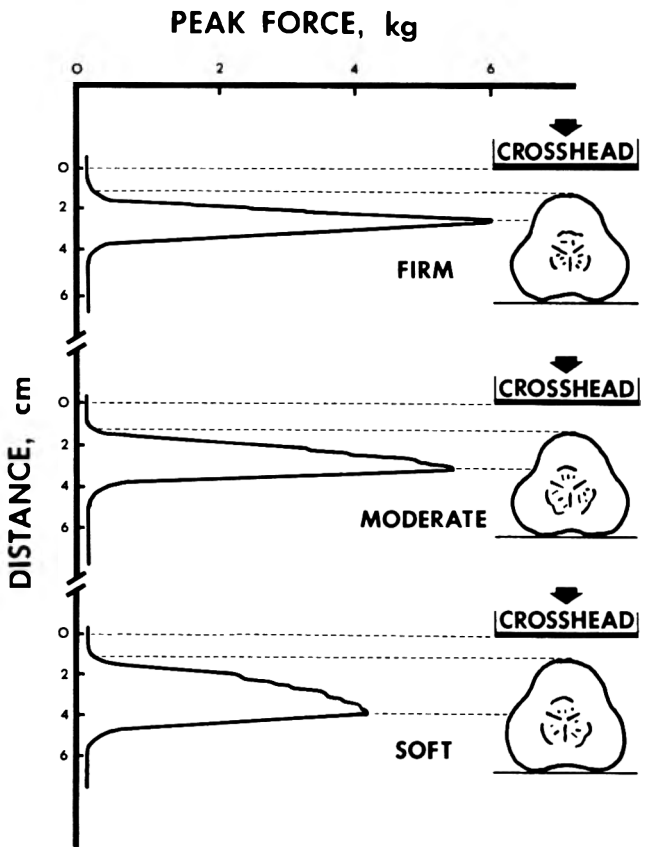


Fig. 1—Typical force-distance curves for textural evaluation of green-stock cucumbers in relationship to the Instron crosshead position and cucumber firmness.

cucumbers were placed in duplicate 2-qt Mason jars and maintained in dark temperature controlled cabinets. A constant flow rate of the air was maintained by capillary regulators. One ml gas samples were taken at entrance and exit ports of the sealed jars and analyzed using a Carle GC-8700 gas chromatograph equipped with a thermal conductivity detector (Carle Instruments, Inc., Fullerton, CA). Respiration rates were calculated in the unit of ml CO_2/kg/hr as follows:

$$\text{ml } CO_2/\text{kg/hr} = \frac{\%CO_2}{100} \times \frac{\text{Flow rate (ml/min)}}{\text{cucumber wt (g)}} \times \frac{60 \text{ (min/hr)}}{0.001 \text{ (kg/g)}}$$

Salt-stock pickle analyses

Thirty brine-stock cucumbers out of each pail were cut longitudinally and evaluated for bloater damage and soft center development. The cut stock was categorized as no damage, honeycomb, lens, balloon, and soft center. Types of bloaters were determined according to the "Bloater Chart" of Etchells et al. (1974). A tally of total soft pickles among all classes was also recorded.

The FPT fitted with 7/16 inch diameter tip (D. Ballauf Mfg. Co., Washington, DC) was employed to evaluate salt-stock firmness (Bell et al., 1955). Firmness was measured as the force (lbs) required to puncture the upper carpel wall of a pickle. Ten pickles were tested out of each pail. All the FPT measurements were made by the same operator.

The Instron was also used to evaluate salt-stock firmness. Ten pickles from each pail were measured and each was punctured once through the side wall in the center of the pickle.

RESULTS & DISCUSSION

Green-stock cucumber analyses

Mean values for chemical composition of green-stock cucumbers held at various temperatures for up to six days prior to brining are presented in Table 1. Size 3B cucumbers with average length of 11.2 ± 0.9 cm and width of 4.8 ± 0.3 cm were used for analyses.

Significant main effect differences in specific gravity were detected by analysis of variance for holding temperture and time. In general, cucumbers held at higher temperatures had significantly lower specific gravities than those held at lower temperatures. The specific gravities of cucumbers held at different temperatures significantly decreased in a linear manner with increasing holding time. This may be associated with the increased moisture loss of cucumbers during increased holding temperature and time without concomitant loss of tissue volume. Tukey mean separation indicated that the specific gravities of cucumbers held at 5°C for up to 6 days, 20°C for up to 2 days, and 30°C for 1 day showed no significant differences from specific gravity measured immediately after harvesting. No significant differences were shown for cucumbers held at 20 and 30°C for 4 and 6 days. Marshall (1975) reported that specific gravity of cucumbers was an important factor in determining potential salt-stock damage. Results indicated that cucumbers with decreased specific gravity produced more bloater damaged stock.

Cucumbers held at 5°C had significantly lower pH values than those held at 20 and 30°C; however, no significant differences were found between cucumbers held at 20 and 30°C.

No significant differences were shown in the soluble solids content of cucumbers among the control and all treatments; however, a significant decreasing linear trend was detected for holding time.

Significant differences in the total acidity of cucumbers were detected for holding temperature and time. Cucumbers held at 5°C had significantly higher percent total acidity than those held at 20°C. No significant differences were detected between control and holding temperature treatments. Total acidity of cucumbers for 5 and 20°C each did not significantly differ among holding days; however, a linear decreasing response to holding time was shown when cucumbers were held at 30°C.

Textural measurements of the green stock cucumbers are shown in Table 2. The average values of slice punch forces obtained from four consecutive slices from the central region showed no significant differences among treatments.

Each of the piece crush measures (Table 2) was obtained by taking the mean value for the two pieces from each cucumber tested. These mean values were used to compare textural characteristics among environmental holding treatments.

Firm cucumbers required higher forces to cause flesh cracking or breakage than did soft cucumbers. Significant main effect differences in piece crush forces were detected for holding temperature and time and their interactions. No significant differences in piece crush forces were detected between the control and 5°C, or between 20 and 30°C. Cucumbers held at 20 and 30°C had significantly lower piece crush forces which decreased linearly with time. No significant differences were shown for piece crush work among all treatments.

Significant differences were detected in piece crush deformation for both holding temperature and time.

Table 1—Chemical composition of green-stock cucumbers held at 5, 20, and 30°C for up to six days prior to brining[a,b]

Treatment temp, time (°C, day)		Specific gravity	pH	Soluble solids (°B)	Total acidity (%)
Control		0.984 ± 0.013^{def}	5.9 ± 0.1^{ab}	3.9 ± 0.5^{a}	0.06 ± 0.01^{ab}
5	1	0.986 ± 0.006^{ef}	5.8 ± 0.1^{a}	4.2 ± 0.4^{a}	0.07 ± 0.01^{b}
	2	0.966 ± 0.024^{cdef}	5.9 ± 0.1^{ab}	4.4 ± 0.2^{a}	0.07 ± 0.01^{b}
	4	0.969 ± 0.007^{cdef}	5.9 ± 0.1^{ab}	4.1 ± 0.2^{a}	0.06 ± 0.01^{ab}
	6	0.963 ± 0.014^{cd}	5.9 ± 0.1^{ab}	3.8 ± 0.2^{a}	0.07 ± 0.01^{b}
20	1	0.987 ± 0.008^{f}	5.9 ± 0.1^{ab}	4.2 ± 0.4^{a}	0.06 ± 0.01^{ab}
	2	0.965 ± 0.011^{cde}	5.9 ± 0.1^{ab}	4.4 ± 0.2^{a}	0.07 ± 0.01^{b}
	4	0.931 ± 0.016^{ab}	6.1 ± 0.2^{ab}	3.7 ± 0.4^{a}	0.06 ± 0.01^{ab}
	6	0.915 ± 0.030^{a}	6.6 ± 0.5^{c}	3.7 ± 0.2^{a}	0.05 ± 0.02^{a}
30	1	0.983 ± 0.010^{def}	5.9 ± 0.1^{ab}	4.3 ± 0.1^{a}	0.07 ± 0.01^{b}
	2	0.952 ± 0.013^{bc}	6.0 ± 0.1^{ab}	4.2 ± 0.3^{a}	0.07 ± 0.01^{b}
	4	0.913 ± 0.038^{a}	6.3 ± 0.3^{bc}	3.7 ± 0.3^{a}	0.06 ± 0.01^{ab}
	6	0.910 ± 0.044^{a}	6.1 ± 0.3^{ab}	3.8 ± 0.5^{a}	0.06 ± 0.01^{ab}

[a] Mean values and standard deviations (like letters within each column indicate no significant differences at $P > 0.05$ by Tukey mean separation).
[b] n = 10 (2 replicates/treatment x 5 cucumbers/replicate).

Piece crush deformation of cucumbers did not significantly differ between control and 5°C, or between 20 and 30°C; however, significant differences were detected between these two groups. Percent deformation increased (indicating cucumber softening) in a significantly linear trend for all temperatures as the holding time increased from one through 6 days.

Effect of location within the cucumber on textural evaluations (over temperature and time) by Instron punch and crush is shown in Fig. 2. Slices 2 and 4 were shown to have significantly higher slice punch forces than did slice 1. The stem end had a higher piece crush force than did the blossom end, though not significant, indicating that the stem end may be firmer than the blossom end regardless of the treatments. Piece crush work showed that the stem end was significantly firmer in texture than the blossom end. This result is in agreement with that obtained by Breene et al. (1972) who explained that there is an increased skin thickness toward the stem end and a greater proportion of flesh relative to seed cavity tissue; however, no significant difference in percent deformation was shown between ends.

Generally, slice punch force was poorly correlated to any of the piece crush measurements in the evaluation of cucumber texture; however, piece crush force was significantly correlated to piece crush work (r = 0.53**) and

Table 2—Textural characteristics of green-stock cucumbers held at 5, 20, and 30°C for up to 6 days prior to brining[a]

Treatment temp, time (°C, day)		Slice punch[b] Force (kg)	Piece crush[c] Force (kg)	Piece crush[c] Work (kg-cm)	Piece crush[c] Deformation (%)
Control		0.5 ± 0.1[a]	6.3 ± 1.4[c]	4.7 ± 1.4[a]	31.3 ± 6.9[a]
5	1	0.4 ± 0.1[a]	6.1 ± 1.7[c]	4.8 ± 1.7[a]	29.0 ± 5.7[a]
	2	0.4 ± 0.1[a]	6.5 ± 1.2[c]	5.3 ± 1.1[a]	29.8 ± 5.9[a]
	4	0.4 ± 0.1[a]	5.7 ± 0.9[bc]	6.4 ± 2.4[a]	34.2 ± 4.5[ab]
	6	0.4 ± 0.1[a]	6.0 ± 1.2[c]	6.1 ± 1.5[a]	34.2 ± 6.9[ab]
20	1	0.4 ± 0.1[a]	6.1 ± 1.7[c]	5.7 ± 1.9[a]	32.8 ± 7.4[a]
	2	0.4 ± 0.1[a]	6.0 ± 1.5[c]	5.9 ± 1.7[a]	33.6 ± 8.4[a]
	4	0.4 ± 0.1[a]	5.8 ± 1.3[bc]	6.3 ± 2.2[a]	35.5 ± 8.3[ab]
	6	0.4 ± 0.3[a]	3.2 ± 1.7[a]	4.0 ± 3.5[a]	43.2 ± 6.5[bc]
30	1	0.3 ± 0.1[a]	5.2 ± 0.9[bc]	5.5 ± 1.2[a]	32.8 ± 6.4[a]
	2	0.4 ± 0.1[a]	5.7 ± 1.1[bc]	5.9 ± 1.8[a]	36.3 ± 7.8[abc]
	4	0.4 ± 0.1[a]	4.7 ± 1.9[abc]	4.5 ± 2.1[a]	36.5 ± 7.5[abc]
	6	0.4 ± 0.2[a]	4.0 ± 1.7[ab]	4.8 ± 2.4[a]	45.1 ± 5.7[c]

[a] Mean value and standard deviations (like letters within each column indicate no significant differences at P > 0.05 by Tukey mean separation).
[b] n = 40 (2 replicates/treatment x 5 cucumbers/replicate x 4 slices/cucumber).
[c] n = 20 (2 replicates/treatment x 5 cucumbers/replicate x 2 pieces/cucumber).

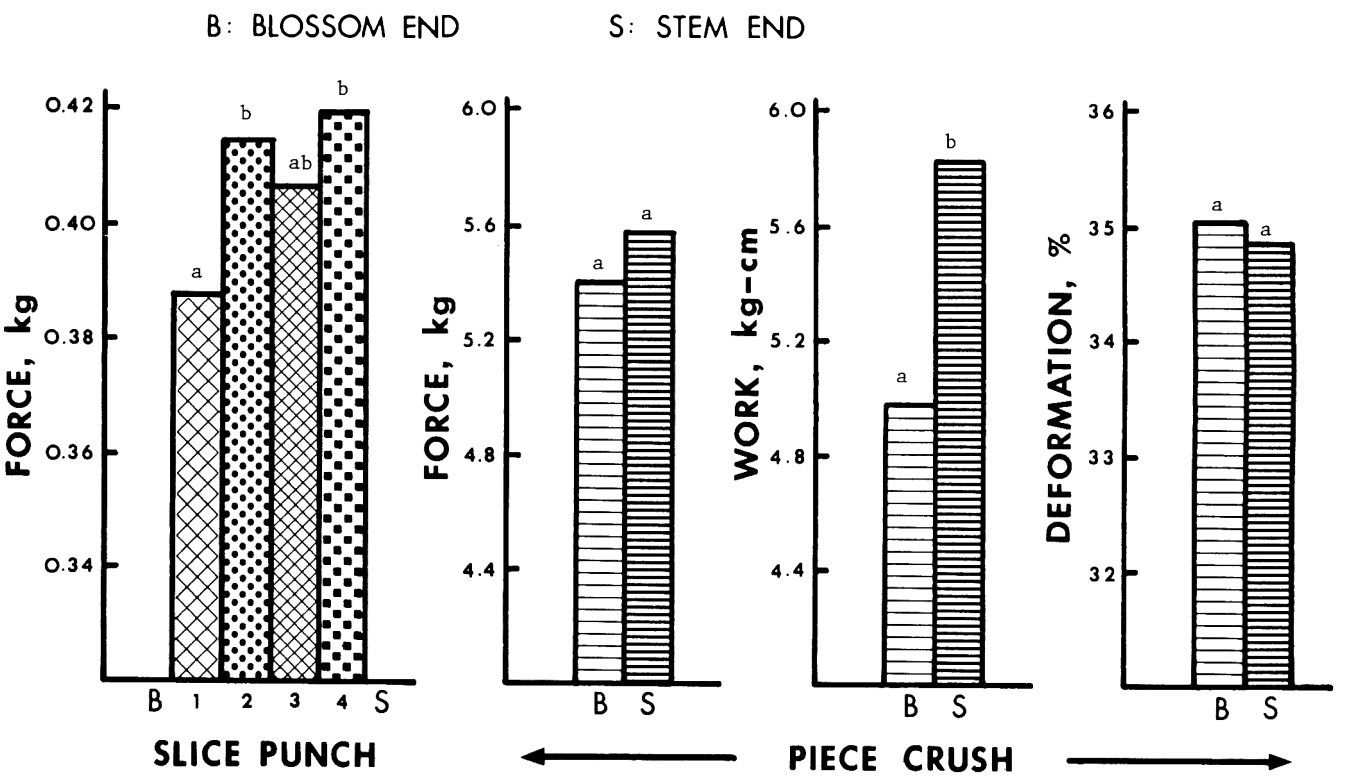


Fig. 2—Effect of cucumber slice and piece locations on mean textural measure values (over temperature and time) for cucumbers held at 5, 20, and 30°C for up to six days prior to brining (like letters within each group indicate no significant differences at P > 0.05 by Tukey mean separation).

piece crush deformation (r = −0.76**). Cucumber slice punch measure was indicative of carpel strength which may be associated with bloater defects; however, it did not correlate well with other firmness measures. It was judged that side crushing of pieces was a meaningful measure of cucumber firmness and percent deformation was assessed to be a valuable parameter.

Respiration rates of green-stock cucumbers during post-harvest holdings are shown in Fig. 3. Cucumbers held at 5°C exhibited an increasing linear and quadratic response to increased holding time; however, at 20 and 30°C respiration rates increased rapidly during the first few holding days and then declined. An increased respiration rate of cucumbers during holding results in a higher consumption of the sugar, thus, sugar may be limited for the curing process during fermentation. Therefore, cucumbers with higher respiration rates were expected to yield salt-stock of poor quality.

The effect of the holding time and temperature treatment on weight losses of green-stock cucumbers is shown in Fig. 4. Significant differences in main effects for weight loss were detected for both holding temperature and time. Data indicated that higher temperatures caused consistently higher weight losses than did lower temperatures during holding periods. In addition, cucumbers held at 20 and 30°C had significantly linear increases with increases in holding time. An increase in weight loss indicates a decrease in moisture content which may cause shriveling of cucumbers and result in poor salt-stock quality. Visual examination of green-stock following holding at 20 and 30°C after 4 days showed severe mold growth and decreased surface quality.

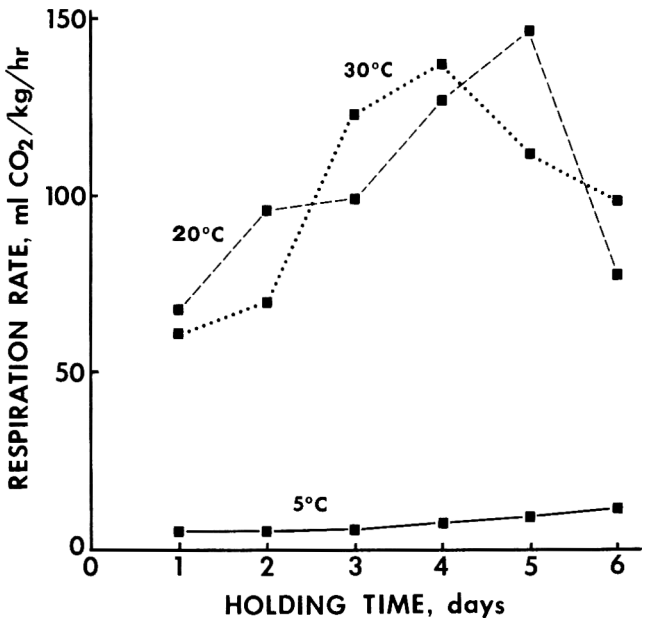


Fig. 3—Respiration rates of green-stock cucumbers held at 5, 20, and 30°C for up to 6 days prior to brining.

Salt-stock pickle analyses

Mean values for several visual defect classes of salt-stock pickles are outlined in Table 3. Pickles fermented from cucumbers held at 5°C showed no significant differences in any class for holding time.

Generally, the control, immediately brined, yielded the highest percentage of good quality pickles while very significant reductions in the percentage of good pickles were shown with increases in holding temperature. Significant linear decreases in the percentage of good pickles were shown with increases in holding time for 20 and 30°C.

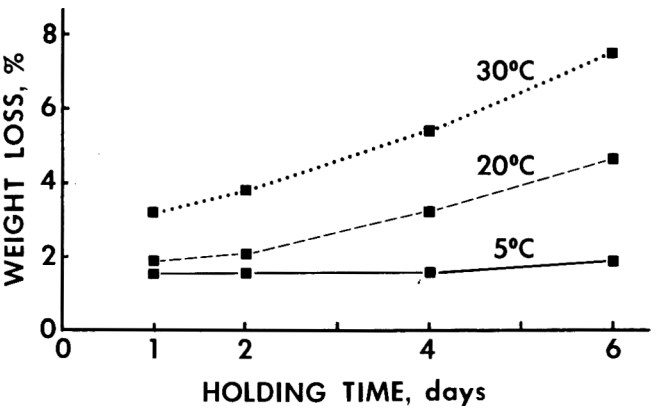


Fig. 4—Percent weight loss (calculated on fresh weight basis) of green-stock cucumbers held at 5, 20, and 30°C for up to 6 days prior to brining.

Table 3—Visual defect classification of salt-stock pickles fermented from cucumbers held at 5, 20, and 30°C for up to 6 days prior to brining[a]

Treatment temp, time (°C, day)		Defect class (%)[b]					Total soft among all defect classes[b] (%)
		No damage	Honeycomb	Lens	Balloon	Soft center	
Control		47 ± 19[b]	37 ± 5[d]	12 ± 16[a]	5 ± 3[a]	0 ± 0[a]	0 ± 0[a]
5	1	40 ± 18[ab]	25 ± 3[bcd]	25 ± 21[a]	10 ± 4[a]	0 ± 0[a]	4 ± 5[a]
	2	40 ± 4[ab]	42 ± 7[d]	13 ± 0[a]	5 ± 3[a]	0 ± 0[a]	0 ± 0[a]
	4	35 ± 7[ab]	25 ± 11[bcd]	32 ± 7[a]	9 ± 12[a]	0 ± 0[a]	2 ± 2[a]
	6	42 ± 2[ab]	24 ± 5[abcd]	17 ± 5[a]	19 ± 2[a]	0 ± 0[a]	7 ± 0[a]
20	1	39 ± 12[ab]	27 ± 5[cd]	14 ± 5[a]	21 ± 2[a]	0 ± 0[a]	5 ± 3[a]
	2	32 ± 7[ab]	27 ± 9[cd]	24 ± 5[a]	19 ± 2[a]	0 ± 0[a]	10 ± 10[a]
	4	11 ± 2[ab]	10 ± 4[abc]	13 ± 0[a]	62 ± 12[bc]	4 ± 5[a]	37 ± 10[b]
	6	0 ± 0[a]	4 ± 5[ab]	15 ± 7[a]	32 ± 12[abc]	52 ± 16[b]	100 ± 0[d]
30	1	34 ± 23[ab]	37 ± 5[d]	20 ± 14[a]	10 ± 4[a]	0 ± 0[a]	9 ± 12[a]
	2	34 ± 9[ab]	23 ± 0[abcd]	17 ± 5[a]	27 ± 5[ab]	0 ± 0[a]	10 ± 4[a]
	4	0 ± 0[a]	12 ± 2[abc]	12 ± 2[a]	64 ± 23[bc]	14 ± 19[a]	64 ± 5[c]
	6	0 ± 0[a]	2 ± 2[a]	5 ± 7[a]	5 ± 3[a]	88 ± 7[c]	100 ± 0[d]

[a] Mean values and standard deviations (like letters within each column indicate no significant differences at P > 0.05 by Tukey mean separation).
[b] Percent of each defect class out of 60 cucumbers (2 replicates/treatment x 30 cucumbers/replicate, n = 60).

Honeycomb and balloon defects showed similar results from all treatments (Table 3). Percentage honeycomb and balloon did not differ significantly between control and 5°C, or between 20 and 30°C; however, significant differences were shown between these groups. At 20 and 30°C percent honeycomb defect showed a linear decreasing response to the days of holding. This could be due to the observed percent increases in the sum of balloon and soft center with increased holding time. At 30°C percent balloon defect showed a quadratic response to the holding time.

In general, total bloater formation was found to increase with increased holding temperature and time. Previous discussion showed that holding cucumbers at high temperatures for long periods resulted in decreased specific gravity. Therefore there may be an association between bloater defects and specific gravity of cucumbers. Further work in this area appears warranted. These data support that reported by Marshall (1975) who concluded that total bloater formation generally had an inverse relationship with specific gravity of cucumbers.

Percentage of soft centers and total softening of salt-stock pickles is shown in Table 3. The most significant effect observed was the severe softening of salt-stock pickles fermented from cucumbers held for several days at 20 and 30°C. In addition, percent total softening was highly significantly correlated to percent soft center (r = 0.90***).

Data showed that cucumbers held at 20 and 30°C for up to 4 or 6 days resulted in the greatest defects for salt-stock. It was apparent that the occurrence of softening in salt-stock pickles was caused by extended holding time at high temperatures.

Textural evaluation data of salt-stock pickles by the FPT and Instron are presented in Fig. 5. Significant reductions in the texture of cucumbers were found with increased holding temperature. Texture of pickles significantly decreased with increased holding time at 20 and 30°C; however, no significant differences for holding time were detected at 5°C. Pickles fermented from cucumbers held at 20 and 30°C for 6 days were not evaluated due to excessive softness. A highly significant correlation (r = 0.98***) was shown between textural evaluations of pickles by FPT and Instron puncture tests. Either method will provide suitable measurement of resistance to puncture; however, the Instron showed less variability.

Results of this study indicate that postharvest holding conditions affect the chemical and physical composition of green-stock and subsequent salt-stock quality. Cucumbers held at 5°C for up to 6 days showed firm texture, low respiration rates, minimum weight loss, and good quality stock after fermentation. Increasing postharvest holding temperatures and holding times prior to brining resulted in loss of firmness, high respiration rates, increased weight loss, and a decrease in salt-stock quality. Values expressing cucumber texture were generally higher at the stem end than at the blossom end. Salt-stock pickle softening increased dramatically under high temperature/long time holding periods. Postharvest holding of cucumbers before brining is very detrimental to final salt-stock pickle quality. Quality loss is accelerated at temperatures ranging from 20–30°C due primarily to textural degradation and internal softening. Cucumbers can be held at 5°C for up to 6 days or at 20°C for at least 2 days before quality of either green-stock or salt-stock undergoes significant deterioration.

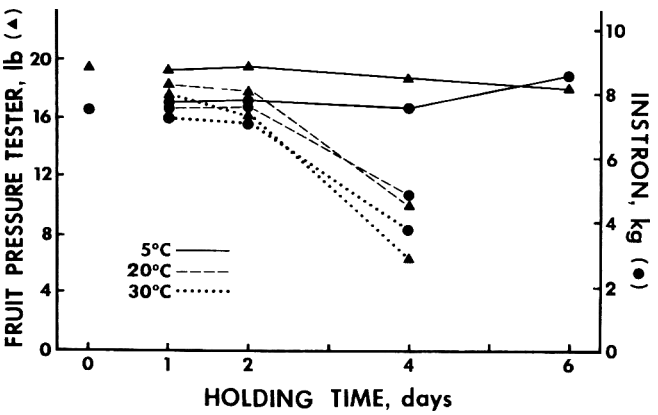


Fig. 5—Center puncture forces of salt-stock pickles fermented from cucumbrers held at 5, 20, and 30°C for up to 6 days prior to brining.

REFERENCES

Apeland, J. 1961. Factors affecting the keeping quality of cucumbers. Internatl. Inst. Refrig. Bul. Sup. 1: 45.

Bell, T.A., Etchells, J.L., and Jones, I.D. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Technol. 4: 157.

Bell, T.A., Etchells, J.L., and Jones, I.D. 1951. Pectinesterase in the cucumber. Arch. Biochem. Biophys. 31: 431.

Bell, T.A., Etchells, J.L., and Jones, I.D. 1955. A method for testing cucumber salt stock brine for softening activity. ARS, USDA. ARS-72-5, 15.

Breene, W.M., Davis, D.W., and Chou, H.E. 1972. Texture profile analysis of cucumbers. J. Food Sci. 37(1): 113.

Breene, W.M., Jeon, I.J., and Bernard, S.N. 1974. Observations on texture measurement of raw cucumbers with the fruit pressure tester. J. Texture Studies 5: 317.

Costilow, R.N., Bedford, C.L., Mingus, D., and Black, D. 1977. Purging of natural salt-stock pickle fermentations to reduce bloater damage. J. Food Sci. 42: 234.

Costilow, R.N. and Uebersax, M.A. 1978. Micrograph Bulletin presented to Pickle Packers Internatl., Inc., St. Charles, IL.

Eaks, I.L. and Morris, L.L. 1956. Respiration of cucumber fruits associated with physiological injury at chilling temperatures. Plant Physiology 31: 308.

Etchells, J.L., Bell, T.A., Costilow, R.N., Hood, C.E., and Anderson, T.E. 1973. Influence of temperature and humidity on microbial, enzymatic, and physical changes of stored, pickling cucumbers. Appl. Microbiol. 26: 943.

Etchells, J.L., Bell, T.A., Fleming, H.P., Kelling, R.E., and Thompson, R.L. 1974. Bloater chart. Published and distributed by Pickle Packers Internatl., Inc. St. Charles, IL.

Etchells, J.L. Borg, A.F., and Bell, T.A. 1968. Bloater formation by gas-forming lactic acid bacteria in cucumber fermentations. Appl. Microbiol. 16: 1029.

Etchells, J.L., Costilow, R.N., Anderson, T.E., and Bell, T.A. 1964. Pure culture fermentation of brined cucumbers. Appl. Microbiol. 12(6): 523.

Fellers, P.J. and Pflug, I.J. 1967. Storage of pickling cucumbers. Food Technol. 21: 74.

Fleming, H.P., Thompson, R.L., Etchells, J.L., Kelling, R.E., and Bell, T.A. 1973a. Bloater formation in brined cucumbers fermented by Lactobacillus plantarum. J. Food Sci. 38: 499.

Fleming, H.P., Thompson, R.L., Etchells, J.L., Kelling, R.E., and Bell, T.A. 1973b. Carbon dioxide production in the fermentation of brined cucumbers. J. Food Sci. 38: 504.

Heldman, D.R., Marshall, D.E., Borton, L.R. and Segerlind, L.J. 1976. Influence of handling on pickling cucumber quality. Trans. ASAE. 19(6): 1194.

Hirose, T. 1976. Effects of degree of maturation of cucumber fruits on the chilling injuries and respiration rates. Sci. Rept. Fac. Agr. Kobe Univ. 12: 15.

Jeon, I.J., Breene, W.M., and Munson, S.T. 1973. Texture of cucumbers: correlation of instrumental and sensory measurements. J. Food Sci. 38: 334.

Jeon, I.J., Breene, W.M. and Munson, S.T. 1975. Texture of salt stock whole cucumber pickles: correlation of instrumental and sensory measurements. J. Texture Studies 5: 411.

Lutz, J.M. and Hardenburg, R.E. 1977. The commercial storage of fruits, vegetables and florist and nursery stocks. USDA Agr. Handbook No. 66, U.S. Government Printing Office, Washington, DC.

Magness, J.R. and Taylor, G.F. 1925. An improved type of pressure tester for the determination of fruit maturity. USDA Dept. Circ. No. 350.

Marshall, D.E. 1975. Density-sorting of green stock pickling cucumbers for brine stock quality and related studies. M.S. Thesis, Mich. State Univ., E. Lansing, MI.

Marshall, D.E., Cargill, B.F., and Levin, J.H. 1972. Physical and quality factors of pickling cucumbers as affected by mechanical harvesting. Trans. ASAE 15(4): 604.

Marshall, D.E., Hooper, A.W., Baker, L.R., and Heldman, D.R. 1975. A method for measurement of carpel strength in cucumbers. Trans. ASAE. 18(4): 752.

Ryall, A.L. and Lipton, W.J. 1979. "Handling, Transportation and Storage of Fruits and Vegetables," 2nd ed., Vol. 1, Avi Publishing Co., Westport, CT.

Ms received 6/22/81; revised 10/6/81; accepted 10/12/81.

Changes in Roasted Peanut Flavor and Other Quality Factors with Seed Size and Storage Time

HAROLD E. PATTEE, JACK L. PEARSON, CLYDE T. YOUNG, and FRANCIS G. GIESBRECHT

ABSTRACT

Peanut seed size and time in storage have been investigated as to their influence on roasted flavor, peanut butter color, blanchability of roasted peanuts, oxidative stability and iodine value of raw peanuts. Seed size had a significant effect on roasted flavor and peanut butter color with constant roasting time and with roasting to a uniform peanut butter color. Blanchability of the seed after roasting was also significantly affected by seed size as well as storage time. Oxidative stability of the raw peanuts showed a general tendency to decrease with seed size and storage time. Iodine values were significantly higher in the smallest seed size but storage time did not affect iodine values. The data suggest that inferior quality is being introduced into marketing channels through use of a 5.95 mm screen size as a minimum for grading U.S. No. 1 Virginia-type peanuts.

INTRODUCTION

THE MARKET GRADES of peanuts and, thus, their market value are based to a large degree upon screen size, i.e., whether the kernel rides or falls through screens of certain sizes. The screen size demarcations between market grades seem to have been based more on economic considerations than on quality. It has been indicated that many current grades and standards do not adequately reflect the characteristics of the desired product (Hinds and Kromer, 1973). Thus, a need exists to understand the relationships between peanut size and quality. A search of the literature suggests a paucity of information relating peanut size to quality. However, recent papers (Pattee et al., 1981a, b) have related concentration of free sugars and free amino acids to seed size.

Research into cold storage effects on peanut quality has been conducted intermittently over the past 50 years (Beattie, 1932; Marzke et al., 1976; Thompson et al., 1941; Woodroof, 1945; Woodroof et al., 1947, 1949). These studies have documented the contributions cold storage can make in delaying quality deterioration of peanuts. However, little if any information is available regarding the degree of quality change which may be occurring during storage and within which seed sizes the greatest changes may be occurring. The objective of this study was to determine if different quality changes were taking place within selected seed sizes of Virginia-type peanuts over storage time.

MATERIALS & METHODS

PEANUTS from the 1978 crop were obtained from a commercial sheller, pregraded into U.S. No. 1, Medium, and Extra Large Virginias. The peanuts were from North Carolina farms. The shelled peanuts were separated over slotted screens into four seed sizes (short diameter dimensions in mm): 5.95 (ride a 5.95 screen, pass a 6.35 screen), 7.14 (ride a 7.14 screen, pass a 7.44 screen), 7.94 (ride a 7.94 screen, pass an 8.34 screen), 8.73 (ride an 8.73 screen, pass a 9.13 screen). The 5.95, 7.14, and 7.94 mm size designations are the minimum seed size in each of the three market grades of Virginia-type peanuts. In meeting seed count standards for marketing Virginia-type peanuts, it is often necessary to combine the next larger size of peanuts into the grade. The 7.14 mm and 7.94 mm size seed allowed us to evaluate the largest seed size generally found in the U.S. No. 1 and Medium grades. All splits were hand picked from the U.S. No. 1 grade and used as a size category.

Although the peanuts were purchased at 8% (wet basis) moisture content, they were screened at 6.3%.

Peanuts packaged in cloth bags (5 kg per bag), were stored in controlled environment rooms set at 4°C and 65% R.H. to simulate commercial peanut storage conditions. At pre-set times, three replicate 5 kg samples were taken from each of the five size categories, and immediately shipped by priority mail to the National Peanut Research Laboratory (NPRL), AR, SEA, USDA, Dawson, GA. At NPRL they were dried to 5% moisture and kept at 1°C until processing or analysis was performed on the samples.

Oxidative stability of raw peanuts was determined as oxygen bomb times by the method of Blankenship et al. (1973). Iodine values (refractometric method) of raw peanuts were determined by tentative methods of the American Peanut Research and Education Association (1971).

Peanuts made into peanut butter for sensory and objective color measurements were roasted in a four-compartment cylinder in a Blue M "Power-O-Matic 60" laboratory oven which had been modified to rotate the cylinder during roasting. Two different roasting regimes were used as treatments. These treatments were termed "Uniform Time" (UT) and "Variable Time (VT). The UT regime was set by the time required to roast a one-kg sample batch, containing an equal weight of each seed size category, to medium roast (Hunter L = ca. 49). The conditions established were 167°C for 22 min. This roasting regime was then used on all seed size-storage time combinations. The "splits" category was not used in this roasting regime. For each storage period each replication of the four seed size categories was subdivided into four subsamples. The subsamples from each size category were simultaneously roasted in four separate compartment roasting cylinders. After roasting the subsamples were quickly cooled by forced ambient air. The four subsamples from each sample were then recombined. The VT regime allowed different roasting times at 167°C for each seed size including splits. This VT regime produced from all size categories a peanut butter of a relative uniform medium-roast color as judged by Hunter L, a, and b color measurements.

Blanchability percentages for the roasted peanuts were determined by the method of Barnes et al. (1971). Color measurements were made with a Hunter Lab Color/difference Meter, Model D25D2L on finely ground butter of 100% peanuts (no additives), which had been roasted, blanched and degermed. The peanut butter was produced in 1000g lots using a Bauer Bros. horizontal attrition mill with 3500 rpm, 3 hp, 3-phase, G.E. motor, hand-turned worm-gear feed, and 8 in. diam metal grinding plates adjusted to ca 0.5 mm clearance between the plates. The newly ground butter was sealed in plastic bags and refrigerated overnight or longer before it was brought to room temperature and emptied into 10 cm square containers with frosted plastic sides and clear bottoms, through which the three color-reflectance parameters were read.

Sensory evaluation

Peanut butter flavor scores were obtained using a 10-member experienced flavor panel. Panelists are given a preliminary orientation on flavor evaluation and then evaluated over six test sessions. The selected panelists are able to distinguish small flavor differences consistently. They receive additional training consisting of

Author Pattee is with the USDA-SEA-AR and Dept. of Botany, Author Young is with the Dept. of Food Science, and Author Giesbrecht is with the Dept. of Statistics, North Carolina State Univ., P.O. Box 5906, Raleigh, NC 27650. Author Pearson is with the USDA-SEA-AR, National Peanut Research Laboratory, Dawson, GA.

two 1-hr sessions on physiological and pyschological principles of flavor response and major causes of variation in flavor and appearance of peanuts–raw, roasted and ground. Sample testing is conducted using individual booths with red and blue masking lights. Presentation of the sample is made on individual plastic spoons. The number of samples presented per sitting depends on the design of the experiment but may not exceed 12. In evaluating the flavor of the peanut butter samples, panelists tasted the samples in order of their randomized code numbers listed on an evaluation ballot and rated the sample on a 5-category scale (excellent–1, good–2, fair–3, poor–4, or very poor–5.

Statistical analysis

Data were analyzed using the Statistical Analysis System of Barr and Goodnight (1972) and the least significant difference test described by Steel and Torrie 1960).

RESULTS & DISCUSSION

THE RELATIONSHIP between roasted product flavor and selected Virginia-type peanut seed sizes was of major interest in this study. The results in Table 1 evidence the significant differences found in both flavor and color of peanut butter from the 5.96 mm and the 7.14 mm and larger size seeds of Virginia-type peanuts when all seed sizes are subjected to the same roasting conditions (UT). The magnitude and direction of these differences suggest that the 5.95 mm seeds have distinctly different roasting characteristics and that the flavor of the peanut butter produced from these seeds is less desirable than the flavor of other seed sizes. Since this difference in flavor existed when all seed sizes were roasted to the same color (VT), inferior flavor quality was not simply the result of over-roasting the smaller seeds but must be caused by different inherent flavor precursor composition of the 5.95 mm seeds (Table 1).

The potential of chemical composition to influence roasted flavor may be evaluated by comparing the reported concentrations of free amino acids and sugars (Pattee et al., 1981a, b) in these peanuts. Although the average concentration of the free amino acids related to typical roasted flavor (aspartic acid, glutamic acid, histidine, and phenylalanine) was 32.3% lower for 7.14 mm than the 5.14 mm size seeds the average concentration of those related to atypical roasted flavor (threonine, tyrosine, lysine, and arginine) was 59.8% lower. Glucose, the carbon source precursor for roasted flavor (Koehler et al., 1969), did not change with seed size. Sucrose, a source for glucose, is the predominant sugar present in peanuts and is highest in the 5.95 mm seeds. This high sucrose level may be involved in the darker roast color formation of these small seed via the caramelization reaction. However, elimination of this darker roast effect through the VT treatment did not improve the flavor rating for the peanut butter produced from these peanuts (Table 1). Future studies to delineate possible off-flavor factors in the 5.95 mm seed will involve the use of an expanded scoring system and a professional flavor profiling panel.

Table 1–Flavor scores and Hunter L color values of peanut butter from peanuts of varying seed size

Seed size (mm)	Flavor score[a] (UT)[a]	Color[a] (UT)	Flavor score[a] (VT)[c]	Color[a] (VT)
5.95	4.0	44.4	4.0	49.3
7.14	2.5	50.9	3.0	49.4
7.94	2.7	52.8	2.8	49.1
8.74	2.5	54.3	2.4	49.1
splits	–	–	3.0	47.5
L.S.D.$_{95}$	0.6	0.6	0.7	0.4

[a] Values are an average across the 0, 3, 6, and 9 month storage times for each seed size
[b] Uniform roasting times
[c] Variable roasting times

Storage time showed no significant effect on the roasted flavor or color of the peanut butter from any of the size categories. However, delay times of up to 9 months beyond the original storage periods for conducting the flavor analyses may have allowed additional changes to occur in the samples which negated any differences resulting from the initial storage periods even through the samples were stored at 5% moisture content and 1°C.

Storage had an effect on oxidative stability and iodine values of raw peanuts and blanchability percentages of roasted peanuts. In general, there was a decrease in oxidative stability of the peanuts with storage time but not in a uniform manner across all seed sizes (Table 2). A significantly better oxidative stability was observed for 5.95 mm seeds than for the larger size seeds. The greater loss in oxidative stability with storage time of these seeds might be related to their significantly higher level of unsaturated fatty acids as indicated by their iodine values. Among the larger seed sizes there was no significant difference in iodine value, nor did storage time have any effect on their iodine values.

The last quality factor, blanchability of roasted seeds, also pointed to the 5.95 mm seeds as an inferior product source, which may result in an undesirably skin-flecked butter without rigorous after-blanch hand picking. The first tests for blanchability were conducted six months after initiation of storage and the unblanched seeds averaged 7.7% in the 5.95 mm seeds. Twelve months after initial storage commenced the unblanched value had dropped to 3.9%. This value was still significantly higher than the mean values for 7.14 mm, 7.94 mm, or 8.74 mm seeds which were 1.1, 0.5, and 0.4%, respectively. The least significant difference is 0.6 for these values at P = 0.05. Storage period had no significant effect on the blanchability of these larger size seeds.

–Continued on page 460

Table 2–Oxidative stability and iodine values of raw peanuts of varying seed size and storage time

Seed size (mm)	Oxidative stability[a] (min) Storage Time (months) 0	3	6	9	Mean	Iodine value[b] Storage time (months) 0	9	Mean
5.95	135.5	110.3	117.5	115.8	119.8	98.4	98.2	98.3
7.14	109.7	105.0	110.2	98.8	105.9	96.7	97.2	97.0
7.94	114.0	102.2	106.3	105.3	107.0	96.5	97.4	97.0
8.74	107.7	108.5	102.2	97.5	104.0	95.7	96.3	96.0
splits	114.0	98.8	99.8	101.5	103.5	–	–	
means	116.2	105.0	107.2	103.8		96.8	97.3	

[a] S.D. (Means) = 8.8; L.S.D.$_{95}$ (Seed size) = 5.1; L.S.D.$_{95}$ (Time) = 4.6.
[b] S.D. Means) = 0.75; L.S.D.$_{95}$ (Seed size) = 0.6; L.S.D.$_{95}$ (Time) = 0.4.

Evaluation of Sesame Flour as a Complementary Protein Source for Combinations with Soy and Corn Flours

ODOARDO J. BRITO and NERLEY NÚÑEZ

ABSTRACT

Sesame products in combination with soy flour, were evaluated as a protein source. Proximate analyses, microbiological examination, determination of vitamins, minerals, selenium content, and nutritional evaluation were carried out. Monagas Aceitera variety flours are similar to other sesame proteins in composition but are higher in lysine and methioine. Enrichment with soy increased PER value for all sesame products. At commonly used fortification levels, sesame-soy blends may be as useful as soy alone for addition to corn-based foods. Some sesame flours and isolates might contain toxic amounts of selenium.

INTRODUCTION

"AJONJOLI," the Spanish name for sesame (*Sesamun indicum* L.) is one of the first, if not the earliest, condiments used, and is the oldest oilseed known to man. Sesame is cultivated because of its oil and it has been named the "Queen of the oilseeds crops" because of its high oil yield, mildness and pleasant taste (Johnson et al., 1979).

Sesame is grown mainly in the developing tropical and subtropical areas of Asia, Africa, South and Central America. It has been adapted also to grow in semiarid regions. The increasing demand for inexpensive sources of protein in under-developed countries has given importance to growing sesame.

Sesame flour has been used traditionally for animal feed. Its use as food for humans has been restricted to sweetmeats, sesame butter, candies, halva, garnish on specialty breads and tropical refreshments (Johnson et al., 1979). This restriction is due to the high content of oxalates and fiber in the hulls and the presence of selenium.

Sesame flour is important as a source of proteins because it is rich in sulfur amino acids, especially methionine, a fact which separates this seed from other oil seeds (Lyman et al., 1956; Block and Weiss, 1957; Evans and Bandermen, 1967). It is also rich in tryptophan and deficient only in lysine and isoleucine (Lyon, 1972; Johnson et al., 1979) as shown in Table 1.

This investigation was undertaken to evaluate sesame products as a potential source of proteins for less developed countries.

EXPERIMENTAL

SAMPLES of commercial sesame cake and flour were furnished by BRANCA, a Venezuelan edible oil company. Cake refers to the residue from the expeller oil extracting process while flour is defined as solvent-extracted cake.

In order to assess possible damage to sesame proteins during the industrial oil extraction process, a control flour was prepared from commercial dehulled sesame seeds, variety *Monagas aceitera* obtained from a commercial distributor in Maturin, Monagas, Venezuela, were mechanically dehulled by the "Fundación Centro de Investigaciones del Estado para la Producción Agroindustrial (CIEPE) San Felipe, Edo. Yaracuy, Venezuela. The dehulled seeds were cracked and ground using a Wiley mill in order to increase their surface area and to facilitate the oil extraction. The resulting paste was extracted with hexane in a Soxlet apparatus at room temperature for 10 hr. The meal was dried in a vacuum oven at 25°C for 12 hr and an 18 mesh sieve was used to separate any remaining whole seeds from the resulting flour.

Sesame protein isolate was prepared from the hexane-extracted flour by the procedure shown in Fig. 1. Defatted flour was mixed

Authors Brito and Núñez are affiliated with the Universidad Simón Bollvar, Departamento de Tecnología de Procesos, Biológicos y Bioquímicos, Caracas, Venezuela.

Table 1—Amino acid composition of protein in selected flours: Sesame (Monagas aceitera variety), corn, soy, and a blend of 50:50 sesame-soy

Amino acid[a]	Sesame (Monagas)	Corn	Soy	Blend	FAO reference
Methionine	3.7	1.5	1.3	2.8	2.2
Cystine	2.2	1.0	1.2	1.6	2.0
Lysine	3.8	2.1	6.7	5.5	4.2
Threonine	4.0	2.5	3.7	3.9	2.6
Leucine	7.1	9.8	7.8	7.0	4.8
Isoleucine	4.1	2.7	4.9	4.5	4.2
Valine	4.7	4.0	5.1	4.8	4.2
Phenylalanine	6.0	3.5	5.2	6.1	2.8
Histidine	2.3	1.7	2.6	2.7	2.4
Arginine	9.3	2.8	8.0	8.4	2.0
Alanine	5.1	5.0	4.3	4.9	–
Glutamic Acid	14.0	14.6	19.5	15.0	–
Glycine	7.3	2.4	4.1	6.1	–
Serine	4.0	3.6	4.5	4.5	–
Tyrosine	5.1	2.7	3.6	4.0	–
Aspartic acid	7.3	4.5	12.7	9.1	–

[a] Values are given in grams per 16 g of Nitrogen.

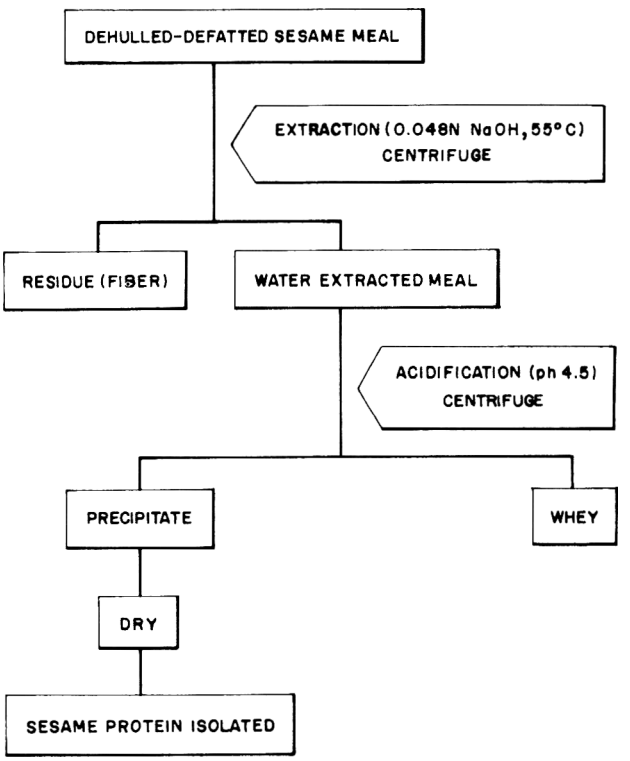


Fig. 1—Scheme for sesame protein isolation.

with 20 volumes of 0.048N NaOH at 55°C for 45 min with moderate agitation. After centrifugation, the aqueous alkaline extract was adjusted to pH 4.5 by addition of 1N HCl and the precipitate collected by centrifugation and dried at room temperature in a vacuum oven.

Soybean and corn flours were bought locally from commercial distributors. 2,3-Diamino naphthalene was obtained from Co. "Photrex" grade cyclohexane was obtained from J.T. Baker Chemical Co. Chemicals used in this study were reagent grade or better.

Chemical and microbiological analysis

Moisture content of samples was determined according to AOAC (1975). Crude fat was measured by hexane extraction according to AOAC (1975). Crude protein was determined by the Kjeldahl method (AOAC, 1975), using % N x 6.25 for all samples. Ash and crude fiber were also determined according to AOAC (1975). Iron, calcium and phosphorous were determined following standard methods for cereal foods of the AOAC (1975) described in method 14.011, 14.014 and 14.015, respectively. Oxalic acid was determined using permanganate titration (AOAC method 32.032). Thiamin and riboflavin were determined fluorometrically (AOAC method 43.024 and 43.039, respectively) while niacin was determined colorometrically (AOAC method 43.044).

Microbiological examination of sesame products were carried out according to the methods described by the International Commission on Microbiological Specifications for Foods (ICMSF, 1978). The test of Mackenzie et al. (1948) was used to determine the presence of fecal coliforms.

Determination of Selenium

The method used was that of Michie et al. (1978), which involves the total destruction of organic matter in the presence of a mixture of concentrated nitric and percloric acids. Following this treatment a complex between selenium (IV) and 2,3-diamino napthalene is formed. This complex is extracted with cyclohexane and the selenium content of the extracts is measured fluorometrically.

Table 2—Gross composition of corn flour, soy flour, and a blend of sesame and soy flours (percent)[a]

Item	Protein	Crude fiber	Fat	Ash	Moisture	Nonprotein[b]
Commercial press cake	35.7	12.1	2.0	5.0	8.0	37.2
Commercial flour	43.7	8.3	1.2	7.8	7.9	31.1
Control flour	51.3	7.9	5.2	6.5	7.6	21.5
Sesame flour, (Monagas)	45.6	13.1	1.7	9.9	8.4	21.3
Sesame (Monagas) - Soy Flour (50:50)	43.0	6.9	1.0	7.0	8.8	33.3
Corn flour	7.4	0.4	0.8	0.2	10.6	80.6
Soy flour	41.6	2.9	1.0	5.4	8.1	41.0

[a] Means of triplicates
[b] Estimated by difference

Table 3—Vitamins, minerals and oxalic acid contents of Monagas aceitera variety sesame seed

Component	Seed	Dehulled seed	Flour
	(mg/100g)		
Iron	21.9	22.0	47.3
Calcium	1,121.0	204.0	401.0
Phosphorous	611.5	613.0	1,287.3
Oxalic acid	1,822.0	80.0	176.0
Thiamin	1.5	–	2.7
Riboflavin	0.25	–	0.42
Niacin	6.0	–	10.1

Nutritional evaluation

White, 28 day old, Sprague Dawley rats, obtained from the Venezuelan National Institute of Nutrition, were used. Six rats (three males and three females) of 50g average weight were used in each experiment. The rats were kept in individual galvanized steel cages with removable, 5 cm deep, stainless steel pans. The diets consisted of 10% protein, 3.5% U.S.P. XIX salt mixture, 1% vitamin mixture (AOAC, 1975), 6% of a mixture consisting of 83 parts commercial corn oil and 17 parts of commercial codfish oil, and corn starch to 100%. Casein, obtained from Merck Cofasa (a local supplier), was used for the control diets. The rats were fed food and water ad libitum during a 3 day acclimation period and during the assay period.

In order to evaluate the complementary effect of sesame and proteins, protein content of the diets was kept constant at 10% while mixing sesame and soy in different proportions (Bressani, 1975).

Amino acid analysis

Amino acid analysis was carried out by the method of Spackman et al. (1958), using a Beckman model 120 B amino acid analyzer.

RESULTS & DISCUSSION

TABLE 2 shows the analytical data of various products used in this study. The results are generally comparable with those reported in the literature. *Monagas aceitera* variety flour has a protein concentration similar to the other sesame flours used in this study and a composition similar to that reported by other authors for sesame flour (Laughman and Rodríguez, 1945; Carter et al., 1961; Jaffe and Chavez, 1971; Yermanos et al., 1971.)

The experimental sesame-soy flour contained 43% protein, which is comparable to that of either soy or sesame flour. Its crude fiber content, 6.9%, was higher than soy flour and lower than sesame flour. The other constituents of the blend (fat, ash, moisture, and nonprotein material) were similar to the component flours.

The vitamin and mineral composition of sesame, *Monagas aceitera* variety, is shown in Table 3. This variety contains more thiamin and niacin than most other oil seeds, whereas the riboflavin content is about the same as the other seeds. The iron and phosphorous contents are similar to those of other oilseeds.

Oxalic acid and calcium occur as calcium oxalate in the outer epidermis of the seed removed during dehulling, thus their content is greatly reduced during this step (Table 3). Data in Table 4 show that the mesophile, enterobacteriaceae, yeast and mold counts of sesame products are comparable to those of commercial corn flour. It should be remembered that these sesame flours as well as the corn flour receive additional heat treatment before use. The amino acid compositions of sesame (*Monagas aceitera*) flours, corn flour, soy flour and 50:50 sesame-soy blend are shown in Table 1. As in corn, lysine appears to be the limiting amino acid of sesame protein, while methionine is the limiting amino acid for soybean protein. *Monagas aceitera* variety flour has a better amino acid pattern than other sesame

Table 4—Mesophiles, enterobacteriaceae, yeast and mold counts of sesame products and corn flour

Sesame products	Mesophiles counts/g	Enterobacteriaceae counts/g	Yeast & mold counts/g
Commerical cake	2.2×10^3	5.7×10^3	6.5×10^3
Commerical flour	1.4×10^3	4.5×10^2	3.3×10^3
Monagas variety flour	1.6×10^3	5.0×10^3	4.8×10^3
Corn flour	1.0×10^4	2.5×10^3	5.5×10^3

varieties. It is higher in lysine, 3.8g/16g N, than the reported average literature values of 2.5–3.0g/16g N. (Lyman et al., 1956; Johnson et al., 1979) for other sesame flours. This variety is also richer in methionine (3.7g/16g N), which might be important when sesame is used in combination with soy bean.

A blend of flours consisting of equal amounts of sesame and soy protein should provide sufficient amounts of these two amino acids, since both amino acids are present in higher concentrations in a 50:50 mixture than in Food and Agriculture Organization of the United Nations (FAO) reference protein. As shown in Fig. 2, commercial flour and commercial cake had lower PER values than those of flours prepared in the laboratory when these products were the only protein in the diet. The control flour has a PER approximately 0.3 greater than that of the commercial flour. Avoiding high temperatures during processing probably accounts for the higher PER values of the products prepared in the laboratory. Commercial flours are prepared by pressing the seeds at temperatures above 110°C; temperatures during the laboratory process never exceeded 55°C.

The *Monagas aceitera* variety flour had the highest PER value (1.40); addition of 0.2% L-Lysine HCl to the *Monagas acitera* variety flour increased its PER value to that of casein (2.5). This suggests that sesame protein is deficient in lysine and that lysine-fortified flour could be used as a protein supplement.

The PER values of diets containing both sesame and soy are also shown in Fig. 2. Increasing the amount of soy protein in the diet raised the PER of all the sesame products. An optimal combination was reached at the 50:50 level. Combinations with higher soy protein did not increase the PER values significantly and, in the case of flour prepared in the laboratory, further increases in soy content may decrease PER values.

Although sesame and soy do not have amino acid profiles as complementary as soy and corn (Bressani, 1975) two sesame products (Monagas and control flours), when combined with equal parts of soy flour, have PER values of 2.45 and 2.38, respectively, which are not significantly different from that of casein, (2.50). All PER values were adjusted on the basis of 2.5 for the casein reference.

Fig. 3 shows the PER values for diets in which protein was derived from different combinations of corn flour and a 50:50 blend of sesame (*Monagas aceitera*) flour and soy flour. Increasing the protein of the sesame-soy blend raised the PER to a maximum of 2.3 at 40% corn plus 60% blend. The PER of combination increased to 2.6 with the addition of 0.2% L-Lysine HCl. This suggests that the 40% corn:60% sesame-soy combination of flours is deficient in lysine, which is to be expected as both corn and sesame protein are low in lysine. While combinations of corn and soy can give PER values higher than combinations of corn plus sesame soy-blend (Cravioto and Cervantes, 1965; Bressani, 1975), is seems likely that at the levels of fortification commonly used (8–10%) sesame – soy blends may be useful as soy alone for addition to corn-based foods. This would be particularly true if there are any significant economic or sociological advantage to using sesame rather than soy.

Selenium is usually found in plant materials in association with protein as selenamino acids (Olson et al., 1970). This is of importance for the preparation of protein isolates and concentrates from sesame. As can be seen in Table 5 the current commercial manufacturing process leads to a final product with high selenium content. On the basis that chronic selenium toxicity is observed in animals after weeks, or perhaps months, of ingesting plants with 5 ppm of the mineral, selenium intoxication would be expected in human beings after long-term consumption of 5 ppm daily (National Academy of Sciences, 1976). The selenium

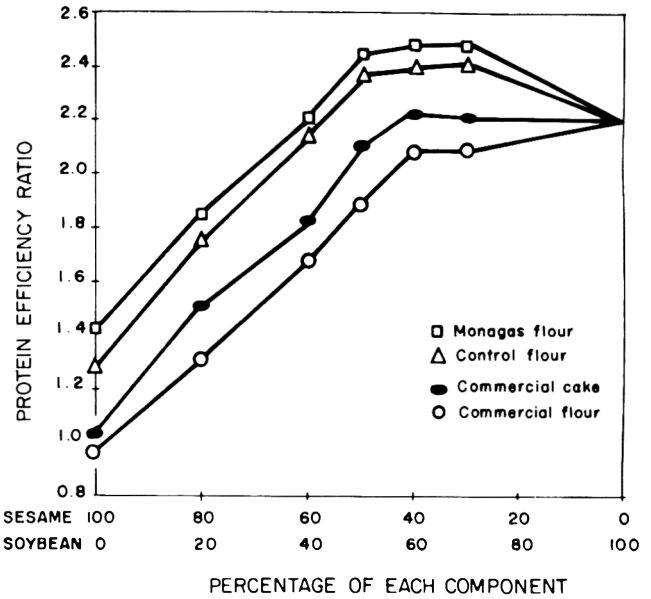


Fig. 2–Protein efficiency ratio of combinations of various sesame products with soybean flour.

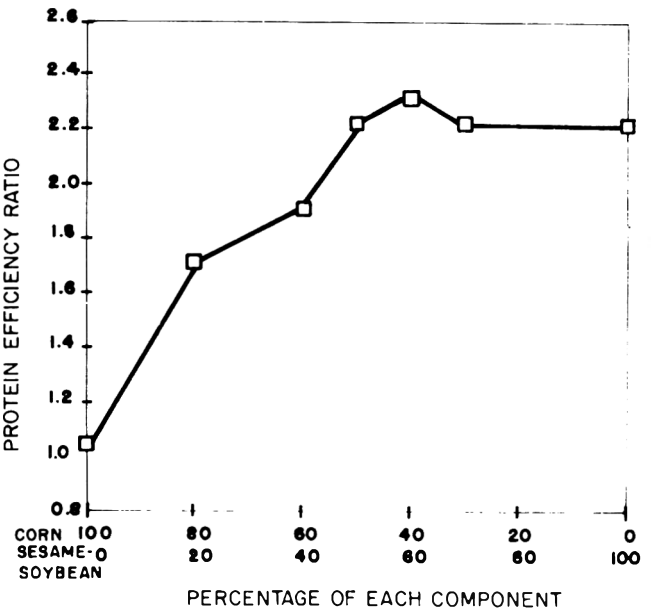


Fig. 3–Protein efficiency ratio of combination of corn flour with a 50:50 blend of sesame (Monagas aceitera variety) flour and soy flour.

Table 5–Selenium and protein content of sesame products

	Commercial seed		Commercial flour		Monagas Aceitera seed	
	Selenium (ppm)	Protein (%)	Selenium (ppm)	Protein (%)	Selenium (ppm)	Protein (%)
Whole seed	5.3	25.6	N.D.	N.D.	0.6	23.6
Hulls	N.D.*	28.6	N.D.	N.D.	N.D.	27.1
Dehulled seed	5.9	25.9	N.D.	N.D.	0.6	25.6
Flour	11.9	51.3	9.9	43.7	1.3	45.6
Residue fiber	N.D.	0.5	N.D.	N.D.	N.D.	0.8
Isolates	36.1	87.2	25.3	81.0	3.0	86.4
Whey	N.D.	1.3	N.D.	1.8	N.D.	1.5

*N.D. = none detected

content of commercial seeds is 5.3 ppm and the protein content is 25.6%. As the seeds are processed to make flour the protein content increases to 51.3%, and the selenium content increases to 11.9 ppm. Further processing produces isolates with a protein content of 87.2% and a selenium content of 36 ppm. The by-products of this process (hulls, oil, residue and whey) contain no appreciable selenium. Commercial flours follow the same pattern beginning with 9.9 ppm selenium and 43.7% protein and ending with 25.3 ppm selenium and 81.0% protein at the isolate level, with no appreciable amounts of selenium in the oil, residue and whey.

It is clear that using seeds with high selenium content to prepare protein concentrates or isolates will result in a final product with unacceptable high levels of the mineral. Sesame grown in Venezuela in the state of Monagas should present no problem in this respect, since soil selenium level have been reported to be quite low (Mondragon and Jaffé, 1971).

Our results with *Monagas aceitera* variety sesame seed are consistent with this observation. The seed sample used in these experiments had a low selenium content (0.6 ppm), the flour contained only 1.3 ppm and the isolate contained 3.0 ppm.

REFERENCES

Association of Official Analytical Chemists. 1975. "Official Methods of Analysis." The association, Washington, DC.

Block, R.J. and Weiss, K.W. 1967. "Amino Acid Handbook," p. 141. Thomas, New York, NY.

Bressani, R. 1975. Nutritional contribution of soy protein to food systems. J. Am. Oil. Chem. Soc. 52: 254A.

Carter, F.L., Vidabelle, C.O., and Allen, L.E. 1961. Effect of processing on the composition of sesame seed and meal. J. Am. Oil Chem. Soc. 38: 148.

Cravioto, R.O. and Cervantes, M. 1965. Eficiencia proteica de la harina de masa enriquecida con harina de soya y de la adicionada con proteina de ajonjolí. Ciencia Mex. 24(3): 159.

Evans, R.J. and Bandermer, S.L. 1967. Nutritive value of some oilseeds proteins. Cereal Chem. 44: 417.

Food and Agriculture Organization of the United Nations. 1979. FAO Production Yearbook. Rome.

International Commission of Microbiological Specifications for Foods of the International Association of Microbiological Societies. 1978. "Microorganisms in Foods," Vol. 1 Univ. of Toronto Press, Toronto, Canada.

Jaffe, W.G. and Chavez, J.F. 1971. El posible uso de la harina de ajonjolí para fines comestibles. Arch. Latinoam. Nut. 11: 31.

Johnson, L.A. Suleiman, J.M., and Lusas, E.W. 1979. Sesame protein: A review and prospectus. J.A. Oil Chem. Soc. 56: 463.

Laughman, D.C. and Rodriguez, M. 1945. Elajonjolí (Sesamun endicum) su cultiro, explotación y mejoramiento. Bol. 2, Ministerio de Agricultura y Cría. Venezuela.

Lyman, C.M., Kui K.A., and Hale. F. 1956. Essential amino acid content of farm feeds. J. Agri. Food Chem. 4: 1008.

Lyon, C.K. 1972. Sesame: Current knowledge of composition and use. J. Am. Oil Chem. Soc. 49: 245.

Mackenzie, E.F.W., Taylor, E.W., and Gilbert, W.E. 1948. Recent experiences in the rapid identification of Bacterium coli typi i. J. Gen. Microbio. 2: 195.

Michie, N.D., Nixon. E.J., and Bunton, N.G. 1978. Critical review of AOAC fluorometric method of determining selenium on foods. 61(1): 48.

Mondragon, M.C. and Jaffe, W.G. 1971. Selenio en alimentos y en orina de escolares de diferentes zonas de Venezuela. Arch. Latinoam. Nut. 21(2): 185.

National Academy of Sciences. 1976. "Selenium and Health." Food & Nutrition Board, NAS, Washington, DC.

Olson, O.E., Novacek, E.J., Whithead, E.I., and Palmer I.S. 1970. Investigations on selenium in wheat. Phytochem. 9: 1181.

Spackman, D.H., Sten, W.H., and Moore, S. 1958. Automatic recording apparattus for use in the chromatography of amino acids. Anal. Chem. 30: 1190.

Yermanos, D.M., Salebb, E., and Cavanagh, G.E. 1971. The sesame plant as a source of protein and other nutrients. J. Am. Oil Chem. Soc. 48: 831.

Ms received 3/17/81; revised 8/12/81; accepted 8/14/81.

CHANGES IN ROASTED PEANUT FLAVOR . . . From page 456

The data presented here have shown relationships between seed size and quality which suggests that seeds of inferior quality are being brought into market channels through use of a 5.95 mm screen size as the miminum for grading U.S. No. 1 Virginia-type peanuts. The consistently inferior nature, except for oxidative stability, of the 5.95 mm seed points to the need for a cooperative type study across several locations to ascertain the minimum screen size allowable in Virginia-type peanuts to assure the consumer the highest possible quality within reasonable economic limits. Although significant differences in important quality parameters were observed in the samples analyzed, the limited nature of the samples does not yet allow broad general conclusions to be drawn.

REFERENCES

Amer. Peanut Res. Educ. Assoc. 1971. Tentative Methods B-1 and B-3. J. Amer. Peanut & Res. Ed. Assoc. 3: 245.

Barnes, P.C. Jr., Holaday, C.E., and Pearson, J.L. 1971. Device to measure ease of skin removal from peanuts. J. Food Sci. 36: 405.

Barr, A.J. and Goodnight, J.H. 1972. "A User's Guide to the Statistical Analysis System." North Carolina State University, Raleigh, NC.

Beattie, J.H. 1932. Effect of cold storage and age on seed germination and yield of peanuts. U.S. Dept. Agric. Circ. 233.

Blankenship, B.R., Holaday, C.E., Barnes, P.C. Jr., and Pearson, J.L. 1973. Comparison of oxygen bomb methods for measuring oxidative stability of peanuts and peanut products. J. Amer. Oil Chemists' Soc. 50: 380.

Hinds, M.K. and Kromer, G.W. 1973. Peanut marketing. In "Peanut Culture and Uses," p. 657. Amer. Peanut Res. & Educ. Assoc., Stillwater, OK.

Koehler, P.E., Mason, M.E., and Newell, J.A. 1969. Formation of pyrazine compounds in sugar-amino acid model systems. J. Agric. Food Schem. 17: 303.

Marzke, F.O., Cecil, S.R., Press, A.F. Jr., and Harein, P.K. 1976. Effects of controlled storage atmospheres on the quality, processing, and germination of peanuts. U.S. Dept. Agric., Agric. Res. Serv. ARS-S-114.

Pattee, H.E., Young, C.T., and Giesbrecht, F.G. 1981a. Free amino acids in peanuts as affected by seed size and storage time. Peanut Sci. 8: 113.

Pattee, H.E., Young, C.T., and Geisbrecht, F.G. 1981a. Free amino and storage effects on carbohydrates of peanuts. J. Agric. Food Chem. 29: 800.

Steel, N.G. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., New York.

Thompson, H. Cecil, S.R., and Woodroof, J.G. 1941. Storage of edible peanuts. Georgia Agric. Expt. Sta. Bull. 268.

Woodroof, J.G. 1945. Why moisture in peanuts should be controlled. Food Industries 17: 1302.

Woodroof, J.G., Thompson, H.H., and Cecil, S.R. 1947. Refrigeration of peanuts and peanut products. Refrig. Eng. 53: 124.

Woodroof, J.G., Thompson, H.H., and Cecil, S.R. 1949. How refrigeration protects quality of peanuts. Food Industries 21: 15.

Ms received 8/26/81; revised 10/19/81; accepted 10/22/81.

Paper Number 7002 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27650.

Use of trade names of specific materials does not constitute endorsement by the North Carolina Agricultural Research Service or the U.S. Dept. of Agriculture to the exclusion of others which may be available.

Development, Utilization and Protein Quality of Potato:Soy:Egg Flakes

C. A. HARGETT, A. I. NELSON, K. E. WEINGARTNER, and J. W. ERDMAN JR.

ABSTRACT

Various ratios of potato:full-fat soy:whole egg (P:S:E) were combined and drum dried to yield flakes which were rehydrated and formed into baked or fried patties. Methods were developed to utilize either fresh or dehydrated potatoes for flake production. Sensory evaluations showed 60:34:6 P:S:E fried patties to be more generally accepted than baked patties. Freezing of the uncooked dough prior to frying or baking was shown to be a good storage method. P:S:E flakes (60:34:6) were also substituted for 12% of patent wheat flour in bread. P:S:E supplementation improved the protein efficiency ratio (PER) of patent flour wheat bread. PER's for 60:34:6 P:S:E flakes and baked patties were equivalent to casein.

INTRODUCTION

MARASMUS AND KWASHIORKOR are prevalent in developing countries where food production is not keeping pace with population growth. The diets consumed in these nations are frequently cereal based and therefore low in quantity and quality of protein as well as calories.

Potatoes, because of their high yield of protein and calories per hectare, have definite potential in alleviating food shortages, especially in the developing countries (Bennett, 1975). Potato protein, though low in quantity is of high nutritional quality compared to most plant proteins because of its overall superior amino acid balance. It is rich in lysine and therefore an excellent supplement for lysine-poor protein sources such as cereal.

Soybean protein is also considered an ideal supplement for cereals because of its high protein content and favorable amino acid pattern. Soybeans contain approximately 20% oil which serves as a good source of calories, and like potatoes, soybeans are widely available and economical.

Little research has been reported concerning the effect of combining soy protein with potatoes. A combination of soybeans and potatoes may provide a better balance of nutrients than either alone. The addition of whole egg to a potato soy combination should further round out the amino acid balance and increase the level of fat soluble vitamins, and other micronutrients.

The objective of this work was to develop a flake containing potato, full-fat soy, and whole egg, to incorporate this material into different food systems, and to evaluate the nutritional quality of these food products.

EXPERIMENTAL

Preparation of flakes

Three flake materials were produced by varying the ratio (dry basis) of potato, soy, and egg, and by utilizing different potato products and processing methods. In all three methods whole soybeans (Bonus Variety) were heated for 20 min at 98.9°C (210°F) in an air dryer (Proctor and Swartz, Inc., Philadelphia, PA). A spinning drum plate apparatus was used to split the cotyledons, thus loosening the hulls. Hulls and split cotyledons were separated by running them twice through a forced air conveyor belt system. The resulting cotyledons were blanched in tap water at a rolling boil for 20 min. After blanching the beans were drained, cooled with tap water, and twice passed through a Rietz Desintegrator (Rietz Company, Westchester, PA) using a 0.023 inch mesh screen. For all products large Grade A eggs were beaten to homogeneity and added to the soy slurry.

Method I (Fig. 1) utilized French's Big Tate dehydrated potato flakes which were milled at medium speed for 5 min in a Waring Commercial Blendor (Model 31 BL 79, Dynamics Corp. of America, New Hartford, CT) and added to the soy egg slurry. The slurry was mixed for 5 min using a Hobart mixer (Hobart Mfg. Co., Troy, OH). Water was added during mixing until the proper consistency was reached, and the slurries were drum dried on a double drum dryer (Mathis Machine Corp., South Bend, IN) with a space setting of 0.01 inch between drums and a steam pressure of 40 psig. The material came off the drum dryer as rather fine flakes. However, the occasional clumps were broken up by passing the flake material through a 1/8 inch mesh screen. This was accomplished by gentle rubbing of the material through the screen.

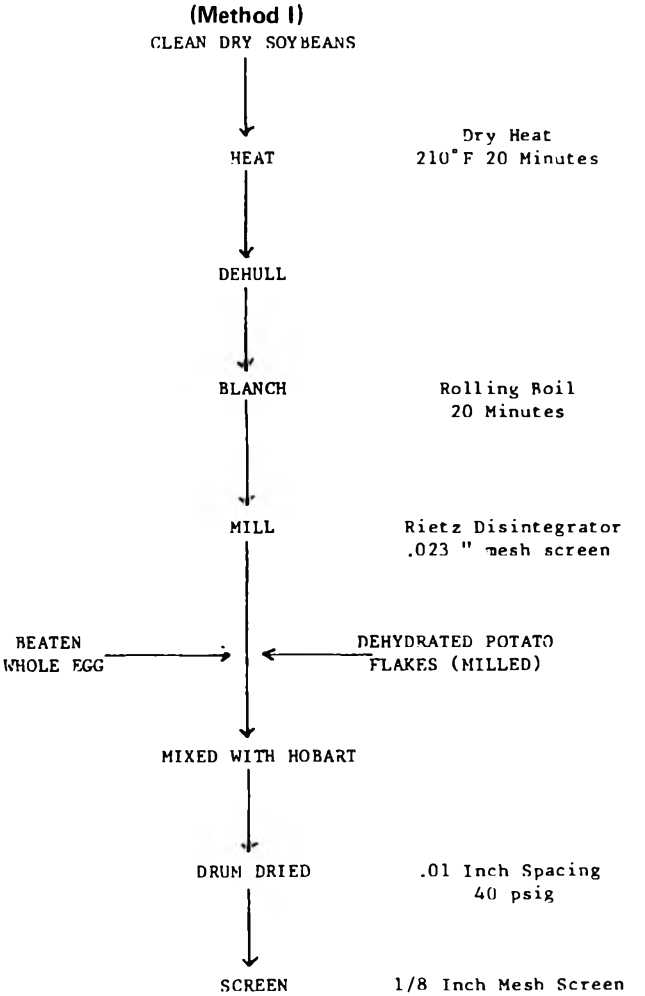


Fig. 1—Preparation of product containing dehydrated potato flakes.

Authors Nelson, Weingartner, and Erdman are with the Dept. of Food Science, 567 Bevier Hall, 905 S. Goodwin Ave., University of Illinois, Urbana, IL 61801. Author Hargett, formerly with the Univ. of Illinois, is now with General Mills, Inc., Washington & Town Road, West Chicago, IL 60185.

Method II (Fig. 2) utilized whole, peeled Russet Burbank potatoes which were sliced into 5/8 inch slabs, washed, and pre-cooked in 73.9°C (165°F) water for 30 min. The pre-cooked slabs were cooled by immersing in ice water for 16 min and cooked in steam at atmospheric pressure for 30 min. Immediately after cooking the hot potato slabs were mashed, added to the soy-egg slurry, mixed in the Hobart mixer for 5 min and drum dried (0.01 inch spacing, 40 psig). This procedure was followed to give minimal sticky starch characteristics to the potato (Cording et al., 1957).

Method III (Fig. 3) was the same as Method II without the pre-cooking and pre-cooling stages. All drum dried products were passed through the 1/8 inch screen prior to use.

All materials used in PER and storage studies were processed by Method III. Protein, fat, and moisture were determined for flakes according to standard AOAC (1975) methodology.

Determination of potato:soy:egg (P:S:E) ratio

Three P:S:E ratios (60:34:6, 40:48:12, and 34:60:6 [d.b.]) were prepared by Method I. Fried patties were formed by mixing a variable amount of NaCl (for taste) with 30g of dry flakes and rehydrating with 34 ml of water to a firm dough. The dough was scaled to 83g and shaped into patty form using a hamburger press (Heuck Co., Cincinnati, OH). Each patty was fried 5 min on each side in 10 ml of Crisco pure vegetable oil at 215.5°C (420°F) (Model RC 33 Frying pan, Sunbeam Corp., Chicago, IL). The patties were then evaluated for flavor and texture by a taste panel made up of graduate students and staff.

A hedonic scale ranging from 1–9 was used to detect differences between fried patties made with flakes containing various ratios of potato:soy:egg processed by Method III and to detect differences between fried patties made from 60:34:6 P:S:E material processed by Methods II and III. The panel consisted of the same ten members with each test being repeated twice.

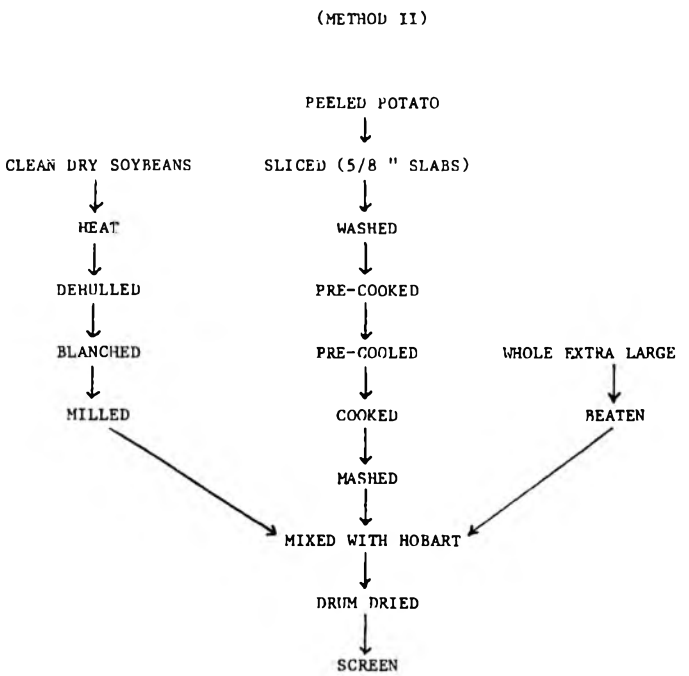


Fig. 2—Preparation of product utilizing pre-cooked and pre-cooled Russet Burbank potatoes.

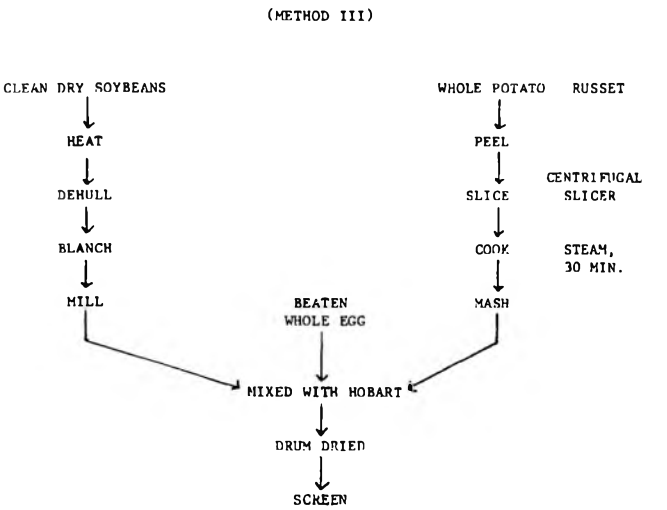


Fig. 3—Preparation of product utilizing Russet Burbank potatoes that were not pre-cooked or pre-cooled.

Storage

Formed patties were divided into three groups. Group I was baked, group II was fried, and group III was not cooked. The patties were then vacuum sealed (Cyrovac Co., Cambridge, MA) in polyethylene bags and stored at −18°C. After 1 or 2 months' storage, frozen baked, fried, and uncooked dough patties were removed from the freezer. The uncooked frozen dough patties were divided into two groups, one which was baked and the other fried. Patties which had been fried or baked prior to freezing were baked. The frozen patties were not allowed to thaw prior to cooking. The patties which had been frozen then baked or fried and those which had been baked or fried prior to freezing and then reheated, were each hedonically ranked for general acceptability after 30 or 60 days' storage. Fresh baked and fried patties were hedonically ranked for general acceptability at 0 days. The taste panels were repeated three times on different days with the same nine panel members.

Bread preparation

Enriched patent wheat flour (compliments of Purity Sunbeam Baking Co., Champaign, IL) was supplemented with 12% of 60:34:6 P:S:E flakes. A modification of the Kansas-State process for making high protein bread (Tsen and Tang, 1971) was followed.

White flour, P:S:E material, shortening, and salt were thoroughly mixed (Table 1). Compressed yeast and sugar were dissolved in warm water (40°C) and added to the above mixture. Water was added and the dough kneaded at low speed for 1 min in a Hobart Kitchen Aid model K5-A mixer (Troy, OH) followed by medium speed for 4 min. The dough was scaled into 510g pieces and allowed to ferment at 30°C (86°F) and 80% R.H. in a fermentation chamber (National Manufacturing Company, Lincoln, NE). The doughs were then moulded, panned, and proofed under the same conditions until the top of the dough was 1.5 cm above the top of the pan. Baking was at 220°C (428°F) for 25 min.

Protein evaluation

Protein efficiency ratio studies were conducted to determine the protein quality of flakes, patties, and bread made using 60:34:6 P:S:E. All flours were produced using Method III. Diets were prepared according to AOAC (1975) recommendations for PER studies. Fifty male weanling Sprague Dawley rats (Harlan Industries, Indianapolis, IN) were individually housed in stainless steel cages in a temperature controlled room. A rat chow diet was fed to all animals for a three day adaptation period after which they were weight averaged into five groups. Each group received a 10% protein diet contributed by either casein, P:S:E, ground bread or ground patties.

Table 1—Bread formula

Ingredient	Amount (g)	% of Patent flour
Patent flour (14% H_2O Basis)	700	100
Substituted flake (d.b)	91	12
Yeast	35	5
Salt	14	2
Sugar	35	5
Shortening	Variable	Variable
Water	Variable	Variable

Feed consumption and weight gain were recorded while animals consumed diet and water ad libitum. After 28 days PER's were calculated for each animal.

Table 2—Proximate analysis of various ratios of potato:soy:egg (P:S:E) flake

Ratio of potato:soy:egg (d.b.)	% Moisture[a]	% Protein[b] (d.b.)	% Lipid[a] (d.b.)
60:34:6	6.1	23.6	10.3
34:60:6	5.6	33.7	17.0
40:48:12	6.3	31.1	17.8

[a] Calculated as the mean of three samples
[b] Calculated as the mean of two samples

Table 3—Effect of various potato:soy:egg ratios upon the acceptability of fried patties[a]

Potato:soy:egg (d.b.)	Texture	Flavor	Crust
60:34:6	Smooth pasty	Good potato flavor	Browned nicely
40:48:12	Granular pasty (Not as pasty as 60:34:6)	Soy flavor	Browned nicely
34:60:6	Granular pasty	Distinct soy flavor	Browned nicely

[a] All patties contained 30g P:S:E flake, 34 ml water, 750 mg salt.

Table 4—Effect of milling potato flakes upon the acceptability of 60 potato:34 soy:6 egg, fried patties[a]

Potato flake % Milled	Properties of dough	Properties of patty
25	Cohesive, firm	Light & flaky appearance, not pasty, crust separation
50	Not very cohesive and sticky	Not as flaky as 25% milled, pasty, crust separation
75	Less cohesive than 50% milled, very sticky and hard to handle	Pasty, crust separation
100	Less cohesive than 50% milled, very sticky and hard to handle	Pasty, crust separation

[a] All patties contained 30g P:S:E flake, 40 ml water, 880 mg salt.

Statistical analysis

All PER and sensory data were subjected to an analysis of variance and the least significant difference test was applied when appropriate (Steel and Torrie, 1960).

RESULTS & DISCUSSION

The proximate composition of selected test material appears in Table 2. As expected the 60:34:6 P:S:E ratio contained the lowest percent protein (23.6%) while the 34:60:6 ratio was the highest with 33.7% protein. The percent fat varied with the amount of soy and egg added.

Fried patties made from various ratios of P:S:E prepared by Method I were evaluated by the taste panel on the basis of texture and flavor (Table 3). The 40:48:12 and 34:60:6 P:S:E patties were described as granular in texture with a soy flavor, while the 60:34:6 P:S:E patty was described as smooth in texture with a good potato flavor. The potato flavor was more acceptable than the soy to these panelists. The granularity and soy flavor present in the first two samples may be attributed to their containing a greater percentage of soy. All patties were described as pasty, and had crusts which browned nicely.

In order to eliminate the pastiness of the fried patties, materials containing 60:34:6 P:S:E were prepared by varying the ratio of milled to unmilled potato flakes (Table 4). The 60:34:6 P:S:E ratio was used because of the smooth texture and potato flavor of fried patties in the previous study. The taste panel judged the fried patties to be more pasty and not as flaky and light in texture as the percent milled to unmilled potato flakes increased. The ratio of 25% milled to 75% unmilled was the only combination that yielded a cohesive firm dough and a fried patty that was not pasty.

It was concluded from the above studies that a drum dried P:S:E material could be produced utilizing dehydrated potato flakes. The flour could be rehydrated and the dough fried to form a patty with acceptable taste and texture.

The next area investigated was the production of flour and patties using whole fresh potatoes instead of dehydrated potato flakes. Table 5 shows the results of panel evaluaton for flavor, general acceptability, and off-flavor when samples of fried patties were prepared from materials processed by Methods II and III. There were no significant differences between methods as regards flavor or off-flavor. The 40:48:12 product prepared by Method III scored significantly lower in general acceptance with a mean score of 6.20. All scores were considered acceptable. The two 60% potato products scored highest. This may be explained because the panelists preferred potato over soy flavor. This is supported by examining the hedonic means for flavor evaluation.

Sensory results for off-flavor showed no difference among samples. All samples were considered to be good regarding the absence of off-flavor.

Method III (potatoes not pre-cooked and pre-cooled) was less time consuming than Method II. Panelists did not prefer patties made from product processed by one method

Table 5—Sensory analysis of fried potato:soy:egg patties[a]

Sample	60:34:6 Method II	60:34:6 Method III	34:60:6 Method III	40:48:12 Method III
Flavor[b]	7.00 ± 0.57	7.20 ± 0.28	6.55 ± 0.35	6.20 ± 0.57
General acceptability[b]	6.95 ± 0.07a	7.15 ± 0.21a	6.70 ± 0.14a	6.20 ± 0.14b
Off-flavor[b]	7.65 ± 0.07	7.50 ± 0.42	7.70 ± 0.42	7.25 ± 0.35

[a] Data points represent mean ± S.D. Means not sharing a common letter are different at $P \leq 0.05$.
[b] P:S:E hedonic means. Flavor and General Acceptability: 9 = like extremely; 5 = neither like nor dislike; 1 = dislike. Off-flavor: 9 = lack of off-flavor; 1 = off-flavor.

over the other. Therefore, method III was considered the process of choice when fresh potatoes were utilized.

Results for the storage study can be found in Table 6. Patties fried prior to and/or after freezing were considered generally more acceptable than patties which had been baked. Results of the statistical analysis confirmed that there was a significant difference in general acceptability between baked and fried patties at $P \leq 0.05$. A significant difference was also found between fresh and frozen patties fried on the day of the evaluation and those which had been fried prior to freezing. Patties fried the day of the evaluation had the highest hedonic means and were preferred over those which had been fried prior to freezing.

No significant differences in general acceptability were found between samples frozen for 30 or 60 days indicating no adverse effects due to storage for this period. Fresh fried patties made on day 0 were shown to be statistically equivalent in general acceptability to patties in which the dough had been frozen 30 or 60 days then fried, indicating that freezing the uncooked dough was a good storage method.

The results of feeding studies conducted to evaluate products made from 60:34:6 P:S:E (Method III) appear in Table 7. The ground patty and flake diets resulted in growth statistically equivalent to each other and to that of the casein group. The ground patty diets made from 60:34:6 flakes showed that baking did not significantly reduce protein quality. Neither of the bread diets were able to support growth equal to that of the casein group. Other studies have shown that available lysine decreases with baking time as does a bread's ability to support growth (Jansen et al., 1964a, b). The 100% wheat bread diet was significantly inferior to all diets in growth supporting ability. The PER (0.48) obtained was slightly higher than results obtained by some researchers and lower than results obtained by others. Reported PER's for white bread were 0.30 (O'Connor et al., 1979), 0.79 (Tsen et al., 1977) and approximately 1.0 (Hoover, 1974). The discrepancies may have been due to researchers employing different baking times and temperatures. The PER for bread fortified with 12% P:S:E was significantly better than the 100% wheat bread. Tsen et al. (1977) and Hoover (1974) obtained similar improvements with soy fortified bread. The corrected PER of 12% supplemented P:S:E bread (1.08) in this study was similar to the PER (1.03) obtained from 12% supplemented soy, whole egg bread (O'Connor et al., 1979).

Table 6—Sensory results for general acceptability in the two month frozen storage study

Sample	Day	Hedonic Mean[a,b]
Fresh fried	0	7.63 ± 0.17a
Frozen dough fried	30	7.78 ± 0.00a
Frozen dough fried	60	7.51 ± 0.13a
Fresh baked	0	5.55 ± 0.30c
Fried frozen baked	30	6.04 ± 0.64bc
Fried frozen baked	60	6.29 ± 0.42b

[a] Mean ± S.D. of daily panel averages (N = 3).
[b] Means not sharing common letters are significantly different at $P \leq 0.05$.

CONCLUSIONS

POTATO:SOY:EGG drum dried products were produced from both fresh and dehydrated potatoes. The 60:34:6 potato:soy:egg combination from dehydrated potatoes was incorporated into fried or baked patties and bread. Studies showed that fried patties were generally more acceptable than baked patties and that freezing of uncooked dough prior to baking or freezing was a good storage method. Protein efficiency ratios revealed that the flakes, baked patties and 12% supplemented potato:soy:egg bread were of good protein quality.

REFERENCES

AOAC. 1975. "Official Methods of Analysis." Association of Official Agricultural Chemists, Washington, DC.
Bennett, T.A. 1975. The potato as a world food source. Canadian Farm Economics 10: 1.
Cording, J., Willard, M.J., Eskew, R.K., and Sullivan, J.F. 1957. Advances in the dehydration of mashed potatoes by the flake process. Food Technol. 11: 236.
Hoover, W.J. 1974. Use of soy products in cereal products. J. Am. Oil. Chem. Soc. 51: 186A.
Jansen, G.R. Ehle, S.R., and Hause, N.L. 1964a. Studies on the nutritional loss of supplemental lysine in baking. 1. Loss in a standard white bread containing 4% nonfat dry milk. Food Technol. 18(3): 109.
Jansen, G.R., Ehle, S.R., and Hause, N.L. 1964b. Studies on the nutritional loss of lysine in baking. 2. Loss in water bound and in breads supplemented with moderate amounts of nonfat dry milk. Food Technol. 18(3): 114.
O'Connor, M.P., Erdman, J.W., and Nelson, A.I. 1979. Baking characteristics and protein quality of soy-whole egg, soy-egg yolk, and soy-egg white supplemented breads. J. Food Sci. 44: 839.
Steele, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw Hill Book Co., Inc., New York, NY.
Tsen, C.C., Reddy, P.R.K. and Gehrke, C.W. 1977. Effects of conventional baking, microwave baking, and steaming on the nutritive value of regular and fortified bread. J. Food Sci. 42: 402.
Tsen, C.C. and Tang, R.T. 1971. K-state process for making high protein breads. 1. Soy flour bread. Baker's Dig. 45(5): 26.
Ms received 8/5/81; revised 9/14/81; accepted 9/18/81.

The authors thank Dr. B.P. Klein and Dr. L.S. Wei for their suggestions. This was presented in part at the 40th Annual IFT meeting in New Orleans, 1980. This work was supported in part by the Univ. of Illinois Agricultural Hatch Grant #30-15-50-352.

Table 7—Protein efficiency ratios of flake, baked patty, and breads[a,b]

Protein source	Food consumed (g)	Weight gain (g)	Corrected[c] PER
100% Wheat Bread	338.4 ± 74.5	20.1 ± 4.6	0.48 ± 0.11a
88% Wheat, 12% 60:34:6 PSE Bread	334.1 ± 48.8	47.1 ± 11.8	1.08 ± 0.18b
Patty (60:34:6	626.6 ± 40.2	187.7 ± 17.3	2.23 ± 0.09c
Flake (60:34:6)	605.0 ± 53.8	182.9 ± 26.2	2.38 ± 0.21c
Casein	519.6 ± 88.9	168.6 ± 29.1	2.50 ± 0.16c

[a] Figure represents average of 10 animals.
[b] Mean ± S.D.
[c] Means not sharing common letters are significantly different at $P < 0.01$.

Purification and Concentration of Betalaines by Ultrafiltration and Reverse Osmosis

Y. N. LEE, R. C. WILEY, M. J. SHEU, and D. V. SCHLIMME

ABSTRACT

Beet juices prepared by solid-liquid extractions were processed by ultrafiltration (UF) and reverse osmosis (RO) to 30°Brix at 20°C. De Danske Sukkerfabrikker (DDS) UF/RO Lab Module-20 plate-and-frame system was used having a 0.72-m^2 effective membrane area and pressures from 5–40 bar. After prefiltering, pectinase treated juices were sequentially processed through 20,000 and 6,000 (UF) molecular weight (MW) cut-off membranes. The UF purified products were then concentrated by RO processes on several types of cellulose acetate (CA) membranes, one with a 500 MW cut-off and 70% NaCl permeability provided colorants that were separated from a majority of soluble solids. Addition of invertase to pectinase-treated juices decreased flux but yielded a three-fold increase in betalaine concentration on a dry weight basis. Betalaine concentration by UF and RO processes also halved nitrate level and greatly reduced flavor.

INTRODUCTION

VERSATILE UNIT OPERATIONS such as ultrafiltration (UF) and reverse osmosis (RO) have attracted the food industry to apply these techniques for concentrating liquid food products. These processes are especially beneficial for products which would be adversely affected by elevated temperatures. In addition, the UF and RO processes are energy saving. Brennan et al. (1976) estimated the electricity cost for removing water by RO was only about one tenth of that used by conventional evaporation techniques. Applications of UF and RO for concentrating liquid food products were initiated and generalized in the dairy industry in the 1960s after Breton and Reid (1957) proposed RO for desalination of sea water. Marshall et al. (1968) studied concentration of cottage cheese whey solids by RO as an alternate method for whey disposal, while Madsen (1974) reported industrial scale applications of UF and RO to concentrate whey solids and skim milk in several European countries. Other studies using UF and RO concentrating techniques on various liquid foods such as maple syrup, egg white, fruit and vegetable juices, and on plant pigments such as anthocyanin, have been reported (Willits et al., 1967; Lowe et al., 1969; Merson and Morgan, 1968; Matsuura et al., 1973; Woo et al., 1980). All researchers concluded that their techniques, when modified, could be applied to the food industry.

Betalaines, consisting of betacyanines and betaxanthines, the pigments found in red table beet tissue (*Beta vulgaris* L.), are important food colorants which have potential to serve as substitutes for synthetic organic dyes. About 75–95% of the betacyanines are betanine and the majority of the betaxanthines are vulgaxanthine-I. Both betanine and vulgaxanthine-I are heat-labile plant pigments having relatively short half-life value (von Elbe et al., 1974; Saguy, 1979; Singer and von Elbe, 1980).

Authors Wiley, Sheu, and Schlimme are with the Food Science Program, Dept. of Horticulture, Univ. of Maryland, College Park, MD 20742. Author Lee, formerly affiliated with the Univ. of Maryland, is now with Research Centre, Borden Inc., 600 N. Franklin St., Syracuse, NY 13204.

Commercial manufacture of beet pigments, i.e., betalaines, depends to a great extent on the continuous availability of highly pigmented cultivars, efficient processing procedures, concentrating techniques and/or spray drying processes. Wiley et al. (1979) showed an efficient method of recovering the betalaines from raw beet tissue by using a solid-liquid extraction procedure. Although higher recoveries were found by using this method as compared with conventional hydraulic expression techniques, the resultant juices were somewhat diluted with a water-citric acid solvent. It was observed that only 0.5–1.0% of the extracted beet juice solids were betalaines, the rest being sucrose, protein, minerals, flavor components, etc. In other research related to beet colorants, Adams et al. (1976) reported that the fermentation of ultrafiltered beet juices by yeast, *Candida utilis*, under partial anaerobic conditions was able to substantially reduce non-pigment moieties and resulted in a five- to sevenfold increase in the betacyanine content on a solids basis. However, the resultant aqueous fermented mixture was diluted and needed concentration.

It appeared that efficient concentrating techniques must be developed to remove a substantial portion of water present in betalaine containing beet juices which may be produced from solid-liquid extractions, hydraulic expressions and/or fermentation processes. The objectives of this study were to determine the feasibility of using UF and RO at comparatively low temperatures to purify and concentrate beet soluble solids in liquid systems, to separate non-pigment solids from beet colorants as an approach to increasing the pigment/solids ratio, and to reduce some of the objectionable components in beet extracts such as natural beet flavors and nitrates.

EXPERIMENTAL

Preparation of beet juices

The beet juices for UF and RO processes were prepared by using a solid-liquid extraction procedure. The extraction procedures were similar to those developed by Wiley and Lee (1978) and later modified by Wiley et al. (1979). Raw beets, *Beta vulgaris* L. cv. Ruby Queen grown in New York State, were washed, trimmed, and crinkle-sliced at 3 mm thickness for extraction process. The acidity of the liquid phase in the diffuser was controlled at pH 5.2 by introducing an acid solvent (0.5% citric acid solution) into the feed hopper. The initial diffuser fill temperature was controlled at 85°C; the sliced beets were held for 10 min at this initial temperature, and then the temperature was reduced to 75°C until the end of the extraction process.

The collected beet juices were immediately treated with pectinase (Clarex, Miles Laboratory, Inc., Elkhart, IN; or Klerzyme, GB Fermentation, Inc., Des Plaines, IL) or with invertase (Maxinvert, GB Fermentation, Inc., Des Plaines, IL) in addition to the pectinase. The pectinase was added at a 0.5 ml/L juice level and the invertase was added at a 1.25 ml/L juice level. The enzyme treated juices were held at room temperature (25°C) for 6 hr and then stored at 1°C for 42 hr.

The juices were then prefiltered through a stainless-steel Seitz pressure filter (Republic Seitz Filter Co., Mildale, CT) at 2.5 kg/cm^2 and 5°C using Celite 535 (Johns Manville, N.Y., NY) as a filtering aid. At least 85% of the Celite 535 particles had a diameter less than 35μ.

—Continued on next page

Apparatus

The De Danske Sukkerfabrikker (DDS) Lab Module-20 (Copenhagen, Denmark) was used to conduct the UF and RO processes. The module can be used either in the UF or RO mode depending on the types of membranes installed. A schematic diagram of the UF/RO system and cross-section view of the plate-and-frame of the DDS equipment is shown in Fig. 1. The module (A) is constructed as a plate-and-frame system and is generally used with 0.36 or 0.72 m^2 effective membrane areas. The membrane support plates (L) and membrane spacers (M) are assembled and compressed by a hydraulic pump (C). The liquid to be concentraed flows radially through a thin channel (0.3–0.5 mm) in a rather short pass (50mm).

The juice stored in the reservoir is pumped by suction pressure (D) into the positive pump (B) and then becomes a high pressure stream (E) and enters the module. The positive pump has a capacity up to 12 l/min and the rate is controlled by a variator (J). The feeding fluid flows upwards through the 0.3–0.5 mm channel exiting from the outlet (G) where a pressure regulator (I) is located. The pressure inside the module is controlled by the pressure regulator and can be operated up to 100 bar. The maximum pressure the UF and RO membranes will tolerate is determined by the characteristics of the specific membranes. The fluid permeated through the membranes (N) flows inside the thin channels of the membrane spacers (L) and passes out from the permeate tubes (K).

Membranes and operating conditions

The specifications of UF and RO membranes (General Dairy Equipment Co., Minneapolis, MN) and the operating conditions used in this study are listed in Table 1. Synthetic great resistant (GR) membranes resistant to acid and base were used in the UF processes and cellulose acetate (CA) membranes were used in the RO processes. The cleaning procedures for UF process after each operation were followed by acid-and-base CIP (Clean-in-place), while those for RO process were followed by enzymatic solutions (1% Terg-a-zyme solution, De Danske Sukkerfabrikker, Copenhagen, Denmark).

Analytical

Betacyanines and betaxanthines. The UF and RO experiments were conducted at 20°C during processing; the relative betacyanine

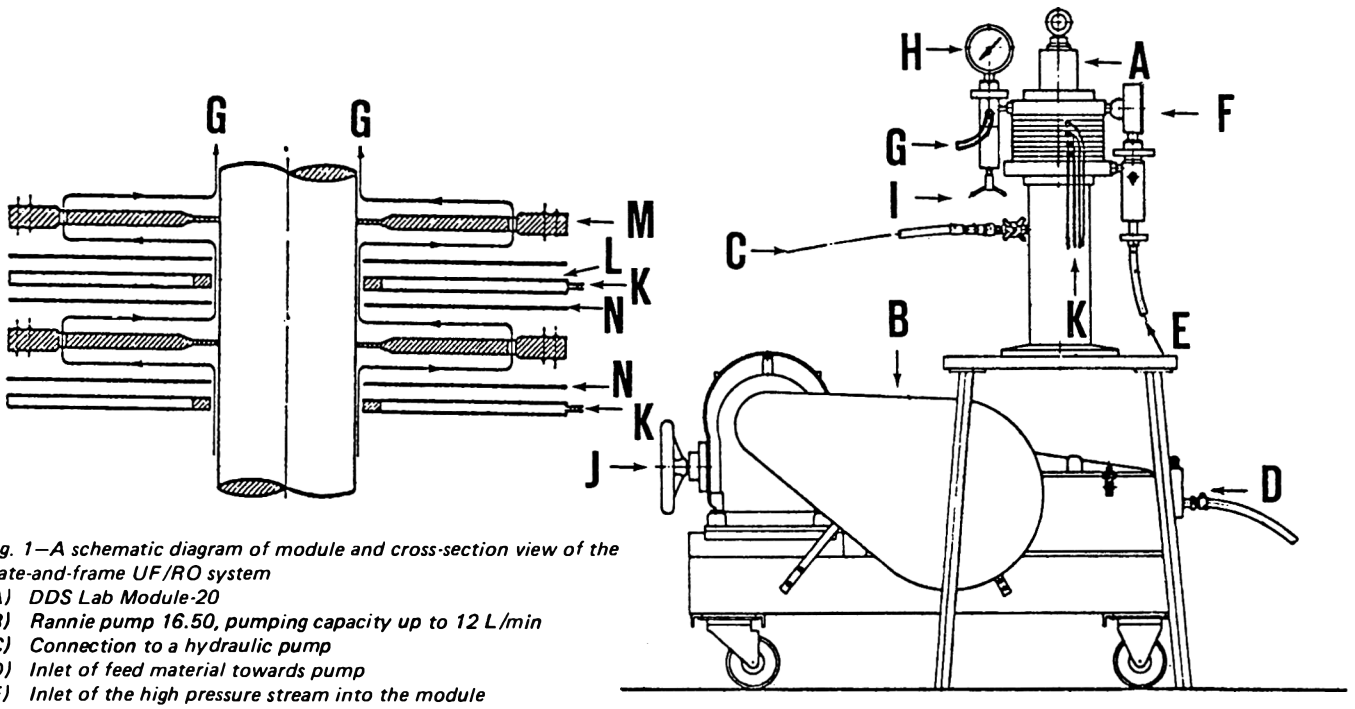


Fig. 1—A schematic diagram of module and cross-section view of the plate-and-frame UF/RO system

(A) DDS Lab Module-20
(B) Rannie pump 16.50, pumping capacity up to 12 L/min
(C) Connection to a hydraulic pump
(D) Inlet of feed material towards pump
(E) Inlet of the high pressure stream into the module
(F) Inlet pressure indicator
(G) Outlet of concentrated fluid
(H) Outlet pressure indicator
(I) Pressure regulator
(J) Flow rate variator
(K) Permeate tube
(L) Membrane support plate
(M) Membrane spacer
(N) Membrane

Table 1—Specifications of UF and RO membranes and operating conditions

Type	Permeability	Approx. cut-off MW value	Recommended operating conditions			Experimental operating conditions			
			pH	°C	bar	pH	°C	bar	effect. area (m^2)
Ultrafiltration	%Lactose								
GR-61P	98	20,000	0–14	0–80	0–15	5.0	15	5	0.72
GR-81P	95	6,000	0–14	0–80	0–15	5.0	15	5	0.72
Reverse osmosis	%NaCl								
CA-865	70	500	2–8	0–30	0–40	5.0	20	30–40	0.36
CA-975	25	500	2–8	0–30	0–40	5.0	20	30–40	0.36
CA-990	10	350	2–8	0–30	0–50	5.0	20	30–40	0.36
CA-995	4	180	2–8	0–30	0–60	5.0	20	40	0.36

and betaxanthine concentrations in the initial feeds and in the final concentrates were determined by the spectrophotometric method developed by Nilsson (1970) and later modified by Wiley et al. (1979) using the McIlvaine's buffer solutions at appropriate pH. This method provided a quick scanning and determination of colorants in the samples. Absorbances at 476, 537, and 600 nm were measured in a Beckman Model 25 spectrophotometer.

Percent total solids and percent soluble solids. The percent of total solids was determined by weighing 20g juice samples and drying in a 70°C vacuum oven until a constant weight was obtained. The percent of soluble solids was determined by using a Bausch & Lomb Abbe 3L (Rochester, NY) refractometer (sensitivity 0.1%) at 20°C calibrated with distilled water as 0°Brix. The difference between % total solids and % soluble solids was regarded as % insoluble solids.

Sugar composition analysis. Major sugar composition, i.e., glucose, fructose, and sucrose, was determined by high performance liquid chromatography (HPLC) according to the procedures of Solomos and Warman (1982). A carbohydrate column (Water Associates, Milford, MA) was used for sugar separation, and an acetonitrile/water (82/18) mixture was used as the elute solvent.

Nitrates. The nitrate level in the samples was determined by following the method of Cataldo et al. (1975). Betalaines present in the juice samples were removed by activated charcoal. An aliquot sample, 0.2 ml containing from 1.0–200 μg nitrate, was mixed with 0.8 ml of 5.0% (w/v) salicyclic acid in concentrated sulfuric acid (H_2SO_4). After 20 min, 19 ml of 2.0N NaOH were added, and the absorbance at 410 nm was measured. Absorbances were correlated to a standard curve which was prepared from reagent grade sodium nitrate.

Process and data collection

A schematic diagram of complete process procedures is shown in Fig. 2.

In the UF processes, 100–120L of prefiltered beet juice were processed in each operation. The recoveries of solute (x), i.e., betacyanines, betaxanthines, and soluble solids, through the operation were measured as a function of volume ratio, which was computed as follows:

$$\text{Vol. ratio} = \frac{\text{Throughput vol. of UF purified juices } (V_t)}{\text{Initial vol. of prefiltered beet juices } (V_i)}$$

In the RO processes, 50–60L of UF purified juice were concentrated in each operation. The juices were concentrated up to 28–30°Brix on CA-865 membranes, or until flux was decreased to 5.0 L/m^2·hr on CA-975 and CA-990 membranes. Specification for these membranes are presented in Table 1. The concentration ratio in the final concentrate was computed as follows:

$$\text{Conc ratio} = \frac{\text{Conc of solute (x) in the final concentrate } (C_f)}{\text{Conc of solute (x) in the initial feed } (C_i)}$$

The percent solute (x) retention as a function of processing time (t) or feed concentration (°Brix) during RO processes was also determined to evaluate solute transport characteristics on the CA membranes.

$$\text{\% Solute (x) Retention at time (t)} = \frac{\text{\% solute (x,t) in the feed} - \text{\% solute (x,t) in the permeate}}{\text{\% solute (x,t) in the feed}}$$

Flux was used to denote the permeate throughput at a specific processing time (t) during the operation, whereas the throughput or processing capacity was used to denote the total filtrate or permeate obtained in each batch. Both terms have a unit of L/m^2·hr.

Flavor evaluation

Sensory evaluation. To avoid the possibility of visual discrimination of concentrate samples on the basis of color intensity, it was necessary to dilute the final concentrate to the equivalent betacyanine strenght as that of the initial feed (hereafter, referred to as "diluted final concentrate"). Both the inital feed and the diluted final concentrate of the same material were evaluated in pairs, whereas the permeates from all the RO processes were evaluated in a completely randomized order.

Six trained panelists were asked to rank the intensity of beet-like flavor and odor in the samples, using the following six point scale: 6 = very beety, 5 = beety, 4 = moderately beety, 3 = slightly beety, 2 = bland, and 1 = very bland. A sample of freshly prepared solid-liquid extraction material (without enzymatic treatments and UF purifying processes) was used as a reference standard sample to aid detection of differences among the initial feeds. The standard reference was arbitrarily assigned a scale value of 4.0.

Gas chromatography. The total volatile content in the headspace of the samples, including initial feed, diluted final concentrate, and permeate, was analyzed by gas chromatography using procedures similar to those of Dignan and Wiley (1976). About 25 ml of sample were placed in a 50 ml volumetric flask, sealed and incubated in a 70°C water bath.

After a 30 min equilibration time, a 1 ml headspace sample was injected into a 2.4 m × 3.2 mm (o.d.) stainless-steel column packed with 10% Carbowax 20M on chromosorb WAW (60–80 mesh) at 60°C isothermally in a Hewlett Packard Model 5830A GC (Palo Alto, CA) equipped with a flame ionization detector (FID) and a Model 18850A computerized terminal recorder. Other instrumental conditions were: injector 110°C, detector (FID) 250°C, and helium was used as a carrier gas at 30 ml/min velocity.

The total volatile content (volatility) in the sample was determined by summation of the integrated area from each volatile component peak. Volatility values among samples were compared by their respective volatility ratios:

$$\text{Volatility ratio} = \frac{\text{Volatility in the initial feed}}{\text{Volatility in the dilute final conc}}$$

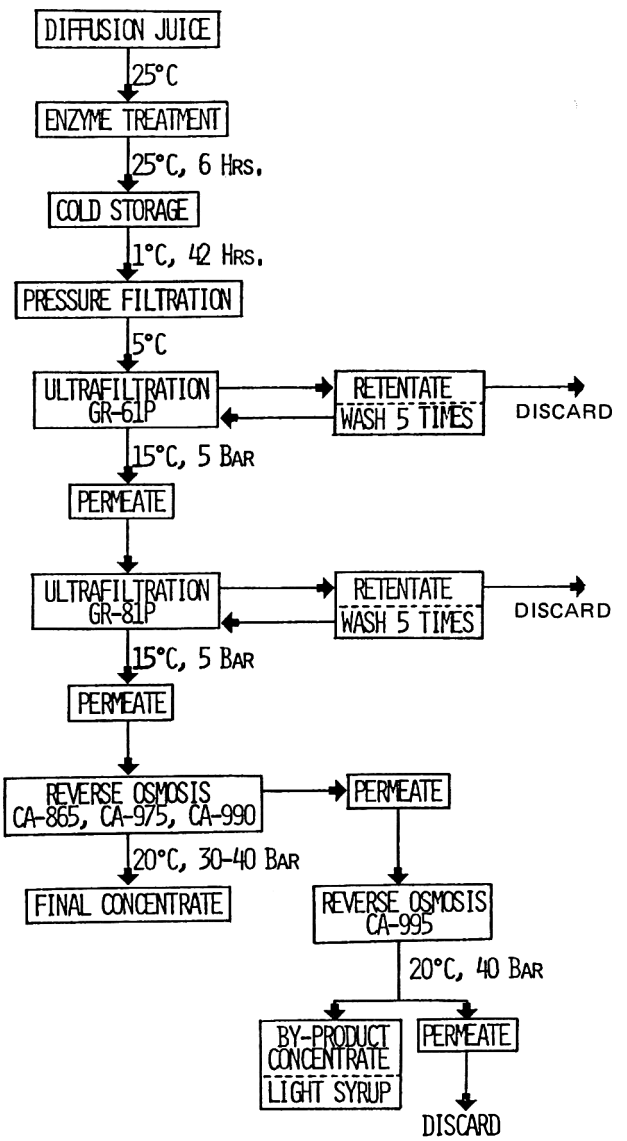


Fig. 2–A schematic diagram of UF and RO processes.

Statistical analysis

Statistical analysis of variance and the Student-Newman-Keul's (SNK) multiple range test for significance was conducted by using UNIVAC 1100/40 computer at the Computer Science Center, University of Maryland.

RESULTS & DISCUSSION

Prefiltration and UF processes

The prefiltering of beet juices in a pressure filter through diatomaceous earth prior to the UF process was difficult due to the presence of pectic substances. Pectic substances formed a colloidal network surrounding the suspended particles and increased juice viscosity. Viscosity is an important factor determining flux and mass transfer characteristics of solutes in the high pressure stream in both UF and RO processes (Pereira et al., 1976). It was found that the addition of pectinase was an essential step for increasing throughput capacity and efficiency in the prefiltering process. Under the experimental conditions of this study, it was observed that no pectic substances remained in the extracted juices after treatment with commercial, food grade pectinase for 6 hr at 25°C. The method used to detect the presence of pectic substances in the enzyme treated juice was an isopropyl alcohol precipitation test. Under these conditions, when invertase was added to pectinase treated juices, the sucrose present in the beet soluble solids was completely inverted to glucose and fructose as analyzed by HPLC.

Lee (1978) observed an increased chroma in the betacyanines in solid-liquid extracted beet juices during 1°C cold storage and postulated that a molecular rearrangement might occur in the red pigments after a heat process and during cold storage. For the purpose of allowing this chroma development in the betacyanines, the enzyme treated juices were held at 1°C for 42 hr before initiating the prefiltering process.

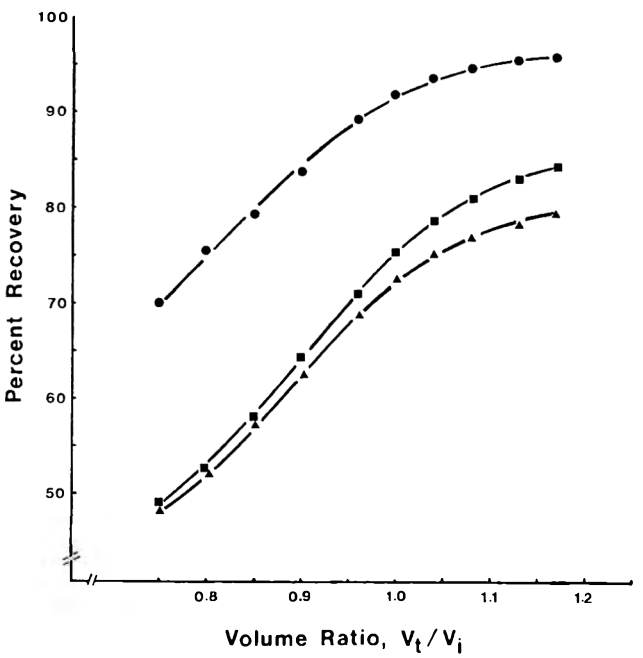


Fig. 3—Percent recovery of betacyanines, betaxanthines and soluble solids as a function of the volume ratio of the throughput UF product to that of the prefiltered juice, using 20,000 MW cut-off (GR-61P) membranes; — ■ — Betacyanines; — ▲ — Betaxanthines, — ● — Soluble solids.

Two types of UF membranes with molecular weight (MW) cut-off of 20,000 (GR-61P) and 6,000 (GR-81P) were used in a dual stage UF process. Fig. 3 shows the recoveries of betacyanines, betaxanthines, and soluble solids from the first stage UF process as a function of the volume ratio of the throughput UF juice (V_t) to that of the initial volume (V_i) using GR-61P membranes. This initial stage of the UF process used 100 L of prefiltered juice (V_i). After 6–8 hr of recirculation, 96L (V_t) of UF purified juice and 4L of retentate were obtained. Only 71, 69, and 89% of the betacyanines, betaxanthines, and soluble solids, respectively, were recovered in the 96L of UF juice. In order to increase the recovery of betalaines, the retentate was washed five consecutive times with 4L of distilled water at each washing. All wash water was added to the 96L of UF purified juice to provide a total of 116L. About 84, 79, and 96% of the betacyanines, betaxanthines, and soluble solids, respectively, were recovered in the 116L. The washed retentate was viscous and consisted mainly of insoluble solids such as soil and intercellular pulp.

The second stage of the UF process used GR-81P membranes and was conducted with the 116L obtained from the first stage process. Betacyanines, betaxanthines, and soluble solids of 66, 60, and 83%, respectively, were recovered in 111.4L of UF filtered juice from the second stage; 4.6L of retentate were obtained. Levels of betacyanine, betaxanthine, and soluble solids were increased to 78, 68, and 92%, respectively, by a washing process similar to that used for first stage UF process. The final volume of the second stage UF purified product and wash water was 135L.

The throughput capacity of the first stage UF process using 6–7°Brix prefiltered juices and GR-61P membranes decreased from 31 to 25 L/m^2·hr after four 8-hr operations. The throughput capacity of the second stage UF process using UF purified product and wash water from the first stage process and GR-81P membranes decreased from 38 to 25 L/m^2·hr after four 8-hr operations. If the prefiltered juices were UF processed directly on the GR-81P membranes, rapid clogging and fouling of the membranes was observed. Moreover, the throughput capacity was decreased from 17 to 12 L/m^2·hr after two 8-hr operations. The juices from the second stage UF process showed high clarity and very low insoluble solids content.

The absence of insoluble solids in the UF purified juices was a significant benefit during subsequent RO processes. According to Matsurra and Sourirajan (1971), insoluble solids suspended in aqueous solutions constitute an immobile phase which blocks the extremely small pores of the CA membranes used in the RO process. It appears that it is necessary for UF processes to be conducted in at least two consecutive steps to promote efficient throughput capacity and UF purified juices of high clarity. A substantial reduction in membrane pore-opening size is a necessary feature of the second step UF process.

Separation and concentration of beet soluble solids in an RO process

This study was conducted in an effort to concentrate betalaines and reduce sugar soluble solids in the final concentrate by manipulating physicochemical RO processing parameters.

Effects of adding chelating agents in the initial feeds. Feed juices treated with EDTA and citric acid yielded only small differences in betacyanine and soluble solids retention in the high pressure stream as compared with control. EDTA and citric acid in the feed were considered to increase electrostatic repulsion and hence increase retention of solutes.

Effects of treating feed juices with invertase. Sucrose, glucose and fructose are nonionized species of organic

solutes in an aqueous solution. The retention intensity of these solutes in the high pressure stream is predominantly governed by the number of polyhydroxylic groups, and by the relative polarity and Taft number and the effectiveness of van der Waals forces among molecules. The majority of the beet soluble solids in the extracted juices was found to be sucrose and varied from 50–70% as analyzed by HPLC. Sucrose has a more negative Taft number than that of glucose and fructose. In general, a more negative Taft number corresponds to more retention intensity on the the high pressure side of the membranes. Furthermore, glucose and fructose in aqueous solution have greater mass transfer and solute transport characteristics than that of sucrose (Pereira et al., 1976).

As indicated in Table 2, the separation of betacyanines from soluble solids benefited from the addition of invertase to pectinase treated juices. The RO final concentrate from pectinase and invertase treated feed juices contained 31.9% of the soluble solids present in the feed juice, whereas the feed juices treated only with pectinase contained 41.6% of the soluble solids in the final concentrate. The data in Table 3 also show that invertase treatment of the feed juice increased the betacyanine level in the final concentrate from 85.8% to 87.9%. Thus, invertase treatment of RO feed juice resulted in a 23.4% reduction of soluable solids and a 2.4% increase of betacyanines in the final concentrate and threefold increase of betacyanine concentration on the dry weight basis.

Flux and processing capacity. Fig. 4 shows the flux as a function of feed concentration during RO processes at 40 bar operating pressure. The addition of invertase to pectinase treated juices caused a slight decrease in flux as compared with those of pectinase treated juices regardless of the type of CA membrane used. The processing capacity of RO process varied from a high of 58.2 L/m^2·hr using CA-865 membranes and pectinase treated initial feed con-

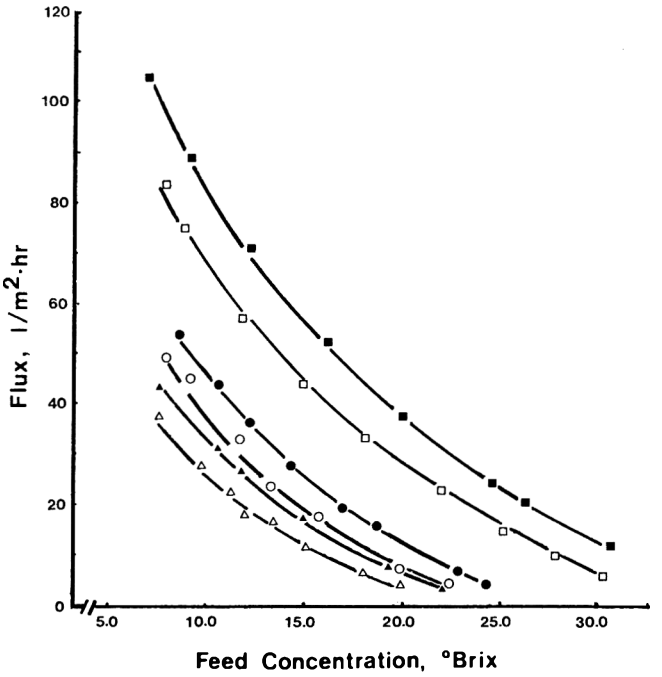


Fig. 4—Flux as a function of feed concentration, using CA-865, CA-975 and CA-990 membranes and operated at 40 bar pressure in the RO process: — ■ — CA-865 membranes, pectinase treated UF product; — □ — CA-865 membranes, invertase and pectinase treated UF product; — ● — CA-975 membranes, pectinase treated UF product; — ○ — CA-975 membranes, invertase and pectinase treated UF product; — ▲ — CA-990 membranes, pectinase treated UF product; — △ — CA-990 membranes, invertase and pectinase treated UF product.

Table 2—Percent of total betacyanines and soluble solids in the final concentrate as influenced by the different enzymatic treatments on the initial feed using CA-865 membranes in the RO process[a]

Enzymatic treatment	Pectinase				Invertase & Pectinase			
Operating pressure (bar)	30	35	40	Avg.	30	35	40	Avg.
Betacyanines	82.2[b]	86.1	89.2	85.8	84.2	88.9	90.5	87.9
Soluble Solids	39.0[b]	41.8	44.2	41.6	30.1	31.7	33.9	31.9

[a] Operating conditions: 0.36 m^2 effective membrane area, 20°C and flow rate 8.0 L/min.
[b] Percent of total betacyanines and soluble solids in the final concentrate, when the amount present in the RO feed is conisdered as 100%. These values represent the percentage of betacyanines and soluble solids that can be recovered in the RO concentrate from the RO feed.

Table 3—Betacyanine, betaxanthine and soluble solids content in the initial feed and final concentrate and their concentration ratio using CA-865 membranes in the RO process[a]

Enzymatic treatment	Pectinase			Pectinase & Invertase		
Operating pressure (bar)	30	35	40	30	35	40
Initial feed						
Betacyanines[b]	460	468	483	455	458	463
Betaxanthines[b]	230	256	252	260	248	246
% Soluble solids	6.1	6.5	6.2	6.3	6.5	6.4
Final concentrate						
Betacyanines[b]	972	962	930	1390	1354	1305
C_f/C_i[c]	2.11	2.06	1.93	3.05	2.96	2.82
Betaxanthines[b]	550	570	572	794	757	725
C_f/C_i[c]	2.39	2.23	2.27	3.05	3.05	2.96
% Soluble solids	28.4	29.3	31.0	28.1	28.9	30.2
C_f/C_i[c]	4.66	4.51	5.00	4.46	4.45	4.72

[a] Operating conditions: 0.36 m^2 effective membrane area, 20°C and flow rate 8.0 L/min.
[b] Concentration of betacyanines and betaxanthines are expressed as mg of betacyanines or betaxanthines per 100g of soluble solids.
[c] Concentration of betacyanines, betaxanthines or soluble solids in the final concentrate over that in the initial feed (C_f/C_i).

centrated at 40 bar operating pressure from 6.2 to 31°Brix to a low of 10.5 L/m²·hr using CA-990 membranes and invertase and pectinase treated initial feed concentrated at 30 bar operating pressure from 7.1 to 16.0°Brix.

Effects of various types of CA membranes. Tables 3, 4, and 5 show the effects of the RO process upon the betacyanine, betaxanthine, and soluble solids content of initial feed versus final concentrate on a dry weight basis as influenced by type of membrane used. The CA membranes with characteristics of 500 MW cut-off and 70% NaCl permeability (CA-865) showed best results of separating betalaines from substantial amounts of soluble solids as indicated by the concentration ratios of the betacyanines and betaxanthines in the final concentrate. Although the other two types of CA membranes (CA-975 and CA-990) showed high retention of all the beet soluble solids, i.e., betalaines and other soluble solids, separation of betalaines from nonpigment soluble solids could not be achieved with these two membranes.

Table 4—Betacyanine, betaxanthine and soluble solids content in the initial feed and final concentrate and their concentration ratio using CA-975 membranes in the RO process[a]

Enzymatic treatment	Pectinase		Pectinase & Invertase	
Operating pressure (bar)	30	40	30	40
Initial feed				
Betacyanines[b]	404	387	430	408
Betaxanthines[b]	227	231	233	221
% Soluble Solids	7.0	7.0	6.9	7.2
Final concentrate				
Betacyanines[b]	457	416	490	444
C_f/C_i[c]	1.13	1.07	1.14	1.09
Betaxanthines[b]	286	272	277	257
C_f/C_i[c]	1.25	1.18	1.19	1.16
% Soluble solids	19.0	23.8	17.2	21.9
C_i/C_i[c]	2.71	3.40	2.49	3.04

[a] Operating conditions: 0.36 m² effective membrane area, 20°C and flow rate 8.0 L/min.
[b] Concentration of betacyanines and betaxanthines are expressed as mg of betacyanines or betaxanthines per 100g of soluble solids.
[c] Concentration of betacyanines, betaxanthines or soluble solids in the final concentrate over that in the initial feed (C_f/C_i).

Table 5—Betacyanine, betaxanthine and soluble solids content in the initial feed and final concentrate and their concentration ratio using CA-990 membranes in the RO process[a]

Enzymatic treatment	Pectinase		Pectinase & Invertase	
Operating pressure (bar)	30	40	30	40
Initial feed				
Betacyanines[b]	430	423	485	445
Betaxanthines[b]	240	236	261	250
% Soluble Solids	6.8	7.1	7.1	6.6
Final concentrate				
Betacyanines[b]	469	463	517	461
C_f/C_i[c]	1.09	1.09	1.06	1.04
Betaxanthines[b]	291	281	294	264
C_f/C_i[c]	1.21	1.19	1.13	1.06
% Soluble solids	19.8	22.4	16.0	20.0
C_i/C_i[c]	2.91	3.15	2.25	3.03

[a] Operating conditions: 0.36 m² effective membrane area, 20°C and flow rate 8.0 L/min.
[b] Concentration of betacyanines and betaxanthines are expressed as mg of betacyanines or betaxanthines per 100g of soluble solids.
[c] Concentration of betacyanines, betaxanthines or soluble solids in the final concentrate over that in the initial feed (C_f/C_i).

The UF purified beet juices treated by pectinase and concentrated on the CA-865 membranes from about 6.5 to 30°Brix showed approximately a ninefold increase in the betacyanine and betaxanthine concentration on a fresh weight basis, i.e., a net twofold increase on a dry weight basis. The addition of invertase to pectinase treated juices effected an approximately 14-fold increase of betacyanine and betaxanthine concentration on a fresh weight basis. This is equivalent to a threefold increase on a dry weight basis.

In one experiment, using invertase and pectinase treated UF purified juice as initial feed material, an operating pressure of 40 bar was used with CA-865 membranes. Initial feed material of 54L at 6.4°Brix was used; 3.5L of final concentrated product at 30.2°Brix and 49.6L of cumulated permeate at 4.6°Brix were obtained in a processing period of 3.1 hr. Approximately 92% of the water present in the initial feed was eliminated from the final concentrate by the RO process. A total of 91, 83, and 34% of the betacyanines, betaxanthines, and soluble solids, respectively, which were present in the initial feed were retained in the 3.5L of final concentrate.

Fig. 5 best exemplifies RO processing data of this experiment. The high retention of the betalaines as compared with soluble solids or sugar solids was probably due to their strong electrostatic repulsion with CA membranes, whereas the soluble solids, primarily glucose and fructose, were to a great extent preferentially sorbed at the membrane-solution interface. The slight increase in solute retention at Brix levels of 6–18° is attributed to molecular attractions among solutes due to van der Waals forces. The marked decrease in the retention of soluble solids and sugar solids when the feed material Brix was higher than 18° was attributed to very high osmotic pressure and therefore a substantially decreased effective pressure (Sourirajan, 1967). The decreased effective pressure caused an increase in solute transport characteristics and/or perhaps a greater effect of the concentration polarization phenomenon which occurs across the membrane phase (Kimura and Sourirajan, 1968a, b).

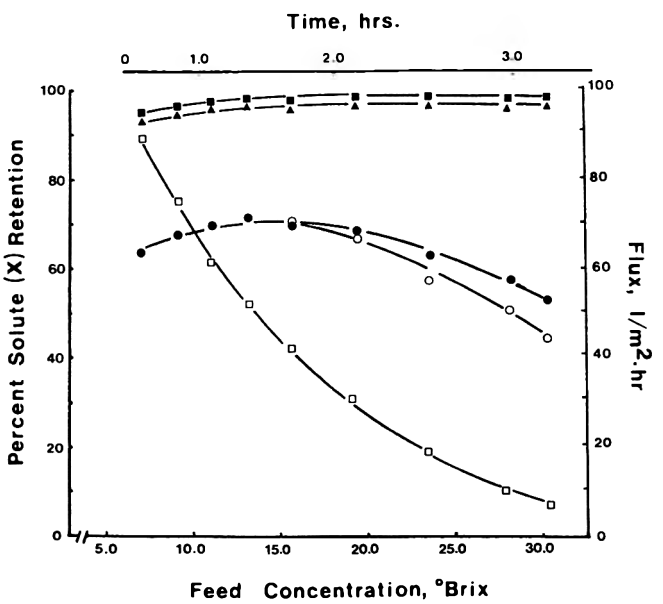


Fig. 5—Processing data on the retention of solutes and flux as a function of feed concentration and processing time, using invertase and pectinase treated UF product, CA-865 membranes, and operated at 40 bar pressure in the RO process: —■— Betacyanines; —▲— Betaxanthines; —●— Soluble solids; —○— Glucose or fructose; —□— Flux.

Flavor evaluations

Sensory evaluation of flavor and odor showed that there was no significant difference between RO process initial feeds and a reference standard of freshly extracted juice. The enzyme treatments, prefilter treatment and/or UF processes did not alter or reduce beet-like flavor and odor characteristics. However, results from sensory evaluation by six trained panelists indicated that there was a significant reduction ($P < 0.01$) of beet-like flavor and odor constituents in the final concentrate vis-a-vis the initial feed when CA-865 membranes were used in the RO process (Table 6). Although the other two types of membranes (CA-975 and CA-990) showed a slight reduction of the beet-like flavor and odor characteristics, statistical analysis indicated this reduction was not significantly different from the flavor-odor characteristics of the initial feed material.

Sensory examination of the permeates from each RO process showed that the beet-like flavor and odor characteristics from the CA-865 membrane permeate were significantly greater ($P < 0.01$) than permeates from the CA-975 and CA-990 membranes.

The total volatility of initial feed and final concentrate samples used for sensory evaluation was also analyzed by gas chromatography. Twelve individual volatile components were detected in each sample tested. Among these, ethanol, dimethyl sulfide and isovaleraldehyde constituted 60–70%, 15–20%, and 5–10%, respectively, of the total volatility. Minor volatile constituents included diacetyl and isobutanol. The volatility ratio varied from a high of 10.7 with an RO process of 40 bar, a CA-865 membrane, and pectinase and invertase treated initial feed to a low of 3.1 with an RO process of 40 bar, a CA-975 membrane and pectinase treated initial feed. In general, a high volatility ratio was obtained when CA-865 membranes were used in the RO process. Analysis of data showed a high positive correlation ($r = 0.86$) between non-pigment soluble solids and total volatility of both initial feed and final concentrate.

Nitrate reduction in concentrates

Concerns regarding the amount of nitrate in processed foods have been increasing in recent years due to the possible carcinogenic effect of nitrosamines which can arise from interactions of nitrates and nitrites with cellular constituents such as proteins (Binkerd and Kolari, 1975). Nitrates are also widely found in vegetables and root crops, i.e., it has been reported that there are 0.4–1.4g of nitrates per 100g of red beet total solids (Peck et al., 1974). The nitrate level of extracted beet juices in this study varied from 0.6–0.9g per 100g soluble solids. After concentrating to 28–30°Brix in the RO process on CA-865 membranes, these levels were reduced to 0.3–0.5g per 100g of soluble solids.

Table 6–Mean score of sensory evaluation by six trained panelists of the diluted final concentrate and the volatility ratio of the initial feed by GC analyses

Enzymatic treatment		Sensory evaluation[a]		GC analyses[b]	
		Pectinase	Invertase	Pectinase	Invertase
Membrane type	Operating pressure (bar)				
CA-865	30	1.6 a	1.7 a	7.0 xy	8.4 x
	40	1.6 a	1.8 a	9.5 x	10.7 x
CA-975	30	4.0 b	4.0 b	3.2 z	4.8 yz
	40	3.6 b	3.2 b	3.1z	4.3 z
CA-990	30	3.3 b	3.5 b	3.4z	4.1 z
	40	3.5 b	3.3 b	3.4z	3.3 z
Initial Feed		4.0 b	4.1 b	–	–
		$P < 0.01$		$P < 0.01$	

[a] Mean score of six trained panelists, numbers followed by different letters are significantly different ($P < 0.01$), by SNK multiple range test.
[b] Volatility ratio = (Volatility in the initial feed/Volatility in the diluted final conc, numbers followed by different letters are significantly different ($P < 0.01$), by SNK multiple range test.

Utilization of permeate containing sugar solids

The permeate fluid from the CA-865 membrane RO process usually had a soluble solids content of 4–5°Brix and contained a substantial amount of nonpigment, beet soluble solids. When this permeate fluid was concentrated up to 20°Brix at 40 bar operating pressure using a CA membrane with characteristics of 180 MW cut-off and 4% NaCl permeability (CA-995, see Table 1), 95% recovery of soluble solids was obtained with an RO processing capacity of 16.7 $L/m^2 \cdot hr$. This CA-995 membrane, RO processed light-sugar syrup contained very negligible betalaine levels. These betalaines could be removed by filtration through activated charcoal to produce a clear sugar syrup, or it could be utilized as a substrate for alcohol production by fermentation.

CONCLUSIONS

These experiments have shown that the UF and RO processes are acceptable methods to obtain highly purified and concentrated betalaines. Pectinase treatment reduced viscosity of extracted juices and increased efficiency of the prefiltration process through diatomaceous earth and later benefited UF and RO processes. Purification by UF in two consecutive steps, using first 20,000, and then 6,000 MW cut-off membranes provided material with clarity and negligible insoluble solids content. The use of RO to concentrate pectinase treated UF purified products from about 6.5 to 30°Brix on 500 MW cut-off and 70% NaCl permeability CA membranes reduced the initial water volume by 92% and gave a two-fold increase in betalaine content on a dry weight basis. This process provided a processing capacity of 58.2$L/m^2 \cdot hr$. However, the addition of invertase to the pectinase treated UF products slightly decreased flux while increasing concentration of betalaines threefold on a dry weight basis. Approximately 70–75% of the betacyanines introduced into the UF processes were recovered in the RO concentrates. The final concentrate was reduced in nitrates and lacked beet-like flavor and odor characteristics as evaluated by trained panelists and analyzed by gas chromatography.

REFERENCES

Adams, J.P., von Elbe, J.H., and Amundson, C.H. 1976. Production of a betacyanine concentrate by fermentation of red beet juice with Candida utilis. J. Food Sci. 41: 78.

Binkerd, E.F. and Kolari, O.E. 1975. The history and use of nitrate and nitite in the curing of meat. Food Cosmet. Toxicol. 13: 655.

Brennan, J.G., Butters, J.R., Cowell, N.D., and Lilly, A.E. 1976. Filtration and membrane separation. In "Food Engineering Operations," Chapt. 6, p. 120, 2nd ed. Applied Science Publishers Ltd., London.

Breton, E.J. Jr. and Reid, C.E. 1957. Report No. 16. Office of Saline Water, Res. and Develop. Progress

Cataldo, D.A., Haroon, M., Schrader, L.E., and Youngs, V.L. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicyclic acid. Commun. Soil Science & Plant Analysis. 6(1): 71.

Dignan, D.M. and Wiley, R.C. 1976. DMS levels in the aroma of cooked frozen sweet corn as affected by cultivar, maturity, blanching, and packaging. J. Food Sci. 41: 346.

Kimura, S. and Sourirajan, S. 1968a. Concentration polarization effects in reverse osmosis using porous cellulose acetate membranes. Ind. Eng. Chem. Process Design Develop. 7: 41.

Kimura, S. and Sourirajan, S. 1968b. Transport characteristics of porous cellulose acetate membranes for reverse osmosis separation of sucrose in aqueous solutions. Ind. Eng. Chem. Process Design Develop. 7: 548.

–Continued on page 475

Characteristics of Frozen Desserts Sweetened with Xylitol and Fructose

J. RUBEN ABRIL, J. WARREN STULL, RALPH R. TAYLOR, ROBERT C. ANGUS, and TERRY C. DANIEL

ABSTRACT

Combinations of xylitol, fructose and 24 DE corn syrup solids (CSS) were the sweetening agents in ice cream-type frozen desserts. A 2^3 factorial experimental design evaluated the most acceptable combinations of xylitol (4% or 6%), fructose (4% and 6%) and CSS (10% and 12%). Based on chemical, physical and sensory analyses of the eight experimental combinations it was demonstrated that a formulation involving 6% xylitol, 6% fructose and 10% CSS gave product acceptability similar to that of an ice cream with conventional ingredient array and composition.

INTRODUCTION

WHILE SUCROSE has been the major sweetener used in food products, the introduction of high-fructose corn syrups (HFCS) has caused shifts in utilization and food formulations. Interest in nonnutritive and other new sweeteners remains active.

One of the problems with some nonnutritive and other sweeteners is their tendency for an afterimage characteristic. The objective of the present research was to use a combination of materials which might impart the same combined sweetness characteristic as provided by sucrose and at the same time to include an ingredient array in frozen desserts that may be suitable for use by many diabetics.

Xylitol is a polyalcohol with a relitive sweetness (RS) approximately the same as sucrose (RS = 100). As compared to sucrose, it does not cause blood glucose elevation during metabolism. Major studies have shown that some diabetics can utilize fructose and starch readily (Brunzell, 1978).

Current standards of identity for ice cream do not provide for the use of xylitol as a sweetener. Regulatory provisions (FDA, 1980) indicate that xylitol may be safely used in foods, however, for special dietary uses provided the amount used is not greater than that required to produce its intended effects. The annual world production of xylitol is about 6000 tons compared to 80 million of sucrose (Counsell, 1978). Improved technology may increase the availability and reduce the cost of xylitol.

In this study, some conventional nonfat dairy ingredients normally used in ice cream were replaced by comparatively inexpensive materials such as cornstarch, CSS and sodium caseinate. This accounts for the use of ice cream-type frozen dessert terminology in reference to the experimental products.

MATERIALS & METHODS

SINCE NO REPORTS were found in the literature involving the use of xylitol as a sweetener in dairy frozen desserts, various preliminary trials were made to assist in identifying the composition parameters to be studied in the final experimental formulations. A 15% sucrose equivalent was used for comparison in the preliminary formulations, the other ingredients being 10% milk fat, 11% milk solids nonfat (MSNF), and 0.3% stabilizer/emulsifier. At first, one-third (5%) of the sucrose was replaced with xylitol. This gave a product comparable to the control. Certain other formulation modifications were made in order to lower the net price per pound of the mixes. Cornstarch and wheat flour were considered at a level of 2% each, but the resultant dessert had a detectable cereal flavor. Next, MSNF was partially replaced by low DE CSS (24DE). Sucrose was totally replaced by xylitol and fructose. Eventually the 2% flour was replaced by sodium caseinate at an equivalent level. Finally, the ingredients used in the experimental desserts were as follows: frozen cream, nonfat dry milk (NFDM), xylitol, fructose, cornstarch, sodium caseinate, corn syrup solids (CSS) (24DE) and stabilizer/emulsifier.

A 2^3 factorial design was used with fructose, xylitol and CSS being the variable ingredients each at two concentrations (Table 1). The following ingredients and concentrations provided the rest of the formula: 10% milk fat, 2% MSNF, 2% sodium caseinate, 2% cornstarch and 0.3% stabilizer/emulsifier (Table 2).

It should be noted that several days after mix processing, an error in weighing dry ingredients for sample B (Table 1) was discovered. Insufficient ingredient supply prevented correction and reformulation. This resulted in a modified formulation for Sample B; namely, 6% fructose and 10% CSS only. All calculations concerning statistics and sensory evaluation were made on the basis that Sample B was formualted as intended in the original ingredient combinations.

Mix preparation. Approximately 40L of each mix were prepared. The dry ingredients were dispersed in the liquid materials by circulation through a high-velocity centrifugal pump. Mixes were heated by a vat process in 40L milk cans to 60°C, homogenized with a Manton-Gaulin, two-stage homogenizer, (first stage at 140 kg/cm^2 and the second at 35 kg/cm^2), pasteurized at 85°C/15 sec and cooled to 4°C.

Freezing. In preparation for freezing, coloring and flavoring (vanilla) were added. The mixes were frozen with a commercial Cherry-Burrell continuous freezer (320L/hr) to approximately 90–100% overrun. The frozen products were packaged in 60 ml individual plastic containers for panel testing, or in 0.47L paper ice cream containers for other observations.

Sensory evaluation. An eighteen-member, untrained sensory panel (9 males, 9 females) of the University faculty, staff and students evaluated the acceptability of the products. Their ages ranged from 18–50 yr. The panelists were instructed about testing and recording details at each session. Panelists were seated in individual booths and each panelist was presented nine samples each day.

Table 1—Treatment variables in frozen dessert mixes

Mix	Xylitol	Fructose	CSS	Glucose (mg/100 ml)[a]
	---------- % ----------			
A	6	4	10	0.7
B	4	4	10	0.7
C	6	6	10	0.7
D	4	6	10	0.7
E	6	4	12	0.84
F	4	4	12	0.84
G	6	6	12	0.84
H	4	6	12	0.84
I	---------- Control ----------			

[a] Calculated on the basis of 7% glucose in 24 DE CSS (MacAllister, 1979).

Authors Abril, Stull and Taylor are with the Dept. of Nutrition & Food Science; Author Angus is with the Dept. of Agricultural Economics; and Author Daniel is with the Psychology Dept., Univ. of Arizona, Tucson, AZ 85721.

Each panelist tasted a total of 27 samples by the end of the study. Containers were labeled with three-digit random numbers for each replication. The presentation order was also randomized at each session. The samples were tempered at −18 ± 1°C for 24 hr prior to evaluation and were presented at a rate of one about every 2 min. Panelists used an unstructured, hedonic rating scale where 9 = "like" and 0 = "do not like." Analysis of data was by the theory of signal detection (TSD) method (Angus and Daniel, 1974; Stull et al., 1974; Young et al., 1980).

A panel of five trained, experienced ice cream judges examined half-gallon containers of each frozen dessert after 1 wk and then once a month for an additional 4 months. The American Dairy Science Association Dairy Products Judging Guidelines were used to score flavor and body/texture. The samples were stored in a commercial freezer at −18 ± 1°C.

Chemical and physical tests. Fat in the final products and in the frozen cream was determined by the Babcock method. A cross reference test was made in the dessert by the Mojonnier Method (Goss, 1953). Solids were determined with the Mojonnier milk tester. Protein was determined in duplicate by the standard Kjeldahl method. Glucose values were calculated on the basis that 24DE CSS contains 7% of that material (MacAllister, 1979). The viscosities of the mixes were determined by the use of a Brookfield viscosimeter. Shrinkage was measured using the method described by Young (1980). Measurements were taken every week for several weeks.

Meltdown determinations were made after 1 month storage in a commercial freezer at −18 ± 1°C. The method was that of Young (1978) and Nickerson and Pangborn (1961), using a 6 mm mesh wire. The shape of the frozen dessert sample was cylindrical, 36 mm in diameter and 70–95 mm long, giving an average weight of 47g. The average room temperature was 28°C.

Calorimetry was performed by first melting the frozen dessert and then dehydration at 100°C in vacuo. Moisture was determined and an average of 5% was calculated for the five samples. Caloric value was determined on one gram aliquots, using a Parr Bomb Calorimeter.

RESULTS & DISCUSSION

CERTAIN CHARACTERISTICS of the desserts are given in Table 3. Fat, total solids, solids-not-fat, and caloric values are not included but they were within calculated ranges.

Viscosities of the experimental desserts were much higher than the normal range of 30–300 cp for ice cream mixes (Arbuckle, 1977). The most probable cause was the combined hydrophilic effect of CSS together with that of starch and stabilizer. Viscosity values could be lowered by stabilizer elimination and substitution of a modified, low-hydrophilic starch for the regular product.

The protein concentration of the experimental mixes ranged from 2.38–2.50%. The control ice cream had 4.17% protein. For example, Kristoffersen and Miller (1976) checked fifteen brands of commercial ice cream and found protein values ranging from 2.49–4.38% (mean 3.41%). Normally a 6.25 conversion factor is used for protein content calculation for many foods, but for ice cream a 6.38 factor should be used (Jones, 1931).

The expected freezing point depression effect related to the lower molecular weight of xylitol, fructose and glucose (in CSS) was not readily apparent in melt-down values. This was probably due to the effect of the CSS, starch and stabilizer. Sample (C), with the highest melt-down (26 ml), had 12% combined xylitol/fructose and 10% CSS. The similar product (G) with identical sweeteners except for

Table 2—Ingredients used in mix formulation

	Sample								
	A	B	C	D	E	F	G	H	I
Ingredients	%								
Fructose	4.0	4.0	6.0	6.0	4.0	4.0	6.0	6.0	–
Xylitol	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	–
CSS	10.0	10.0	10.0	10.0	12.0	12.0	12.0	12.0	–
Cream[a]	24.3	24.3	24.3	24.3	24.3	24.3	24.3	24.3	24.3
MFDM[b]	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	11.0
Starch	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	–
Caseinate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	–
Water	50.7	52.7	48.7	50.7	48.7	50.7	46.7	48.7	49.37
Stabilizer/Emulsifier	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.33
Sucrose	–	–	–	–	–	–	–	–	15.0
Total solids (TS)	36.0	34.0	38.0	36.0	38.0	36.0	40.0	38.0	37.3

[a] 41% fat, 5.4% MSNF
[b] 95% MSNF, 5% H_2O

Table 3—Representative data on finished frozen desserts: protein; meltdown; shrinkage; flavor and body-texture scores by expert panel; mean d_m ratings by untrained sensory panel

Frozen dessert	Protein (%)	Melt-down (ml/50 ± 9g /45 min)	Shrinkage 9 wk Edges (mm)	Shrinkage 9 wk Top (mm)	Shrinkage 13 wk Edges (mm)	Shrinkage 13 wk Top (mm)	Flavor scores (week after freezing) 1	Flavor scores (week after freezing) 13	Body & texture scores (week after freezing) 1	Body & texture scores (week after freezing) 13	Mean d_m ratings
A	2.47	0	3	0	3	2	8	8	3	3	48
B	2.57	0	1	0	2	2	8	8	4	3	9
C	2.38	26	2	0	3	3	8	9	4	4	58
D	2.46	1	2	1	2	3	9	8	3	3	54
E	2.53	<1	1	1	2	2	8	8	3	3	5
F	2.45	7	1	0	2	1	8	8	4	3	11
G	2.46	6	1	3	4	3	8	8	2	3	31
H	2.50	13	3	1	5	5	8	8	3	4	48
I	4.17	18	1	2	2	4	9	8	4	4	–

12% CSS, however, had only 6 ml melt-down. Experimental dessert (H) had a melt-down (13 ml) that was most similar to the control ice cream (18 ml). In addition to the stabilizing effect of various ingredients, processing methods may also have an effect on melt-down (Sperry, 1980).

Each product, including the regular ice cream, demonstrated a slight tendency for shrinkage development at the end of 13 wk storage. Shrinkage predisposition factors are complex (Nickerson and Tarassuk, 1955) and are not readily evident in the ingredient array variations in this work. Sample (H) with a slightly greater tendency for shrinkage, for example, had generally satisfactory characteristics in melt-down, flavor, body and texture.

The most evident flavor defect was cooked which probably originated with the heat treatment method and caseinate used. This could be reduced by a combination of high-temperature-short-time (HTST) pasteurization, higher quality caseinate and a more intense flavor masking effect provided by materials such as chocolate.

Body and texture of the experimental desserts had a tendency for slight gumminess. This was probably due to the CSS and starch ingredients. Some coarseness and weak body characteristics were evident. No sandiness was detected because of the low or insignificant lactose content in the experimental desserts.

The variable used to measure the degree of preference or discrimination by the untrained panel was the dm value (distance metric) (Angus and Daniel, 1974; Stull et al., 1974). A higher dm indicates that the observer can distinguish more clearly between the control and the experimental sample (Young et al., 1980). In the experiment, the most preferred sample was (C) (d_m = 58) with 6% xylitol, 6% fructose and 10% CSS. Product (E) (d_m = 5; 6% xylitol, 4% fructose, 12% CSS) was least preferred. Desserts with 6% fructose (C, D, G, H) were preferred to those with 4% fructose (A, B, E, F). Those with 10% CSS (A, B, C, D) were usually rated higher than ones with 12% CSS (E, F, G, H) but the trend was not as consistent as with the fructose variable. The dessert containing 6% xylitol, 4% fructose and 10% CSS (Sample A) and the one with 4% xylitol, 6% fructose and 12% CSS (Sample H) had identical preference ratings (d_m = 48).

The comparative dm results may be more clearly seen as plots of the data (Fig. 1 and 2).

In desserts with 4% fructose (Fig. 1) and 4% xylitol, increasing CSS from 10% to 12% had no effect on preference (Sample B vs F). By comparison, in dessert formulations involving 4% fructose and 6% xylitol increasing the % CSS (Sample A) had a dm value (48) slightly lower than in the product with 6% fructose and with equal amounts of the other sweeteners (Sample C, Fig. 2). In desserts with 6% fructose and 4% xylitol (Fig. 2), increasing CSS from 10% to 12% (Sample D vs H) resulted in a minor change in d_m values. By comparison, sample C vs G had a marked decrease in preference when CSS was increased from 10% to 12%. In the case of desserts with 4% xylitol, however, increasing CSS from 10% to 12% did not lower the d_m values. In general, the results of the taste panel agree well with the findings of the expert observers. The effect of fructose as a possible masking agent for afterimages may be evident in several samples where the fructose concentration was increased from 4% to 6% (Sample H vs E) (Doty and Vanninen, 1975).

It has been pointed out that a sweet ice cream is generally preferred (Arbuckle, 1977). Certain comparisons are of interest, therefore, regarding sweetness intensity (SI) and preference (d_m) (Fig. 3). [SI = (RS_1) (% concentration) + (RS_2) (% concentration) + etc.] The most preferred sample (C) had a d_m of 58 and a SI of 1717. This was slightly exceeded in SI by that of sample (G) (1726) which had a d_m of only 31. In general, increasing dm values or preference are in direct proportion to increasing sweetness (Fig. 3). The slight decrease in d_m in sample (E) with a higher SI than in product (F), however, is not well understood and is an exception.

As discussed earlier, sample (B) actually contained 6%

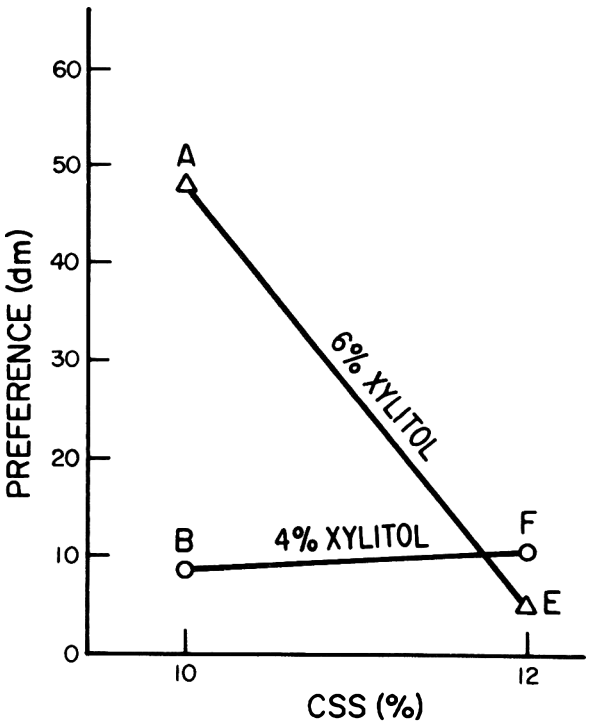


Fig. 1—Mean preference ratings (in d_m) for frozen desserts containing 4% fructose as a function of xylitol and CSS contents. A, B, E, F refer to samples identified in Table 1.

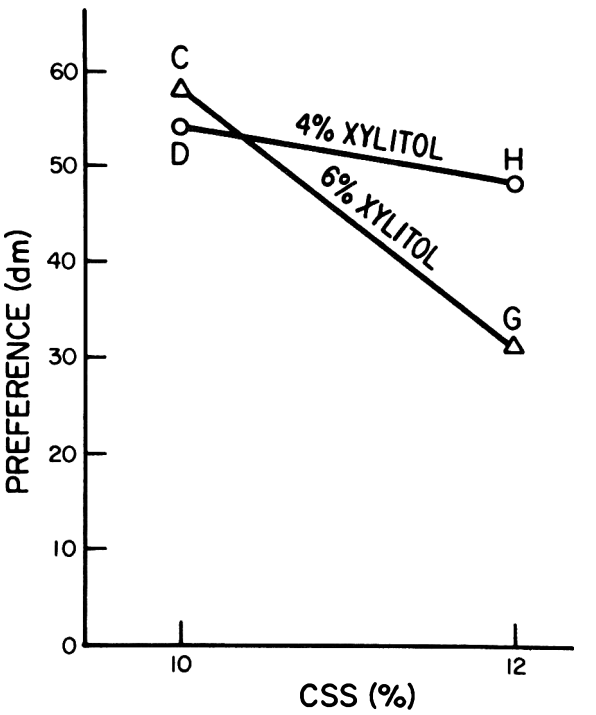


Fig. 2—Mean preference ratings (in d_m) for frozen desserts containing 6% fructose as a function of xylitol and CSS contents. C, D, G, H refer to samples identified in Table 1.

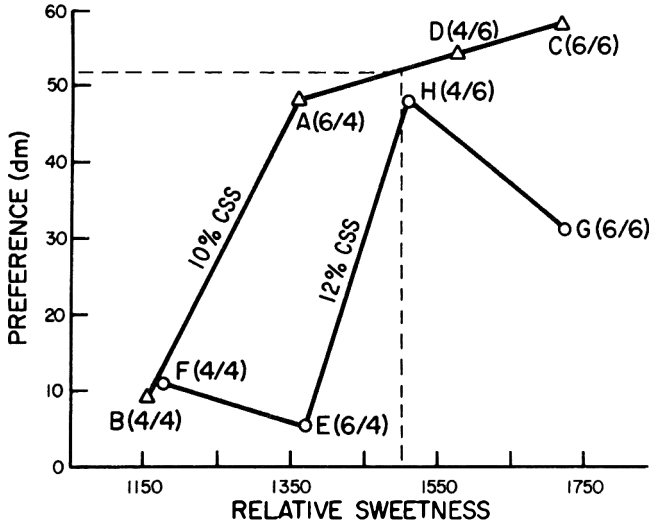


Fig. 3–Preference (d_m) for frozen desserts related to sweetness intensity (SI). Letters refer to samples identified in Table 1.

fructose and no xylitol. Based on SI only, it might be expected that there would be no marked difference in the dm value with the intended 4% fructose and 4% xylitol since the SI is almost identical (Fig. 3; 1126 vs 1161).

The lower protein values in the experimental mixes are due principally to the replacement of MSNF with starch and CSS. The protein concentration could be raised 0.2–0.3% to meet standards (FDA, 1977) by the addition of materials such as caseinates or whey solids.

This study has demonstrated that xylitol, fructose and CSS can be used as the major sweeteners in a frozen dairy dessert having properties similar to those of an ice cream with conventional ingredient array and composition. Minor adjustments in the experimental ingredient combination can be expected to reduce the observed high viscosity. Similarly, a need for a comparatively slight increase in protein is indicated and can be accomplished readily by various options in the selection of nonfat materials.

REFERENCES

Angus, R.C. and Daniel, T.C. 1974. Applying theory of signal detection in marketing: Product development and evaluation. Am. J. Agric. Econ. 56: 573.

Arbuckle, W.S. 1977. "Ice Cream," 3rd ed. Avi Publishing Co., Westport, CT.

Brunzell, J.D. 1978. Use of fructose, xylitol or sorbitol as a sweetener in diabetes mellitus. Diabetes Care 1: 223.

Counsell, J.N. (ed). 1978. "Xylitol." Applied Science Publishers Ltd., London.

Doty, T.E. and Vanninen, E. 1975. Crystalline fructose: Use as a food ingredient expected to increase. Food Technol. 29: 34.

Food & Drug Administration. 1977. Revision of standards of identity for ice cream, ice milk, sherbet, and water ices. Fed. Reg. 42(70): Apr. 1.

Food & Drug Administration. 1980. Food additives permitted for direct addition to food for human consumption. Fed. Reg. 21(23): Apr. 1.

Goss, E.F. 1953. Techniques of Dairy Plant Testing. Iowa State College Press, Ames, Iowa.

Jones, D.B. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. U.S. Dept. Agr. Circ. 183.

Kristoffersen, T. and Miller, J.R. 1976. Protein in vanilla ice cream. Dairy Ice Cream Fld. 159: 38.

MacAllister, R.B. 1979. Nutritive sweeteners made from starch, p. 15. In "Advances in Carbohydrate Chemistry and Biochemistry," Ed. R.S. Tippson and D. Norton. Academic Press, New York.

Nickerson, T.A. and Pangborn, M.R. 1961. The influence of sugar in ice cream. 3. The effect on physical properties. Food Technol. 15: 105.

Nickerson, T.A. and Tarassuk, N.P. 1955. How to control shrinkage in ice cream. J. Dairy Sci. 38: 1305.

Sperry, G.D. 1980. Production of dairy products. The importance of melt-down. NICRA Bulletin.

Stull, J.W., Angus, R.C., Taylor, R.R., Swartz, A.N., and Daniel, T.C. 1974. Rich flavor discrimination in ice cream by theory of signal detection. J. Dairy Sci. 57: 1423.

Young, K.Y. 1978. Neutralized, fluid cottage cheese whey in frozen dairy desserts. M.S. thesis, Univ. of Arizona, Tucson.

Young, K.Y. 1978. Neutralized fluid cottage cheese whey in frozen dairy desserts. M.S. thesis, Univ. of Arizona, Tucson.

Young, K.Y., Stull, J.W., Taylor, R.R., Angus, R.C., and Daviel, T.C. 1980. Acceptability of frozen desserts made with neutralized, hydrolyzed fluid cottage cheese whey. J. Food Sci. 45: 805.

Ms received 6/20/81; revised 10/10/81; accepted 10/12/81.

BETALAINE CONCENTRATION BY UF AND RO . . . From page 471

Lee, Y.N. 1978. Efficiency studies of a solid-liquid extraction method to recover betalaines from red table beets. M.S. thesis, Univ. of Maryland, College Park, MD.

Lowe, E., Durkee, E.L., Merson, R.L., Ijichi, K., and Cimino, S.L. 1969. Egg white/concentrated by reverse osmosis. Food Technol. 23: 753.

Madsen, R.F. 1974. Membrane concentration. In "Advances in Preconcentration and Dehydration of Foods," p. 251. Applied Science Publishers Ltd., London.

Marshall, P.G., Dunkley, W.L., and Lowe, E. 1968. Fractionation and concentration of whey by reverse osmosis. Food Technol. 22: 969.

Matsuura, T., Baxter, A.G., and Sourirajan, S. 1973. Concentration of fruit juices by reverse osmosis. Acta Alimentaria 2(2): 109.

Matsuura, T., and Sourirajan, S. 1971. Physicochemical criteria for reverse osmosis separation of alcohols, phenols, and monocarboxylic acids in aqueous solutions using porous cellulose acetate membranes. J. Appl. Polym. Sci. 15: 2905.

Merson, R.L. and Morgan, A.I. Jr. 1968. Juice concentration by reverse osmosis. Food Technol. 22: 631.

Nilsson, T. 1970. Studies into the pigments in beet root. Lantbr. Hogsk. Annsl. 36: 179.

Peck, N.H., Cantliffe, D.J., Shallenberger, R.S., and Bourke, J.B. 1974. Table beets (Beta vulgaris L.) and nitrogen. Search 4:1.

Pereira, E.N., Matsurra, T., and Sourirajan, S. 1976. Reverse osmosis separations and concentrations of food sugars. J. Food Sci. 41: 672.

Saguy, I. 1979. Thermostability of red beet pigments (betanine and vulgaxanthine-I): Influence of pH and temperature. J. Food Sci. 44: 1554.

Singer, J.W. and von Elbe, J.H. 1980. Degradation rates of vulgaxanthine-I. J. Food Sci. 45: 489.

Solomos, T. and Warman, T.W. 1982. Purification and isolation of table beet root pigments. J. Food Sci. In press.

Sourirajan, S. 1967. Reverse osmosis separation and concentration of sucrose in aqueous solutions using porous cellulose acetate membranes. Ind. Eng. Chem. Process Design Develop. 6: 154.

von Elbe, J.H., Maing, I.Y., and Amundson, C.H. 1974. Color stability of betanine. J. Food Sci. 39: 334.

Wiley, R.C. and Lee, Y.N. 1978. Recovery of betalaines from red table beets by a diffusion-extraction procedure. J. Food Sci. 43: 1056.

Wiley, R.C., Lee, Y.N., Saladini, J.J., Wyss, R.C., and Topalian, H.H. 1979. Efficiency studies of a continuous diffusion-extaction apparatus for the recovery of betalaines from red table beets. J. Food Sci. 44: 208.

Willits, C.O., Underwood, J.C., and Merten, U. 1967. Concentration by reverse osmosis of maple syrup. Food Technol. 21: 24.

Woo, A.H., von Elbe, J.H., and Amundson, C.H. 1980. Anthocyanin recovery from cranberry pump wastes by membrane technology. J. Food Sci. 45: 875.

Ms received 6/5/81; revised 10/26/81; accepted 10/31/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA June 7—10, 1981.

Scientific Article No. 3015, Contribution No. 6078 of the Maryland Agricultural Experiment Station, Dept. of Horticulture (Food Science Program).

The authors thank K. Demaree for assistance in the solid-liquid extraction processing work. Thanks also to De Danske Sukkerfabrikker for providing the Model B diffuser.

Heat Transfer Rates to Liquid Foods During Flame-Sterilization

R. O. TEIXEIRA NETO

ABSTRACT

The influences of headspace, rotational speed of the can, and product viscosity on the rates of heat transfer during flame-sterilization of carboxy-methyl-cellulose (CMC) model solutions, were studied. It was observed that more than 90% of the heat transfer resistance was external, and so the headspace volume and product viscosity had almost no effect on the rates of heat transfer, whilst the can rotational speed was the important parameter that needed to be controlled. An empirical correlation between the Nusselt, Reynolds and Prandtl dimensionless numbers for the external and internal conditions was obtained, in order to describe the heat transfer as a function of the hot air and product thermophysical properties, the can r.p.m. and the diameter. The results will be useful for improving flame-sterilizer utilization.

INTRODUCTION

FLAME-STERILIZATION is a process that achieves sterilization of canned foods by heating the cans through direct contact with a gas flame. Some forced convection in the food can be induced through rotation of the cans. This process is generally four to five times faster than the conventional retort sterilization and is recommended for liquid products, even for those of high viscosities, and for sliced products suspended in brines, syrups or fruit juices.

Flame-sterilization is known to present advantages with respect to texture, color, and vitamin and flavor retention when compared to the conventional processes (Kieseker, 1972; Klepetko and Longworth, 1972; Leonard, 1975; Leonard et al., 1975 a,b,c,d; Leonard et al., 1976). These results support the common observation that from the quality standpoint, fast heating followed by fast cooling is generally desirable, since it will minimize the product exposure to intermediate temperatures that are not so effective for the destruction of microrganisms, but nevertheless contribute, to a significant extent, to the overcooking of the product. This is possible because the rate of microrganism destruction has a much higher activation energy than most of the heat induced reactions which result in loss of desirable nutrients, flavors and colors. Leonard et al. (1975a) reported other technical advantages of the flame-sterilizer as compared to the conventional ones.

One important restriction of the flame-sterilizer is its limitation in only adequately handling cans up to a maximum size of (74 x 110)mm, i.e., 1/2kg cans. Mechanical damage can occur when working with the larger can sizes due to excessive internal pressure developed during processing.

In flame-sterilization cans are heated in contact with a gas flame at about 1000°C. The optimization of the operation results in a compromise between high heat transfer rates to the product and minimum burn problems. Paulus and Ojo (1974), studying the heat transfer properties of bentonite solutions at different concentrations, concluded that the most important variables affecting the performance of a flame-sterilizer were product viscosity, burner arrangement, flame temperature, can rotation speed and headspace volume.

A systematic study of the effect of some of these variables has been done for other continuous cookers. Quast and Siozawa (1974), for instance, presented data on the heat treatment of fluid products in a pilot plant spin-cooker-cooler. They worked with water, sucrose solutions and CMC solutions, and they obtained overall heat transfer coefficients (U) in the range 1370–2270, 180–1910 and 180–2560 Kcal/hr·m^2·°C, respectively. They also reported "U" values found by other authors using conventional retorting and hydrostatic cooking of different products, and these values generally ranged from 180–360 Kcal/hr·m^2·°C. They derived empirical correlations among the Nusselt, Reynolds and Prandtl numbers, for the sucrose and CMC solutions. These correlations were then used to predict the "U" values of food products with physical properties similar to these solutions.

It is known that the "U" values for the flame-sterilizer are far lower than for the conventional sterilizers using saturated steam as the heating medium, this difference being ascribed almost entirely to the superiority of the heat transfer coefficient of the saturated steam compared to the hot gases of the flame. Apart from this, in the flame-sterilization process, since the flame heats only the lower part of the can and the upper part is in contact with gases at lower temperatures (Paulus and Ojo, 1974), a cooling effect would be expected as a function of the rotation speed of the can. This effect was demonstrated by Sherwood et al. (1975). However, despite the small "U" values and heat transfer area, the flame-sterilization process presents a faster heating cycle when compared to conventional sterilization, due to the very high temperature differences between the flame and the product.

The main objective of this work was to investigate the influence of some parameters that were thought to be important in the heat transfer mechanism during flame-sterilization, and also to attempt to correlate the data obtained using dimensionless numbers.

MATERIALS & METHODS

Flame-sterilizer

A pilot scale flame-sterilizer was used to heat only one can at a time. The sterilizer consisted of a metallic chamber with interchangeable top and bottom modules necessary for steam preheating, flame heating and water cooling. The can was supported in the middle of the chamber by two sets of two rollers each (Fig. 1). The rollers could be rotated at different speeds through a varidrive. The flame intensity could be adjusted through a pressure reducing valve and a needle valve in the gas line, coupled to a pressure gauge and a rotameter, respectively.

Viscosity measurement

The viscosity of the solutions was determined with an EPPRECHT viscometer, model TVB, always using spindle no. 2 at 240 r.p.m., to provide apparent viscosity data at the same shear rate. Viscosity data were obtained at four different concentrations and temperatures.

Author Teixeira Neto is affiliated with the Dept. of Food Engineering, Instituto de Tecnologia de Alimentos Campinas, SP Brazil 13110.

Density measurement

A previously calibrated graduated cylinder was filled with different CMC solutions at 20°C and weighed in order to determine the density of the solution. Care was taken to avoid aeration of the solution. Every determination was done in duplicate.

Temperature measurement

Copper-constantan thermocouples were passed through the center of one flat end of the can, and then connected to a special rotary joint that was wired to an automatic temperature recorder. The measurement point inside the can was the geometric center of the same.

Experimental procedure

CMC solutions at four different concentrations were prepared and poured into the cans at 25°C to different headspace volumes. The thermocouples were mounted and the cans were sealed under a vacuum of 640mmHg in order to avoid excessive internal pressure build up during heating. The cans used were 1/2 kg in capacity.

The thermocouples were connected to the temperature recorder and the can was placed in the heating chamber. The desired rotation speed was set and the preheating step was started using saturated steam injection through the top of the chamber. When the can temperature reached 90–95°C the steam was purged from the chamber and a flame, previously adjusted, was positioned underneath the rotating can. After the can temperature rose to 121°C, the burner was removed, and three water spray nozzles positioned at the upper part of the chamber were employed to cool the can rapidly. Hot water was drained from the bottom of the chamber through an appropriate outlet.

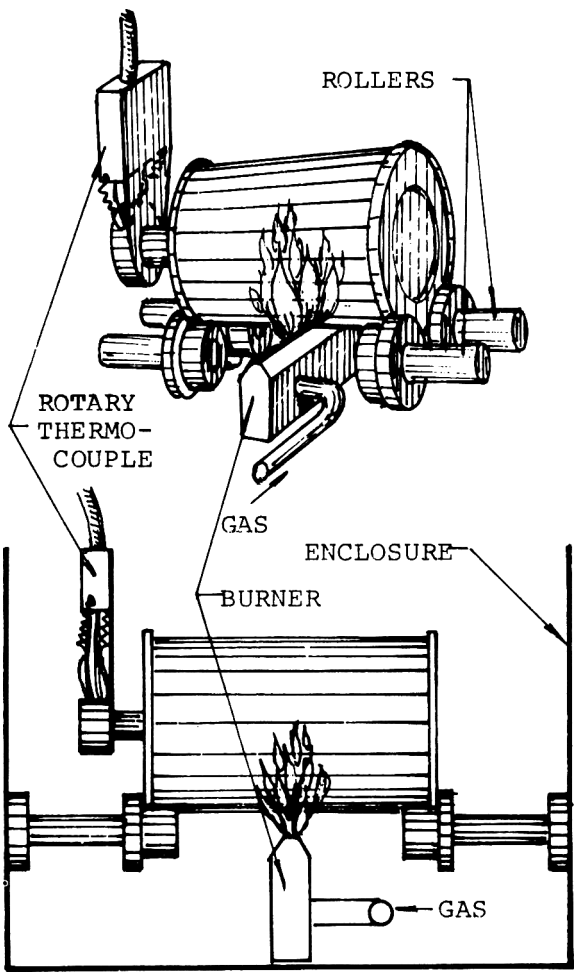


Fig. 1—Schematic view of the can and burner in a pilot flame-sterilizer.

The temperatures in the cans were registered every 15 sec. Only the temperatures corresponding to the flame heating step were used to calculate the heat transfer parameters. The flame condition was maintained constant in all the experiments: The gas pressure was 8psi, the gas flow rate 60g/hr, and the distance from the burner to the can surface 10 mm.

Some extra tests were done in which, besides the thermocouple at the geometrical center of the can, another flexible thermocouple was soldered at the internal wall of the can. The temperatures at these two points were registered every 15 sec, as in the other tests.

Determination of the overall heat transfer coefficient

The equation derived by Leonard et al. (1975a), especially developed for the flame-sterilizer, was used in calculating the heat transfer coefficients.

$$\theta = \frac{mC\,(T - T_i)}{UA(T_f - T_i)} \qquad (1)$$

Where, θ = time of heating (hr); m = mass of can plus contents (kg); C = average specific heat of can and contents (Kcal/kg·°C); U = overall heat transfer coefficient (kcal/hr·m^2·°C); A = heat transfer area of the can (m^2); T = temperature of product at time "θ" (°C); T_i = initial temperature of product (°C); T_f = average flame temperature (°C).

According to this equation, the "U" values can be obtained from the time-temperature data, since the slope of a straight line drawn through the recorded points is given by $mC/UA(T_f - T_i)$.

The average specific heat of can and contents was calculated using the following "C" values: Can = 0.115Kcal/kg·°C, CMC = 0.32 Kcal/kg·°C, and water = 1.0Kcal/kg·°C. The mass of can and contents are given in Tables 1, 2, 3 and 4. The temperatures T_i and T_f were 90 and 900°C, respectively. As the flame does not completely envelop the can and the temperature is not uniform, the heat transfer area has to be estimated, and we used a value of 46.5 cm^2 for this area (half of the lateral area of a cylinder of h=4.0 cm and ϕ=7.4cm). The "U" values obtained using these assumptions are average "U" values for the system, because in reality it varies from point to point in the can.

Photographs of the internal agitation of the product

In order to assist the data analysis, photographs were taken using a glass flask with similar dimensions to the 1/2 kg can. The flask was not heated and was filled with a 2.5% CMC solution at ambient temperature. The headspace volume was measured with a headspace rule and the can rotation speed was controlled using the speed variator of the equipment. We used the same four headspace volumes and can rotation speeds as used in the 70 tests presented in Tables 1, 2, 3, and 4.

Dimensionless correlations

We used the same equations used by Quast and Siozawa (1974) for the Nusselt, Prandtl and Reynolds numbers. These authors used the overall heat transfer coefficient (U) in the place of the film coefficient (h), because in their study they were quite similar. This is not true in our case. However, as it was easier to obtain "U" values instead of "h" values, and since we were attempting to correlate factors influencing the overall heat transfer, we used the same substitution as mentioned above. The Nusselt number thus becomes a modified Nusselt number (Nu_m). Besides this, Quast and Siozawa used an average specific heat of the can and its contents to calculate the Prandtl number, whereas we used the specific heat of the contents alone.

$$Nu_m = \frac{U.D}{k} \qquad (2)$$

where U = overall heat transfer coefficient; D = can diameter; and k = thermal conductivity of can contents.

$$Re = \frac{D.v.\rho}{\mu} \qquad (3)$$

where v = velocity of the can wall (π.D.rotational speed); ρ = density of air or of can contents; and μ = viscosity of air or of can contents.

$$Pr = \frac{\mu.C}{k} \qquad (4)$$

where C = specific heat of can contents.

—Continued on next page

Besides the "U" values, we needed the magnitudes of thermal conductivity, density, specific heat and viscosity for the CMC solutions and air at defined temperatures, in order to use the dimensionless equations. For the CMC solutions the temperature was defined as the average arithmetic temperature between 90 and 120°C, this being the range of temperatures registered at the geometrical center of the cans during the flame heating cycle. We avoided using the film temperature because we registered the temperature at the internal wall of the can for only a few experiments, but enough to see that it has a very complex behavior as a function of the can rotational speed and the other variables of the process. For the air, by looking at the temperature distribution outside the can presented by Paulus and Ojo (1974) for a flame of about 1100°C, we estimated an average temperature of 150°C.

RESULTS & DISCUSSION

THREE VARIABLES were studied in this work: headspace volume, can rotation speed, and product viscosity. The data are summarized in Tables 1, 2, 3 and 4. In these tables the values of "U" were obtained from the slopes of straight lines drawn through 20 to 30 points in plots of "T" versus "θ." The lower correlation coefficient (r) found for these lines was 0.989, but from the 70 tests done only two gave values of "r" lower than 0.995, and these were tests done at 50 r.p.m. (no. 43 and 61) and higher viscosities, indicating some problems of homogenization of the can contents.

Influence of the headspace volume in heat transfer

Looking at Tables 1, 2, 3 and 4 one can see that the "U" values at the same r.p.m., for different headspaces, are not too different. At most they differ from each other by about 10%, which is within the experimental error of this study. Thus, even though the photographs taken indicated more intense agitation for the larger headspaces (Teixeira Neto, 1980), we were not able to detect any increase in "U" values for the CMC solutions used in the present investigation.

Influence of can rotation speed in heat transfer

Tables 1, 2, 3 and 4 show that the "U" values usually decrease with an increase in the can rotation speed. The observed trend was significant and in contrast to the results obtained by Quast and Siozawa (1974) using steam as the heating medium. However, this may be explained by the fact that in the case of the flame-sterilizer there is some heat escape from the can wall to the ambient, and this loss increases as the rotation speed of the can increases, since the drag of ambient air between the flame and the can wall and probably the turbulence as well, also increase.

The interpretation of the photographs with respect to the internal agitation is complex. On one hand it could be seen that for the 2.5% CMC solution, faster speeds had the

Table 1–Data from flame-sterilization of 1.0% CMC solutions

Assay no.	Headspace (mm)	Mass of CMC soln (kg)	Mass of can + lid (kg)	Can rotation speed (r.p.m.)	Heating rate (°C/hr)	U (Kcal/ /hr·m^2·°C)	Re_i ($\times10^{-3}$)	Pr_i	Re_e ($\times10^{-3}$)	Nu_m
1	6	0.408	0.101	50[a]	323	35.8	4.78	18.2	0.50	4.49
2	6	0.408	0.101	100	296	32.8	9.57	18.2	1.00	4.11
3	6	0.408	0.101	150	266	29.4	14.35	18.2	1.50	3.69
4	6	0.408	0.101	200	236	26.1	19.13	18.2	2.00	3.27
5	10	0.402	0.100	50	308	33.6	4.78	18.2	0.50	4.21
6	10	0.402	0.100	100	296	32.3	9.57	18.2	1.00	4.05
7	10	0.402	0.100	150	289	31.5	14.35	18.2	1.50	3.95
8	10	0.402	0.100	200	263	28.7	19.13	18.2	2.00	3.60
9	14	0.380	0.103	50	334	36.4	4.78	18.2	0.50	4.57
10	14	0.380	0.103	100	315	34.3	9.57	18.2	1.00	4.30
11	14	0.380	0.103	150	285	31.1	14.35	18.2	1.50	3.90
12	14	0.380	0.103	200	278	30.3	19.13	18.2	2.00	3.80
13	18	0.364	0.099	50	338	33.5	4.78	18.2	0.50	4.20
14	18	0.364	0.099	100	315	31.2	9.57	18.2	1.00	3.91
15	18	0.364	0.099	150	296	29.3	14.35	18.2	1.50	3.67
16	18	0.364	0.099	200	274	27.1	19.13	18.2	2.00	3.40

[a] Buckled can

Table 2–Data from flame-sterilization of 1.5% CMC solutions

Assay no.	Headspace (mm)	Mass of CMC soln (kg)	Mass of can + lid (kg)	Can rotation speed (r.p.m.)	Heating rate (°C/hr)	U (Kcal/ /hr·m^2·°C)	Re_i ($\times10^{-3}$)	Pr_i	Re_e ($\times10^{-3}$)	Nu_m
17	6	0.412	0.096	50	353	39.3	0.56	154	0.50	4.93
18	6	0.412	0.096	100	323	35.9	1.13	154	1.00	4.50
19	6	0.412	0.096	150	319	35.5	1.69	154	1.50	4.45
20	6	0.412	0.096	200	304	33.8	2.26	154	2.00	4.24
21	10	0.394	0.097	50	349	37.2	0.56	154	0.50	4.67
22	10	0.394	0.097	100	311	33.1	1.13	154	1.00	4.15
23	10	0.394	0.097	150	323	34.4	1.69	154	1.50	4.31
24	10	0.394	0.097	200	326	34.7	2.26	154	2.00	4.35
25	14	0.378	0.097	50	375	38.4	0.56	154	0.50	4.82
26	14	0.378	0.097	100	345	35.3	1.13	154	1.00	4.43
27	14	0.378	0.097	150	326	33.3	1.69	154	1.50	4.18
28	14	0.378	0.097	200	338	34.6	2.26	154	2.00	4.34
29	18	0.363	0.096	50	368	36.2	0.56	154	0.50	4.54
30	18	0.363	0.096	100	368	36.2	1.13	154	1.00	4.54
31	18	0.363	0.096	150	345	33.9	1.69	154	1.50	4.25
32	18	0.363	0.096	150	356	35.0	1.69	154	1.50	4.39
33	18	0.363	0.096	200	364	35.8	2.26	154	2.00	4.49
34	18	0.363	0.096	200	356	35.0	2.26	154	2.00	4.39

tendency to direct the air bubbles to the axis of the flask and to its ends, not favoring good agitation. On the other hand these faster speeds favored a more frequent renovation of the material in contact with the bubbles, allowing better agitation. Which mechanism prevails is difficult to say, and will depend on the rotational speed. However, from a global point of view, this effect would be nullified if the external resistances were greater as compared to the internal ones.

It is also important to mention that of the 70 tests done, three presented problems of mechanical deformation of the can, and these were all tests done at 50 r.p.m. This deformation is evidently due to problems of internal agitation at this rotation speed, generating cold and hot spots inside the can that were responsible for the high pressure developed. Kato (1975) observed a similar phenomenon.

Influence of product viscosity on heat transfer

The data in Tables 1, 2, 3 and 4 do not show much variation in "U" values amongst the CMC solutions at different concentrations (viscosities), when compared under the same conditions of headspace volume and can rotation speed. This observation does not agree with the data presented by Quast and Siozawa (1974), when they used steam as the heating medium. Also, we worked with CMC solutions in the range of viscosities from 20 to 440 cP at 25°C, while Quast and Siozawa worked in the range of zero to 100 cP at 27°C.

Table 5 shows data obtained in seven tests in which the temperature at the internal wall of the can was measured. Some calculations with these data gave us values of external resistances about 50 to 900 times larger than the internal. The values of these resistances are also presented in Table 5. This fact is responsible for the lack of influence of the headspace volume and the viscosity in the "U" values of all tests. Also, it explains why the values of "U" presented by Quast and Siozawa (1974), for steam heating, were about 5 to 90 times larger than the values obtained in our tests with the flame-sterilizer.

It must be said that although in the calculations above we took into account the resistance to heat conduction through the can wall, it was very small and so neglected in the approach of the dimensionless correlations to be presented.

—Continued on next page

Table 3—Data from flame-sterilization of 2.0% CMC solutions

Assay no.	Headspace (mm)	Mass of CMC soln (kg)	Mass of can + lid (kg)	Can rotation speed (r.p.m.)	Heating rate (°C/hr)	U (Kcal/ /hr·m^2·°C)	Re_i ($\times 10^{-3}$)	Pr_i	Re_e ($\times 10^{-3}$)	Nu_m
35	6	0.407	0.097	50[a]	308	33.8	0.48	181	0.50	4.24
36	6	0.407	0.097	100	308	33.8	0.96	181	1.00	4.24
37	6	0.407	0.097	150	285	31.2	1.43	181	1.50	3.91
38	6	0.407	0.097	200	251	27.5	1.91	181	2.00	3.45
39	10	0.395	0.096	50	371	39.5	0.48	181	0.50	4.95
40	10	0.395	0.096	100	334	35.5	0.96	181	1.00	4.45
41	10	0.395	0.096	150	300	31.9	1.43	181	1.50	4.00
42	10	0.395	0.096	200	296	31.5	1.91	181	2.00	3.95
43	14	0.381	0.098	50	315	32.4	0.48	181	0.50	4.06
44	14	0.381	0.098	100	338	34.7	0.96	181	1.00	4.35
45	14	0.381	0.098	150	293	30.1	1.43	181	1.50	3.78
46	14	0.381	0.098	200	289	29.7	1.91	181	2.00	3.73
47	18	0.365	0.097	50	383	37.8	0.48	181	0.50	4.74
48	18	0.365	0.097	100	356	35.1	0.96	181	1.00	4.40
49	18	0.365	0.097	150	319	31.5	1.43	181	1.50	3.95
50	18	0.365	0.097	200	308	30.4	1.91	181	2.00	3.81

[a] Buckled can

Table 4—Data from flame-sterilization of 2.5% CMC solutions

Assay no.	Headspace (mm)	Mass of CMC soln (kg)	Mass of can + lid (kg)	Can rotation speed (r.p.m.)	Heating rate (°C/hr)	U (Kcal/ /hr·m^2·°C)	Re_i ($\times 10^{-3}$)	Pr_i	Re_e ($\times 10^{-3}$)	Nu_m
51	6	0.409	0.096	50	323	35.4	0.29	300	0.50	4.44
52	6	0.409	0.096	100	330	36.2	0.58	300	1.00	4.54
53	6	0.409	0.096	100	349	38.3	0.58	300	1.00	4.80
54	6	0.409	0.096	150	323	35.4	0.86	300	1.50	4.44
55	6	0.409	0.096	150	281	30.8	0.86	300	1.50	3.86
56	6	0.409	0.096	200	300	32.9	1.15	300	2.00	4.13
57	10	0.395	0.097	50[a]	360	38.2	0.29	300	0.50	4.79
58	10	0.395	0.097	100	338	35.9	0.58	300	1.00	4.50
59	10	0.395	0.097	150	323	34.3	0.86	300	1.50	4.30
60	10	0.395	0.097	200	300	31.8	1.15	300	2.00	3.99
61	14	0.377	0.097	50	375	38.0	0.29	300	0.50	4.77
62	14	0.377	0.097	100	323	32.8	0.58	300	1.00	4.11
63	14	0.377	0.097	100	338	34.3	0.58	300	1.00	4.30
64	14	0.377	0.097	150	304	30.8	0.86	300	1.50	3.86
65	14	0.377	0.097	200	278	28.2	1.15	300	2.00	3.54
66	18	0.362	0.097	50	461	44.9	0.29	300	0.50	5.63
67	18	0.362	0.097	100	341	33.2	0.58	300	1.00	4.16
68	18	0.362	0.097	150	323	31.5	0.86	300	1.50	3.95
69	18	0.362	0.097	150	304	29.6	0.86	300	1.50	3.71
70	18	0.362	0.097	200	308	30.0	1.15	300	2.00	3.76

[a] Buckled can

Dimensionless correlations

An empirical general dimensionless equation was written in order to correlate data describing the fluid flow characteristics and physical properties of the fluids inside and outside the can,

$$Nu_m = k\ Re_i^a\ Pr_i^b\ Re_e^c\ Pr_e^d \quad (5)$$

The subscripts "i" and "e" mean internal (product) and external (air), respectively. Since the Prandtl number for air is practically a constant (0.68), we simplified the above equation to:

$$Nu_m = k_1\ Re_i^a\ Pr_i^b\ Re_e^c \quad (6)$$

To substitute for the values of Re_i, Pr_i and Re_e we used the equations already given, in which the magnitudes of ρ, k, μ and C were used according to the following considerations: Of the properties referred above, for CMC solutions, viscosity is the most temperature dependent, followed by thermal conductivity, and their magnitudes at 105°C were used. As our CMC solutions were of low concentration, we used the thermal conductivity of water (Silva, 1971) as "k" for all CMC solutions, The viscosities of all CMC solutions obtained experimentally at different temperatures were plotted on a logarithmic scale against the reciprocal of the temperatures in degrees Kelvin (Fig. 2), and the magnitudes at 105°C were calculated by extrapolation of the straight lines. These magnitudes are apparent viscosities measured at a fixed shear rate. As we known, CMC solutions behave as pseudoplastic fluids whose apparent viscosities decrease with increase in the shear rate. It is very difficult to assess accurately the shear rate inside the can as a function of the rotational speed. For this reason, the magnitudes of viscosity are only approximate. As density and specific heat are less temperature dependent, we used their data at 20°C. Table 6 presents the density at this temperature for CMC solutions at different concentrations. The thermophysical properties of air at 150°C were taken from Silva (1971), and the values of "U" were determined from the time-temperature records obtained from each test, as described previously.

In order to get the best values of k_1, a, b and c in Eq 6, we performed several trial and error calculations using different values of "b" and "c" and plotting the data as in Fig. 3. After these trials we obtained a plot of points (intervals) that showed good alignment, with a correlation coefficient of 0.995. The high correlation coefficient shows that the equation found represents the heat transfer characteristics of the system studied very well. The values of "k_1" and "a" were taken from Fig. 3 as the intercept and the

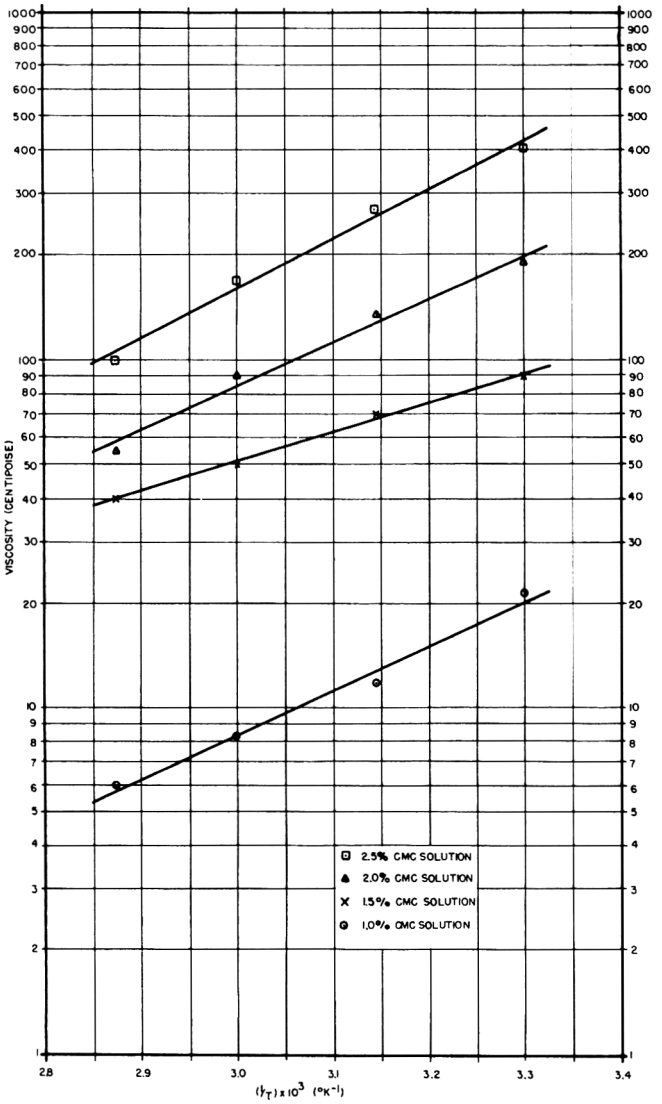


Fig. 2–Viscosity of CMC solutions at different concentrations as a function of the temperature.

Table 6–Density of CMC solutions at different concentrations

CMC solution (%)	Density[a] (g/cc)
1.0	1.001
1.5	1.003
2.0	1.004
2.5	1.004

[a] Average from two samples measured at 20°C

Table 5–Data from flame-sterilization of CMC solutions with temperature measured at the internal wall of the can[a]

Conc. of CMC soln (%)	Head-space (mm)	Can rotation speed (r.p.m.)	Heating[b] rate (°C/hr)	Heating[c] rate (°C/hr)	Initial[d] temp (°C)	External resistance (hr·m²·°C/Kcal)	Internal resistance (hr·m²·°C/Kcal)	E.R. / I.R.
1.5	18	50	295	202	110	0.033	0.00063	52
1.5	18	100	302	284	95	0.032	0.00016	198
1.5	18	150	284	241	95	0.035	0.00012	293
1.5	18	200	274	252	92	0.036	0.00004	882
2.5	10	100	288	169	109	0.031	0.00051	62
2.5	10	150	245	223	101	0.037	0.00046	79
2.5	10	200	234	94	115	0.038	0.00077	50

[a] Tests with some changes in the conditions of flame combustion compared with the other tests
[b] Heating rate measured at the geometrical center of the can
[c] Heating rate measured at the internal wall of the can
[d] Temperature at the internal wall of the can when we started to follow the heating up of the contents; at this time the temperature at the geometrical center of the can was always 90°C.

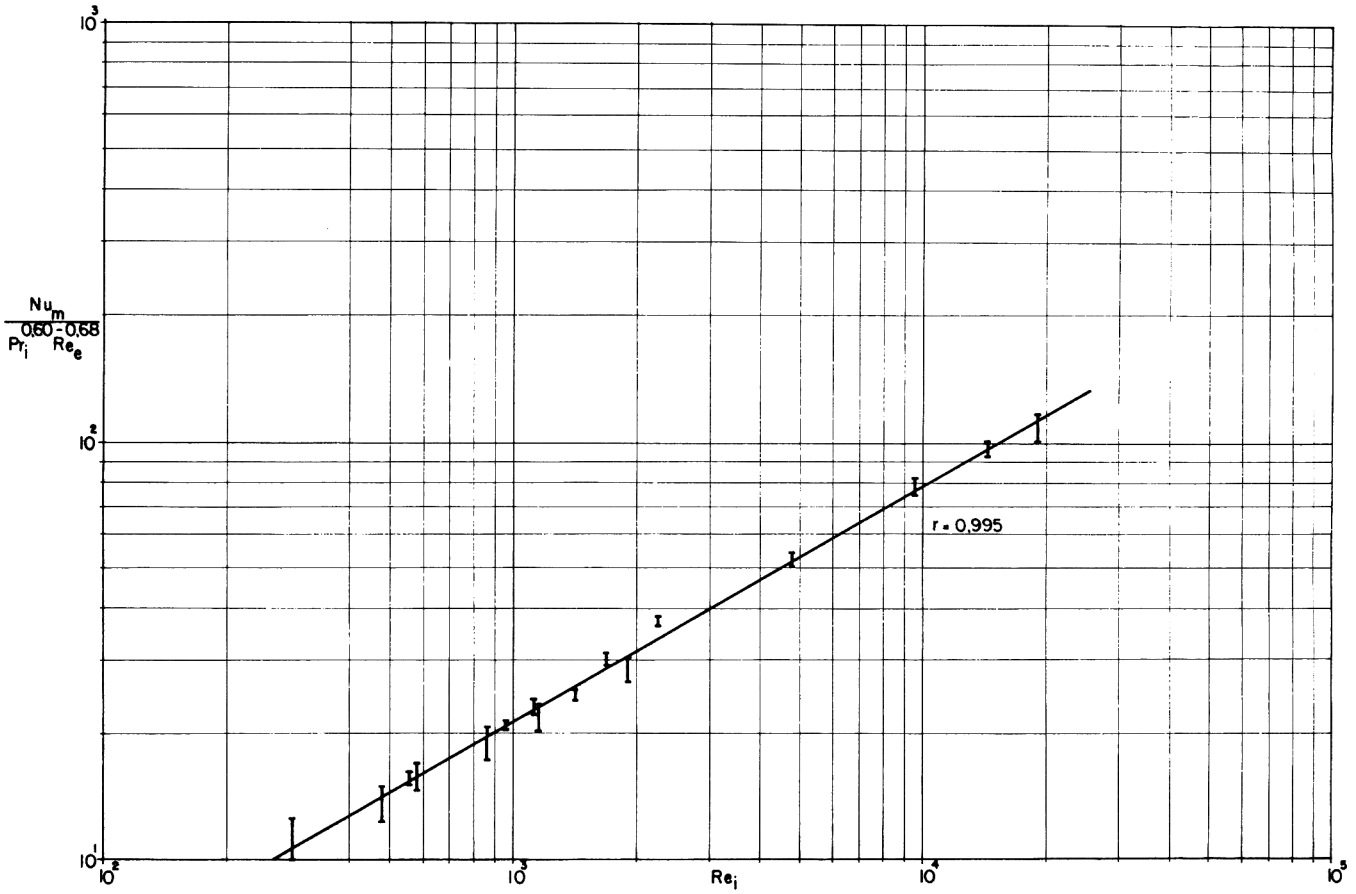


Fig. 3—Dimensionless correlation between data observed and predicted in the heat transfer study in a flame-sterilizer.

slope, respectively. The final form of the correlation equation thus becomes:

$$Nu_m = 0.433\ Re_i^{0.56}\ Pr_i^{0.60}\ Re_e^{-0.68} \qquad (7)$$

CONCLUSIONS

WE CAN SAY that the overall heat transfer to foods sterilized by a high temperature flame, has some differences from the heat transfer in foods sterilized by steam. The main characteristic of flame-sterilization is the much higher external resistance to heat transfer, which is not the case with steam sterilization. Thus, for products sterilized by flame the headspace volume and product viscosity are of minimum interest, since they do not affect the external resistances. The influence of rotational speed is more important and complex, since it affects both the internal and external resistances.

The empirical correlation found in this work will help in understanding the heat transfer phenomenon for foods sterilized by this mean. However, it holds only for the flame conditions fixed here. In order to optimize the design of a real sterilizer much more work is needed, mainly with respect to the influence of the gas flow rate and the distance from the burner to the can wall in the heat transfer rates to the product being sterilized.

REFERENCES

Kato, K. 1975. Personal communication.
Kieseker, F.G. 1972. Methods for sterilization of dairy products. In "Flame Sterilization. Specialist Courses for the Food Industry," No. 2, p. 54. AIFST-CSIRO, North Ryde, N.S.W., Australia.
Klepetko, V.G. and Longworth, I.N. 1972. Flame sterilization of mushrooms. In "Flame Sterilization. Specialist Courses for the Food Industry," No. 2, p. 62. AIFST-CSIRO, North Ryde, N.S.W., Australia.
Leonard, S. 1975. Steriflamme: visão geral. Boletim do ITAL 42: 1.
Leonard, S., Merson, R.L., Marsh, G.L., York, G.K., Heil, J.R., and Wolcott, T. 1975a. Flame sterilization of canned foods: an overview. J. Food Sci. 40: 246.
Leonard, S., Marsh, G.L., Merson, R.L., York, G.K., Fryer, S., Wolcott, T., and Ansar, A. 1975b. Comparative procedures for calculating steriflamme thermal processes. J. Food Sci. 40: 250.
Leonard, S., Marsh, G.L., Merson, R.L., York, G.K., Heil, J.R., and Wolcott, T. 1975c. Chemical, physical and biological aspects of canned whole peeled tomatoes thermally processed by steriflamme. J. Food Sci. 40: 254.
Leonard, S., Marsh, G.L., Merson, R.L., York, G.K., Buhlert, J.E., Heil, J.R., and Wolcott, T. 1975d. Quality evaluation of canned fruit cocktail experimentally processed by steriflamme. J. Food Sci. 40: 257.
Leonard, S., Marsh, G.L., York, G.K., Merson, R.L., Heil, J.R., Wolcott, T., and Ansar, A. 1976. Flame sterilization of some tomato products and fruits in 603 x 700 cans. J. Food Sci. 41: 828.
Paulus, K. and Ojo, A. 1974. Heat transfer during flame-sterilization. Proc. of IV Congress of Food Science and Technology 4: 443.
Quast, D.G. and Siozawa, Y.Y. 1974. Heat transfer rates during heating of axially rotated cans. In "International Congress of Food Science and Technology." Madrid.
Silva, R.M. 1971. Manual de Termodinamica-Transmissão de Calor, p. C7.1 and C11.4, DLP Escola Politecnica da Universidade de São Paulo, São Paulo, Brazil.
Sherwood, T.K., Pigford, R.L., and Wilke, C.R. 1975. "Mass Transfer," p. 239. McGraw Hill, New York.
Teixeria Neto, R.O. 1980. Estudo do transporte de calor no processo de esterilização por chama. Progress report No. 2 to Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), Brasilia, DF, Brazil.
Ms received 6/29/81; revised 9/8/81; accepted 9/11/81.

The author thanks Eng. Alfredo de A. Vitali, Dr. Theo Kieckbusch and Dr. Mentreddi A. Rao for their help and advice throughout the project; the Conselho Nacional de Desenvolvimento Científico e tecnologico (CNPq) for their financial support of this research; and the Instituto de Tecnologia de Alimentos (ITAL) for providing the facilities and also some financial support.

Reactions of Aqueous Chlorine and Chlorine Dioxide with Lipids: Chlorine Incorporation

H. A. GHANBARI, W. B. WHEELER and J. R. KIRK

ABSTRACT

Various lipids were reacted with $HO^{36}Cl$ and $^{36}ClO_2$ in an aqueous medium, and the extent of incorporation of ^{36}Cl into the lipids was determined. Cl incorporation into lipids treated with HOCl was greater than lipids treated with ClO_2. Free fatty acids incorporated more Cl than their corresponding esters. The degree of Cl incorporation was directly correlated with number of double bonds in the lipid. Triglycerides behaved as esters of their component fatty acids. High Performance Liquid Chromatography (HPLC) studies indicated at least two chlorinated products were formed from the reaction of $HO^{36}Cl$ with oleic acid.

INTRODUCTION

AQUEOUS CHLORINE and chlorine dioxide are used in the food industry as antimicrobial and bleaching agents. Both of these compounds are potent oxidizers and under the proper conditions are effective chlorinating agents. Since the safety of some chlorinated compounds is an issue of some concern (Kraybill, 1978; Simon and Tardiff, 1980), it is important to study the possible formation of chloro-organics in compounds of biological origin.

Lipids constitute a class of organic compounds in foods which readily react with chlorine (White, 1972; Ghanbari et al., 1980). The double bonds in the fatty acid moiety of lipids can undergo oxidation and addition in the presence of electrophiles such as HOCl and ClO_2 (NRC, 1979). Cunningham and Lawrence (1976) have shown that when a thin layer of methyl oleate is treated with Cl_2 (g), 85% of the methyl oleate is chlorinated. Leopold and Mutton (1959) reacted Cl_2 (g) and ClO_2 (g) with triolein in $CHCl_3$ and CCl_4 and have reported formation of chlorine containing lipids. Cunningham (1980) prepared chlorinated lipids by reacting chlorine gas with lipids in the neat form and has shown that these chlorinated lipids in the diet of rats reduce weight gain and increase the size of liver, kidney and heart.

In an attempt to simulate aqueous condition in food systems, this study examines the extent of incorporation of chlorine into free fatty acids, their esters, and triglycerides when reacted with HOCl and ClO_2 under aqueous conditions.

EXPERIMENTAL

Materials

Lipids were obtained from Applied Science (State College, PA) and Supelco (Supelco Park, Bellefonte, PA). (^{36}Cl)-Hydrochloric acid was purchased from New England Nuclear, All other chemicals were reagent or analytical grade.

Preparation of chlorine demand free water

Distilled water was first deionized using a Barnstead (Sybron Corporation) water deionizer system and passed through a column of Porapak Q (Waters Associates). Chlorine demand free water was used in preparing all solutions.

Authors Ghanbari, Wheeler and Kirk are affiliated with the Dept. of Food Science & Human Nutrition, Institute of Food & Agricultural Sciences, Univ. of Florida, Gainesville, FL 32611.

Chlorine generation

In order to prepare $HO^{36}Cl$, $H^{36}Cl$ was first neutralized with NaOH and then added in microliter amounts to potassium permanagante in the reaction flask to generate Cl_2 (g). The amount of $Na^{36}Cl$ used was determined by the specific activity (cpm/mg/L) desired in the final solution.

Chlorine gas was generated by the dropwise addition of 2 ml of 3M HCl solution to 1g potassium permanganate in a gas generation apparatus (Ghanbari et al., 1981). The system was closed and under vacuum. Chlorine gas was trapped in 50 ml of 0.02M NaOH solution to yield a final pH of 6–7. Using this sytem, the gas is efficiently trapped predominately as HOCl.

Chlorine dioxide generation

Nonradioactive and radioactive ClO_2 were prepared using two different schemes. Nonradioactive ClO_2 was generated by slowly adding a 2N H_2SO_4 to 1g of $NaClO_2$ in 1 ml H_2O in the gas generating apparatus. The generated chlorine dioxide gas (greenish-yellow color) was passed through a $NaClO_2$ clean-up column and collected in 40 ml of cold H_2O.

Radioactive ClO_2 was prepared from $H^{36}Cl$ as described by Ghanbari et al. (1981). Following neutralization with KOH, $K^{36}Cl$ was converted to $K^{36}ClO_3$ by electrolysis and disproportionation. The $K^{36}ClO_3$ solution was then reduced to $^{36}ClO_2$ using oxalic acid in concentrated H_2SO_4 in the same gas generation apparatus used to generate HOCl. ClO_2 was purified by passage through a Na_2CO_3 column. Contaminants such as CO_2 and Cl_2 are absorbed by the Na_2CO_3.

Chloramine preparation

Chloramine was formed by mixing ammonium hydroxide and hypochlorous acid in a 2:1 molar ratio. The major product of this mixture when incubated at room temperature (pH 7.6) is NH_2Cl (Eaton et al., 1973).

Dispersion of lipids in aqueous medium

All lipids were dispersed in chlorine demand free H_2O using a Polytron homogenizer equipped with an anaerobic probe generator.

Reaction mixtures

Reaction mixtures contained 3.4 mM lipid (1000 mg/L oleate equivalent) and 5.07 mM (180 mg/L) available chlorine equivalent of $HO^{36}Cl$ or $^{36}ClO_2$ in phosphate buffer solution, pH 6.0. The mixtures were incubated at 25°C in a shaking water bath. The reactions were carried out in glass stoppered flasks with about 30% head space; the reaction flasks were wrapped with foil to exclude light. Samples were taken after 0, 5, 15, 30, and 60 min of incubation for extraction of lipids and scintillation counting. For 0 min incubation time, available chlorine was quenched with 10% excess sodium thiosulfate prior to mixing with lipids. All other samples were quenched with sodium thiosulfate at the time of sampling.

Extraction of lipids from the aqueous phase

Preliminary experiments showed that 10–40% of the original radioactivity of $HO^{36}Cl$ was extracted by chloroform from an aqueous mixture of the reagent and the lipids, depending on pH and relative volume of the two phases. It was also noted that chlorine in the chloroform would react readily with lipids if they were present. To eliminate possible reaction between chlorine and lipid during extraction, unreacted $HO^{36}Cl$ in the aliquots of the reaction

mixture was quenched with 10% excess sodium thiosulfate immediately after sampling. Hydrochloric acid was added to adjust pH to approximately 2.0 to assure completion of quenching and facilitate chloroform extraction of the fatty acids. The Cl^- in HCl diluted $^{36}Cl^-$ and minimized $^{36}Cl^-$ contamination of chloroform extracts and acetonitrile eluates. A zero time sample was achieved by quenching the $HO^{36}Cl$ in the reaction mixture with sodium thiosulfate before adding any lipids to the reaction mixture.

A 5 ml aliquot of the reaction mixture (quenched with sodium thiosulfate and acidified to pH 2) was extracted with 10 ml of chloroform. The chloroform fractions were pooled and evaporated to dryness at 60°C under a stream of N_2. The organic compounds remaining in the aqueous phase after chlorform: methanol extraction were recovered using a C_{18} Sep-Pak column (Waters Associates). The aqueous phase was first made to a volume of 10 ml with water and a 5 ml aliquot of the mixture was passed through a conditioned Sep–Pak column and the column was then rinsed with 5 ml 0.1N NaCl. Organic compounds absorbed to the column were subsequently eluted with 5 ml acetonitrile. The acetonitrile fraction was evaporated to dryness at 60°C under a stream of N_2. Both chloroform and acetonitrile extractions were dissolved in Aquasol (New England Nuclear) for scintillation counting.

High performance liquid chromatography

Following incubation of oleic acid and $HO^{36}Cl$, unreacted oleic acid and reaction products were derivatized to UV absorbing phenacyl esters according to the procedure of Borch (1975). Separation and quantitation were carried out using Hewlett Packard model 1284A High Performance Liquid Chromatograph equipped with automatic sampler, solvent programmer, and variable wavelength UV detector. Absorbance was monitored at 240 nm. Separation was accomplished on a 30 cm x 3.9 mm i.d. Bondapak C_{18} reverse phase column (Waters Associates). Elution was accomplished with an acetonitrile: water gradient programmed for 80:20 (v/v) to 100:0 (v/v) at a flow rate of 1.0 ml/min. Eluent fractions were collected using a Gilson Micro-Fractionator model FC-100.

Available chlorine

Available chlorine was measured by iodiometric titration (Franson, 1976; Masschelein, 1979).

Liquid scintillation counting

Radioactivity was quantified using liquid scintillation counting of samples in Aquasol (New England Nuclear) using a Searle Analytic 92 Liquid scintillation system with windows set at 50-800. Counting efficiency was determined by quench curve, and counter reliability was monitored by including standards with each run.

RESULTS & DISCUSSION

Incorporation of chlorine into lipids treated with hypochlorous acid

Data describing chlorine incorporation into lipids reacted with $HO^{36}Cl$ in aqueous suspension are shown in Table 1. The amount of radioactivity per ml of reaction mixture was adjusted to yield a minimum of 1000 counts per min for each 1% ^{36}Cl incorporation. Free fatty acids showed greater incorporation of chlorine than their corresponding methyl esters (oleic acid 10.4%, methyl oleate 1.67). Chlorine incorporation was observed to be directly proportional to the number of double bonds in both free fatty acids and their methyl esters (oleic acid 10.4%, arachidonic acid 29.1), and incorporation of chlorine into triglycerides was predictable from average number of double bonds in the esterified fatty acids. These trends were consistent with the rate of destruction of available chlorine from HOCl reacted with free fatty acids, their methyl esters and various triglycerides.

Examination of the data in Table 1 reveals that the percent chlorine incorporation appeared to decline after 30 min of incubation of the suspensions of free fatty acids containing two or more double bonds. The results for the organics remaining in the aqueous phase after chloroform-methanol extraction are shown in Table 2. These data indicate chlorine was incorporated into water soluble organics produced from lipids treated with $HO^{36}Cl$. The oleic acid-HOCl reaction mixture data showed less than 0.1% incorporation of chlorine into water soluble reaction products after 60 min of incubation (in contrast to 10.4% incorporation into chloroform: methanol extract). Chlorine incorporation into water soluble organics sharply increased in linolenic acid and arachedonic acid experiments. Thus, polyunsaturated fatty acids appear to favor the formation of chlorinated water soluble reaction products. The water soluble organics containing ^{36}Cl were more hydrophilic chlorine derivatives of the fatty acids and/or smaller organic molecules produced by oxidation-chlorination-cleavage of the polyunsaturated fatty acids.

Under the experimental conditions stated in Table 1, no residual active chlorine could be measured after 60 min. of incubation of the HOCl-oleic acid reaction mix-

Table 1—Chlorine incorporation into chloroform:methanol (2:1) extracts of lipids treated with hypochlorous acid. Lipids were suspended in 0.1M phosphate buffer, pH 6.0. Hypochlorous acid (as $HO^{36}Cl$) initial concentration was 5.07 mM (180 mg/L available chlorine) and lipid concentration was 3.4 mM (1000 mg/L oleate equivalent)

Lipids	Total counts in 5 ml aliquot of the reaction mixture (dpm)[a]	Percent chlorine incorporated into chloroform: methanol (2:1) extracts after reaction time[b]					Moles chlorine incorporated per mole of lipid after 60 min incubation[d]
		0 min[c]	5 min	15 min	30 min	60 min	
Oleic acid	1.57×10^5	0	3.22	6.23	8.31	10.4	0.155
Methyl oleate	2.05×10^5	0	0.78	1.03	1.32	1.67	0.0249
Linoleic acid	2.22×10^5	0	9.21	14.2	18.0	16.6	0.247
Methyl linoleate	1.42×10^5	0	2.11	2.62	3.1	3.60	0.0536
Linolenic acid	1.62×10^5	0	18.1	26.1	26.9	26.0	0.387
Methyl linolenate	1.02×10^5	0	3.31	4.54	5.62	6.10	0.0909
Arachidonic acid	1.25×10^5	0	20.2	29.8	31.1	29.1	0.434
Methyl arachidonate	1.00×10^5	0	5.61	7.22	9.11	9.20	0.137
Triolein	1.25×10^5	0	0.61	0.74	1.21	1.56	0.0232
Triglycerides (Olive oil)	1.00×10^5	0	0.30	0.52	0.75	1.31	0.0195
Triglycerides (Wheat germ)	1.58×10^5	0	3.41	3.62	4.06	4.06	0.0605

[a] Amount of radioactivity was determined by scintillation counting of a 0.5 ml aliquot of the reaction mixture for time 0 min.
[b] Average of two to three values each calculated using the following formula: Percent incorporation = $[(dpm_t - dpm_0)/dpm_T] \times 100$ where dpm_0 is counts for total chloroform-methanol extract at time 0; dpm_t is counts for total chloroform-methanol extract at time t (5, 15, 30, and 60 min); dpm_T is total counts in the 5 ml aliquot of the reaction mixture.
[c] Chlorine dioxide was quenched with excess sodium thiosulfate before adding the lipids, hence a true time zero.
[d] These values were calculated using the following formula: Percent chlorine incorporated/100 x molar concentration of HOCl/molar concentration of lipids.

ture. Although all of $HO^{36}Cl$ was destroyed, only 10.4% of the chlorine as ^{36}Cl was incorporated into oleic acid and only 15.5% of the oleic acid molecules were calculated to contain chlorine (based on 1 Cl per molecule of fatty acid). These data appear to suggest that the reaction of HOCl with lipids results in chlorination and possible oxidation of the fatty acid. Similar results were observed with the other lipids used in this study. Compounds in the chloroform-methanol extract of oleic acid-$HO^{36}Cl$ reaction were separated by reverse phase HPLC. Five major peaks were observed by monitoring absorbance at 240 nm, two of which were shown to contain ^{36}Cl by scintillation counting.

Incorporation of chlorine into lipids can best be expressed in terms of moles of chlorine per mole of free fatty acids, methyl ester, or fatty acid residue equivalent of triglycerides. Data represented by the solid line in Fig. 1 shows the relationship between number of double bonds in the free fatty acids and total chlorine incorporation, while the broken line represents the number of double bonds and chlorine incorporation per double bond in fatty acid methyl esters. Total chlorine incorporated (moles chlorine per mole ester) was directly proportional to the number of double bonds. Chlorine incorporation per double bond (mole equivalent) also increased slowly with number of double bonds in the molecule, indicating a synergistic effect for the double bonds in the fatty acids. This is supported by chlorine incorporation data for methyl arachidonate with four double bonds which incorporated chlorine at a level of greater than 4x compared to methyl oleate with one double bond.

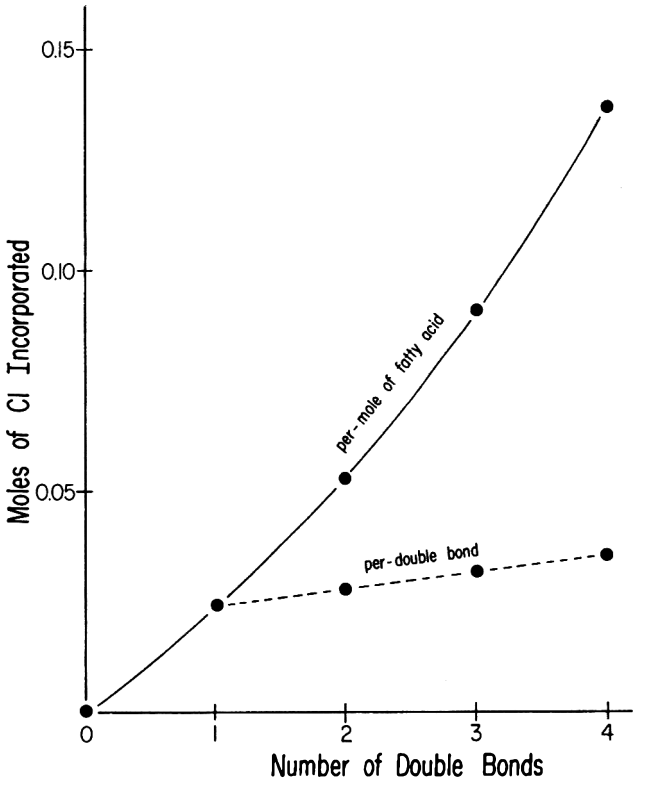


Fig. 1—Relationship between number of double bonds and total chlorine incorporation and number of double bonds and chlorine incorporation per double bond in fatty acid methyl ester treated with $HO^{36}Cl$: Conditions as in Table 1.

Incorporation of chlorine into lipids treated with chlorine dioxide

Experiments involving lipids and $HO^{36}Cl$ and discussed in the previous section were repeated using $^{36}ClO_2$. Experimental conditions and protocols were identical and data were analyzed in the same manner. The data showing chlorine incorporation into chloroform-methanol (2:1) extracted lipids which had been treated with $^{36}ClO_2$ are summarized in Table 3. Chlorine incorporation into lipids treated with $^{36}ClO_2$ followed the same general trends observed for reactions involving $HO^{36}Cl$. As shown by the data in Table 3, the percent radioactivity incorporated into lipids treated with $^{36}ClO_2$ was significantly smaller. Comparison of the moles of Cl incorporated per mole lipid (Tables 1 and 3) shows that, under identical conditions, less chlorinated lipid was formed when ClO_2 was used as the chlorinating agent than HOCl.

Lipids are generally less reactive with ClO_2 as compared to HOCl (Ghanbari et al., 1980); however, this lower reactivity does not fully explain the reduced ^{36}Cl incorporation into lipids treated with $^{36}ClO_2$. Data reported by Ghanbari et al. (1980) showed that arachidonic acid reacted with 70% of ClO_2 and 100% of HOCl after 60 min incubation, but percent radioactivity incorporated into arachidonic acid after 60 min is 7.61 and 29.1 for $^{36}ClO_2$ in $HO^{36}Cl$, respectively (Tables 1 and 3). These data indicate that ClO_2 may favor oxidation rather than chlorination of lipids to a greater extent than chlorine.

Experimental data showed no chlorine incorporation when oleic acid was reacted with chloramine. Similarly there was no chlorine incorporated into oleic acid when NH_4OH was added to the reaction mixture containing $^{36}ClO_2$. One explanation for these latter observations is that the mechanism of chlorination with ClO_2 involves the formation of HOCl which results in electrophilic addition to the double bond; however, in the presence of NH_4OH chloramine is more rapidly formed. Gordon et al. (1972)

Table 2—Chlorine incorporation into water soluble organics produced from lipids when treated with $HO^{36}Cl$. Lipids were suspended in 0.1M phosphate buffer, pH 6.0. Hypochlorous acid (as $HO^{36}Cl$) initial concentration was 5.07 mM (180 mg/L available chlorine) and lipid concentration was 3.4 mM (1000 mg/L oleate equivalent). Water soluble fractions were separated using C_{18} reverse phase column

Lipids	Total counts in 5 ml aliquot of the aqueous phase after chloroform-methanol extraction (dpm)[a]	Percent chlorine incorporation in water soluble organics after reaction time[b]				
		0 min	5 min	15 min	30 min	60 min
Oleic acid	2.58×10^5	0	0.019	0.039	0.058	0.098
Linoleic acid	3.23×10^5	0	0.036	0.071	0.093	0.18
Linolenic acid	3.59×10^5	0	0.56	0.84	1.00	1.06
Arachidonic acid	3.39×10^5	0	1.39	1.56	1.71	1.77

[a] Amount radioactivity was determined by scintillation counting of 1 ml aliquot of the aqueous phase (after chloroform-methanol extraction) for time 0 min.

[b] The following formula was used to calculate these values: Percent incorporation = $[dpm_t - dpm_0)/dpm_T] \times 100$ where dpm_0 is counts for total acetonitrile eluent using aqueous phase of time 0; dpm_t is counts for total acetonitrile eluent using aqueous phase of time t (5, 15, 30 and 60 min); dpm_T is total counts in 5 ml aliquot of the aqueous phase after chloroform:methanol extraction.

Table 3—Chlorine incorporation into chloroform:methanol (2:1) extracts of lipids treated with chlorine dioxide. Lipids were suspended in 0.1M phosphate buffer, pH 6.0. Chlorine dioxide (as $^{36}ClO_2$) initial concentration was 5.07 mM (180 mg/L) available chlorine equivalent and lipid concentration was 3.4 mM (1000 mg/L oleate equivalent)

Lipids	Total counts in 5 ml aliquot of the reaction mixture (dpm)[a]	Percent chlorine incorporated into chloroform: methanol (2:1) extracts after reaction time[b]					Moles chlorine incorporated per mole of lipid after 60 min incubation[d]
		0 min[c]	5 min	15 min	30 min	60 min	
Oleic acid	2.22×10^5	0	1.21	1.85	2.05	2.11	0.00627
Methyl oleate	1.67×10^5	0	0.21	0.82	0.81	1.31	0.00389
Linoleic acid	6.35×10^4	0	2.12	2.06	4.01	4.51	0.0134
Methyl linoleate	2.34×10^5	0	1.26	1.65	1.61	2.52	0.00748
Linolenic acid	1.65×10^5	0	4.06	5.12	5.64	7.01	0.0208
Methyl linolenate	1.60×10^5	0	1.41	2.01	2.06	3.01	0.00940
Arachidonic acid	1.80×10^4	0	5.16	5.89	6.56	7.61	0.0226
Methyl arachidonate	1.26×10^5	0	1.52	2.71	2.61	2.71	0.00805
Triolein	3.46×10^5	0	0.52	0.51	0.65	1.06	0.00315
Triglycerides (Olive oil)	1.94×10^5	0	0.31	0.42	0.65	1.26	0.00374
Triglycerides (Wheat germ)	1.32×10^5	0	1.02	1.37	1.26	2.12	0.00630

[a] Amount of radioactivity was determined by scintillation counting of a 0.5 ml aliquot of the reaction mixture for time 0 min.
[b] Average of two to three values each calculated using the following formula: Percent incorporation = $[(dpm_t - dpm_0)/dpm_T] \times 100$ where dpm_0 is counts for total chloroform-methanol extract at time 0; dpm_t is counts for total chloroform-methanol extract at time t (5, 15, 30, and 60 min); dpm_T is total counts in the 5 ml aliquot of the reaction mixture.
[c] Chlorine dioxide was quenched with excess sodium thiosulfate before adding the lipids, hence a true time zero.
[d] These values were calculated using the following formula: Percent chlorine incorporated/100 x molar concentration of available chlorine/5 x concentration of lipids

have suggested the following mechanism for conversion of ClO_2 to HOCl:

$$ClO_2 + e^- \rightarrow ClO_2^-$$
$$2H^+ + Cl^- + ClO_2^- \rightarrow 2HOCl$$

In conclusion, there were similar trends between chlorine incorporation into lipids reacted with $HO^{36}Cl$ and $^{36}ClO_2$, although HOCl treatment of lipids results in higher chlorine incorporation (10.4% and 2.4% for oleic acid). The lower chlorine incorporation in ClO_2 experiments as compared to HOCl experiments cannot be explained by lower reactivity of ClO_2 alone. Considering these findings and published reports that ClO_2 is more effective than HOCl in bactericidal activity in poultry processing (Lillard, 1979, 1980), use of ClO_2 as an alternative biocide in food processing should be further studied. Data from these experiments confirm the published report (White, 1972) that the increased effectiveness may be due to the lower reactivity of ClO_2 in the presence of organic molecules.

REFERENCES

Borch, R.F. 1975. Separation of long chain fatty acids as phenacyl esters by high pressure liquid chromatography. Anal. Chem. 47(14): 2437.

Cunningham, H.M., and Lawrence, G.A. 1976. A comparison of the distribution and elimination of oleic and chlorinated oleic acids and their metabolites in rats. Fed. Cosmetic Toxicology 14: 283.

Cunningham, H.M. 1980. Toxicology of compound resulting from the use of chlorine in food processing. In "Water Chlorination: Environment and Health Effects," Vol. 3, p. 995. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

Eaton, J.W., Kolpin, C.F., Swofford, H.S., Kjellstrand, C.-M., and Jacob, H.S. 1973. Chlorinated urban water: A cause of dialysis-induced hemolygic anemia. Science 181: 463.

Franson, M.A. (Ed). 1976. "Standard Methods for the Examination of Water and Waste Water," 14th ed. American Public Health Association, Washington, DC.

Ghanbari, H.A., Wheeler, W.B., and Kirk, J.R. 1980. Reaction of aqueous chlorine and chlorine dioxide with lipids. Presented at the Second Chemical Congress of North American Continent, Las Vegas, NV. Aug. 29—Sept. 3.

Ghanbari, H.A., Wheeler, W.B., and Kirk, J.R. 1981. New approaches to the generation of ^{36}Cl-labeled chlorinating agents and the measurement of ^{36}Cl incorporated into biological materials. In "Water Chlorination: Environmental Impact and Health Effects," Vol. 4. (In press)

Gordon, G., Kieffer, R.G., and Rosenblatt, D.H. 1972. The chemistry of chlorine dioxide. Progress in Inorg. Chem. 15: 201.

Kraybill, H.F. 1978. Origin, classification and distribution of chemicals in drinking water with an assessment of their carcinogenic potential. In "Water Chlorination: Environmental Impact and Health Effects," Vol. 1, p. 211. Ann Arbor Science Publ., Inc., Ann Arbor, MI.

Leopold, B. and Mutton, D.B. 1959. The effect of chlorinating and oxidizing agents on derivatives of oleic acid. TAPPI 42(3): 218.

Lillard, H.S. 1979. Levels of chlorine and chlorine dioxide of equivalent bactericidal effect in poultry processing water. J. Food Sci. 44(6): 1594.

Lillard, H.S. 1980. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. Poultry Sci. 59(8): 1871.

Masschelein, W.J. 1979. "Chlorine Dioxide: Chemistry and Environmental Impact of Oxychlorine Compounds." Ann Arbor Science Publ., Inc., Ann Arbor, MI.

NRC. 1979. The chemistry of disinfectants in water, reactions and products. PB-292776, U.S. Dept. of Commerce, Washington, DC.

Simmon, V.F. and Tardiff, R.G. 1980. The mutagenic activity of halogenated compounds found in chlorinated drinking water. In "Water Chlorination: Environmental Impact and Health Effects," Vol. 2, p. 417. Ann Arbor Science Publ., Inc., Ann Arbor, MI.

White, G.C. 1972. "Handbook of Chlorination: For Potable Water, Waste Water, Cooking Water, Industrial Processes, and Swimming Pools." Van Nostrand Reinhold Co., New York, NY.

Ms received 6/11/81; revised 10/21/81; accepted 10/22/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA June 7—10, 1981.

Florida Agricultural Experiment Stations Journal Series No. 3337.

This work was supported in part by National Science Foundation grant number PFR 78-2706.

Dairy and Vegetable Protein Blends by Co-Extraction and Co-Ultrafiltration

DEBRA J. NICHOLS and MUNIR CHERYAN

ABSTRACT

A process was developed for the manufacture of blends of dairy and vegetable proteins by co-extraction and co-ultrafiltration. Defatted soy flour proteins were extracted using either acid or sweet whey as the solvent. The whey/soy slurry was separated in a two stage extraction process using a centrifugal desludger. The proportion of whey protein in the blend was determined by the number of extractions and the total amount of whey used. The combined extracts were processed by ultrafiltration to increase the protein content of the blends. Solubility characteristics of the blends indicate potentially good functional properties.

INTRODUCTION

PROTEINS are added to formulated food products to impart one or more functional characteristics in addition to nutritional fortification. Nonfat dry milk (NFDM) and casein derivatives have provided the food industry with exceptionally versatile and shelf-stable food ingredients for many years. However, the increasing cost and projected limited supplies of both NFDM and casein have created a need for functional food ingredients that can be used as "milk protein replacers" (Craig, 1975; Andres, 1980a; b; Anon., 1979). Emphasis is now being placed on designing blends of dairy and vegetable proteins that display a variety of functional properties. These blends, which combine the low cost of vegetable proteins and the inherent functionality and nutritional value of milk proteins, are used in a variety of processed foods including bakery products, dry mixes, sauces and gravies (Andres, 1980a; b; Anon., 1980; Craig, 1975).

Current commercial methods for producing blends of dairy and vegetable proteins can be summarized as follows: cheese whey or milk (in dry or liquid form) is combined with oilseed flour (usually defatted) and the two ingredients are dry or wet-blended. Chemicals (e.g. salt, alkali) may be added to improve the dispersibility of the proteins or to improve the "flowability" and handling of the final dried product. The wet-blended system is then dried, usually by spray-drying.

This method can be improved when producing whey/soy blends with high protein concentrations. Cheese whey is typically 10–12% protein dry basis and defatted soy flour is about 50% protein dry basis. A 1:1 solids ratio when wet blending results in a blend having approximately 31% protein dry basis, while a 1:2 mixture of soy flour to whey solids will result in a blend having approximately 25% protein dry basis. Thus, while increasing the ratio of whey protein to soy protein improves the nutritional value of the blend, the final protein content of the blend is lowered. This is not a problem for commercial blends (the bulk of which contain 27–35% protein) intended for use as NFDM replacers. However, the production of blends having higher protein content requires the use of the isolate form of the whey and soy protein. This not only involves the added expense of producing the isolates prior to blending but also requires isoelectric or heat precipitation of the proteins which may significantly lower their functionality.

Membrane processing (ultrafiltration and reverse osmosis) is a viable commercial technique for the production of whey protein concentrates (De Boer and Hiddink, 1980; Goldsmith, 1981). Ultrafiltration (UF) is capable of fractionating whey to recover the protein while simultaneously removing lactose and salts without denaturing the heat sensitive whey proteins. Ultrafiltration or reverse osmosis (RO) technology for the manufacture of purified protein isolates from oilseeds is also well-known (Omosaiye and Cheryan, 1979; Nichols and Cheryan, 1981a; Garbutt, 1979; Frazeur and Huston, 1973; Goodnight et al., 1976; Olsen, 1978; Pompei and Maletto, 1974; O'Connor, 1971). This paper reports on an extension of membrane processing technology for the production of dairy/vegetable protein blends. To demonstrate this process, cheese whey was blended with defatted soy flour in a co-extraction process, followed by co-ultrafiltration processing.

EXPERIMENTAL

Preparation of cheese whey/defatted soy flour co-extracts

Fig. 1 is a flow sheet of the overall process. Sweet whey was a by-product of cheddar cheese manufacturing. Acid whey, a by-product of cottage cheese manufacturing, was obtained from Meadow Gold, Champaign, IL. The pH of the sweet whey was 6.2 and that of the acid whey was 4.5-4.6. The whey was pasteurized at 63°C for thirty minutes and then cooled to below 30°C. Food grade alkali (NaOH) was added to increase the pH to 9.0. One part by weight defatted soy flour (Nutrisoy 7B, Archer Daniels Midland Co., Decatur, IL) was suspended with vigorous stirring in nine parts by weight cheese whey. After extracting for 30 min at 30°C, the slurry was fed to a centrifugal desludger (Westfalia Separator-cum-Desludger, Model SAOH). The slurry was separated into an extract containing most of the soluble components (protein, low molecular weight sugars, and ash) and a residue containing the insolubles.

The residue from the first extraction was collected and resuspended in ten parts by weight pasteurized cheese whey and centrifuged as before. The final flour to cheese whey ratio in these experiments was 1:19. The two extracts were pooled and used as feed to the ultrafiltration unit. The pH of the combined extracts was adjusted to 7.0 for ultrafiltration using 6N HCl.

Ultrafiltration of co-extracts

Co-ultrafiltration (co-UF) experiments were performed with a pilot plant size Romicon hollow fiber unit (Romicon, Inc., Woburn, MA). The hollow fiber module used in this study was the HF15-43-PM50 with 1.39m^2 of surface area. The molecular weight exclusion limit for this membrane was 50,000. Operating conditions were 274 kPa (25 psig) inlet pressure, 205 kPa (15 psig) outlet pressure, 50°C and pH 7.0. Details of the process engineering characteristics and optimization of flux for this UF unit are available elsewhere (Nichols and Cheryan, 1981b). Initial water flux, at a particular temperature and transmembrane pressure, was recorded prior to each run and used to monitor cleaning efficiency. The module was cleaned according to the procedure of Nichols and Cheryan (1981a).

Authors Nichols and Cheryan are, respectively, graduate research assistant and associate professor of food engineering, Dept. of Food Science, Dairy Manufactures Building, Univ. of Illinois, 1302 W. Pennsylvania Ave., Ubrana, IL 61801. Address all inquiries to Dr. Cheryan.

The co-UF process combined two modes of operation. During continuous diafiltration (CD), water was added to the feed tank at the same rate that permeate was removed, thus keeping the volume of feed/retentate constant. The rate of removal of permeable solutes was then proportional to volumes diluted (V_D), defined as the volume of water added to the feed tank divided by initial volume of feed. During direct ultrafiltration (UF), the feed was concentrated by volume reduction. The rate of removal of the undesirable, freely permeable solutes was proportional to the volume concentration ratio (VCR), defined as initial feed volume divided by retentate volume.

Analytical methods

Ash and total solids (T.S.) were measured by gravimetric procedures (AOAC, 1970). Nitrogen was determined by the Kjeldahl method (AOAC, 1970). Non-protein nitrogen (NPN) was measured as nitrogen soluble in 13.6% trichloracetic acid (TCA) (Nichols and Cheryan, 1981a). Protein is expressed as either (total N - NPN) x 6.25 or (total N - NPN) x 6.38. Carbohydrate is expressed as difference. Fat content and protein dispersibility index (PDI) were measured by methods described by Lah and Cheryan (1980). Table 1 shows the composition of the raw materials used in this study.

RESULTS & DISCUSSION

Co-extraction of whey and soy proteins

A two-stage extraction process was used in these experiments since earlier results using water as the solvent had indicated this to be the optimum (Nichols and Cheryan, 1981a). Desludging was performed to remove those components in the whey/soy slurry that were neither soluble nor finely suspended. These components were the insoluble carbohydrate, fiber and insoluble protein in the soy flour and the insoluble salts (such as the phosphates and lactates of calcium) in the whey. Tables 2 and 3 show the proximate analyses of the feed (slurry) and product (extracts) streams during co-extraction using acid and sweet whey. The protein content of the first extract was higher than that of the slurry for both the acid and sweet whey extracts due to the removal of insolubles during desludging. The protein content of the second extracts was lower because most of the protein was removed during the first extraction. The recovery of total solids for a single stage process was 71–75% and recovery of protein was 76–83%, while the recovery of total solids for a two stage extraction process was 84–89% and recovery of protein was 91–96%. Thus,

Table 1—Composition of raw material (%)

	Sweet whey[a]	Acid whey[a]	Defatted soy flour
Total solids	5.89	5.84	91.6
Total nitrogen	0.121	0.099	8.2
NPN	0.032	0.037	0.3
Protein[b]	0.57	0.40	49.4
Ash	0.56	0.52	5.8
Other[c]	4.76	4.92	36.4

[a] After pasteurization, pH adjustment and decanting.
[b] (Total N — NPN) x 6.38 for whey; (Total N — NPN) x 6.25 for soy flour.
[c] By difference: includes carbohydrate, fat and peptides.

Table 2—Proximate analysis of feed and product streams during coextraction of soy protein with cottage cheese whey at pH 9.0, 30°C[a]

		Composition (% dry basis)			
	Total solids	Total N	Protein[b]	Ash	Other[c]
Slurry	13.80	6.05	34.8	7.3	54.9
Extract I[d]	12.71	6.51	–	–	–
Extract II[d]	6.63	3.52	–	–	–
Extract I + II[d]	9.49	5.25	28.9	7.6	59.6

[a] Data are means of two replicate runs.
[b] (Total N — NPN) x 6.25.
[c] By difference: includes carbohydrate and residual fat.
[d] Extraction I used 1 part defatted soy flour and 9 parts whey. Extraction II used another 10 parts whey.

Table 3—Proximate analysis of feed and product streams during coextraction of soy protein with cheddar cheese whey at pH 9.0, 30°C[a]

		Composition (% dry basis)			
	Total solids	Total N	Protein[b]	Ash	Other[c]
Slurry	13.90	6.10	35.3	7.8	54.1
Extract I[d]	12.34	6.50	–	–	–
Extract II[d]	6.77	3.22	–	–	–
Extract I + II[d]	8.92	4.96	28.2	8.6	60.4

[a] Data are means of two replicate runs.
[b] (Total N — NPN) x 6.25.
[c] By difference: includes carbohydrate and residual fat.
[d] Extraction I used 1 part defatted soy flour and 9 parts whey. Extraction II used another 10 parts whey.

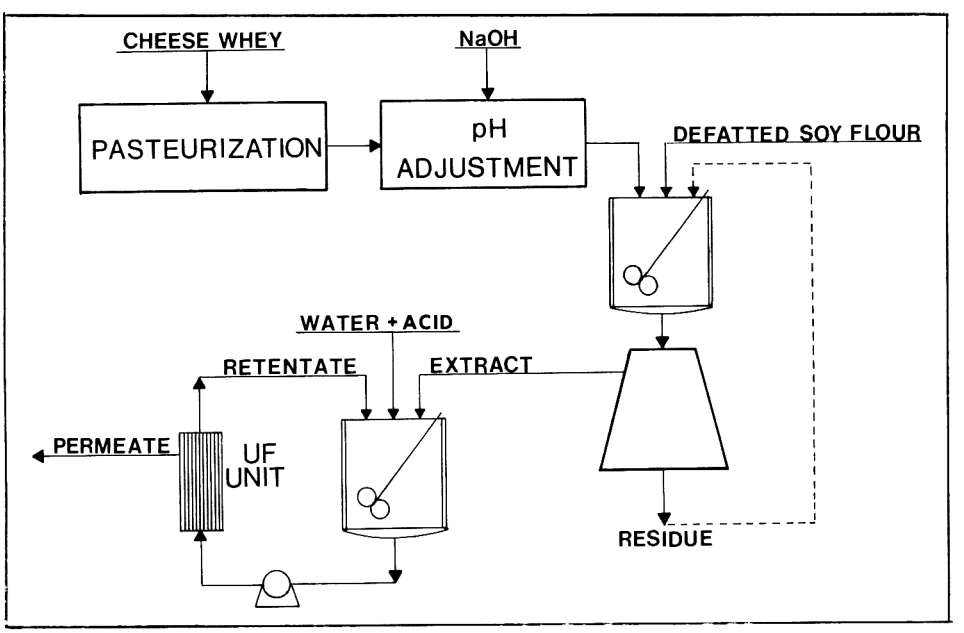


Fig. 1—Flow diagram for production of whey/soy blends by co-extraction and co-ultrafiltration.

including the second extraction stage improved protein recovery by 13–15% and improved the whey to soy protein ratio. A similar two stage process using water to extract the protein in the defatted soy flour resulted in total solids and protein recoveries of 81% and 92.5%, respectively (Nichols and Cheryan, 1981a). However, the protein content (dry basis) of the whey extracts was lower than that of the water extracts due to the addition of nonprotein whey solids.

The proportion of whey protein in the blend can be controlled during co-extraction (Table 4). Increasing the number of extraction stages improves the final ratio of whey protein to soy protein in addition to increasing yield of protein. However, increasing the number of whey extractions also decreases the final protein content of the combined extracts which increases the amount of UF processing that the extracts must undergo to produce a blend with a given protein content.

The co-extracts contained soluble or finely dispersed protein, soluble carbohydrate (in the form of lactose, sucrose, raffinose and stachyose), mineral salts reported as ash and residual fat. The pooled extracts could be evaporated and dried at this point to produce whey/soy protein blends having 28–30% protein, 7–9% ash, 0.5–3.0% fat and 58–60% carbohydrate which is comparable to the composition of many commercially available blends. In this study, however, co-UF was used to fractionate the extracts and improve the protein content.

Co-UF of whey/soy co-extracts

The pH of the co-extracts was first reduced to 7.0 for UF. This pH has been found to be most beneficial for the removal of phytic acid by membrane processing (Osmosaiye and Cheryan, 1979). The temperature during co-UF was high enough to improve flux (i.e., rate of removal of water and undesirable components) and control microbial growth without causing protein degradation. Operating parameters were adjusted to maximize flux (Nichols and Cheryan, 1981b).

A combination of CD and direct UF has been determined to be most suitable for the particular UF unit used in these experiments (Nichols and Cheryan, 1981a). Fig. 2 shows the composition of the retentate during co-UF of the acid whey/defatted soy flour extracts. During CD, the total solids content decreased with increasing V_D due to the loss of permeable solids. Since most of the carbohydrate in the retentate is freely permeable, its concentration decreased significantly. Ash concentration decreased much less dramatically, indicating that not all ash is freely permeable. Protein concentration remained relatively constant during CD. The slight decrease was due to membrane adsorption effects (Nichols and Cheryan, 1981a). The concentration of the freely permeable NPN significantly decreased during CD (Table 5).

When three volumes had permeated (i.e. V_D 3), direct UF processing was begun and continued to VCR 4. Water was no longer added and the retentate was allowed to concentrate. As expected for a concentration process, there was a linear correlation between the concentration of all solutes in the retentate and VCR (Fig. 2). Direct UF served two purposes: it continued to remove undesirable solutes and it concentrated the retained solids (by about fourfold in these experiments), which would substantially reduce the load on any subsequent drying process. Further concentration could have been achieved by continuing UF. However, the degree of concentration achieved would be limited by the high viscosity of the retentate and associated pumping problems resulting from high protein concentrations. This problem could be alleviated by returning to the CD mode of operation.

Fig. 3 shows the composition of the retentate on a dry basis during UF processing of the acid whey/soy flour co-extracts. Since the permeable solutes were mainly lactose and salts, the relative proportion of protein in the retained solids increased throughout processing as non-protein solids were removed from the system. Fig. 4 and 5 show similar results for the experiments performed with the sweet whey/defatted soy flour extracts.

Flux behavior for the hollow fiber UF unit and the whey/soy co-extracts is shown in Fig. 6. The initial flux for the acid whey system was lower than that of the sweet whey system. This could be due to partial precipitation of calcium salts (acid whey contains almost twice as much calcium as sweet whey). These are insoluble at pH 7 and would result in greater fouling of the membrane (Merin and Cheryan, 1980). Muller et al. (1973) also observed higher flux with cheddar cheese whey than acid whey initially. In our system, however, on a long term basis, the acid whey

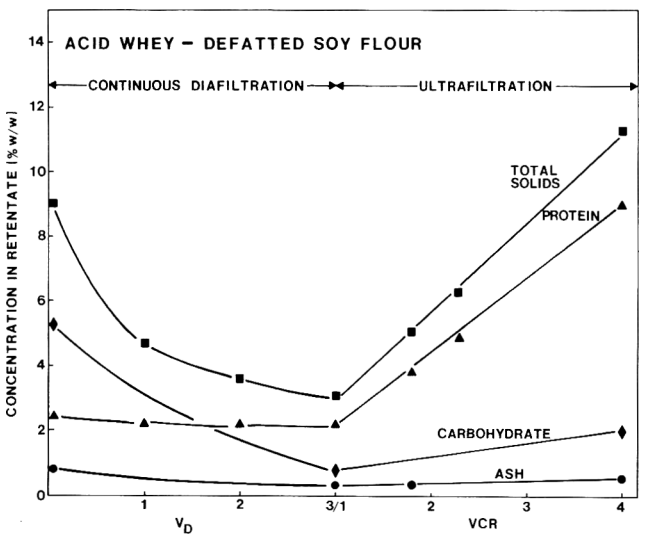


Fig. 2—Composition of retentate during ultrafiltration processing of acid whey/defatted soy flour co-extracts.

Table 4—Effect of number of extractions on protein content of whey/soy co-extracts[a]

Number of whey extractions	Whey protein[b] as per cent of total protein	Protein[b] content (% dry basis)
1	6.8	40.2
2	13.5	30.8
3	19.2	25.6

[a] Based on total solids and protein recoveries of 86 and 94%, respectively. Extraction I uses 1 part by weight soy flour and 9 parts acid whey. Other extractions use another 10 parts by wieght whey to resuspend the residue from the previous extraction.
[b] Protein calculated as (Total N — NPN) x 6.38 for whey and as (total N — NPN) x 6.25 for soy flour.

Table 5—Nitrogen distribution (%) during co-ultrafiltration

	Sweet whey			Acid whey		
	Total N	NPN	Protein[a]	Total N	NPN	Protein[a]
$V_D = 0$	0.497	0.046	2.82	0.443	0.052	2.44
$V_D = 3$	0.458	0.011	2.79	0.345	0.010	2.10
Final retentate	1.420	0.024	8.73	1.366	0.023	8.39

[a] (Total N — NPN) x 6.25.

system gives much higher flux. (Fig. 6). The reasons for this difference in flux behavior are not clear but are probably related to the physical and chemical properties of the acid and sweet whey extracts, since operating conditions were otherwise identical.

Whey/soy protein blends

The composition of the whey/soy protein blends is given in Table 6. The protein content of these blends was quite high and could have been further increased if necessary by modification of the co-UF process. The carbohydrate and ash content of these blends was low as a result of UF processing. The residual (3–5%) fat content in the final dry product could probably be reduced if necessary by separating the fat in the whey prior to co-extraction.

Protein blends are used to improve or provide certain functional characteristics in food products. In order to exert their functional properties, the proteins must generally be in solution. Therefore, protein solubility or dispersibility and its dependence on pH is a key functional property that can be used to evaluate a protein in terms of other functional properties (Lah and Cheryan, 1980). PDI for the co-UF blends is shown in Table 7. PDI for a typical commercial soy isolate (Promine-D, Central Soya Inc., Ft. Wayne, IN) is also shown for comparison. The whey/soy protein blends have high solubilities at the pH extremes of 3.0 and 7.0 and low solubilities in the pH range 4.0–5.5, near their isoelectric points. The acid whey blend had higher PDI values as compared to the sweet whey blend at the pH extremes. Both blends were superior to the commercial soy isolate at all pH values except those in the range 5.0–6.0.

–Continued on next page

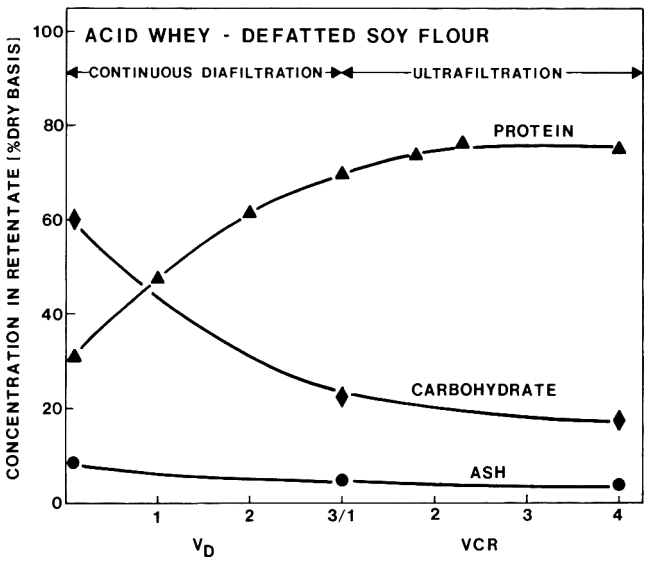


Fig. 3–Composition (% dry basis) of retentate solids during ultrafiltration processing of acid whey/defatted soy flour coextracts.

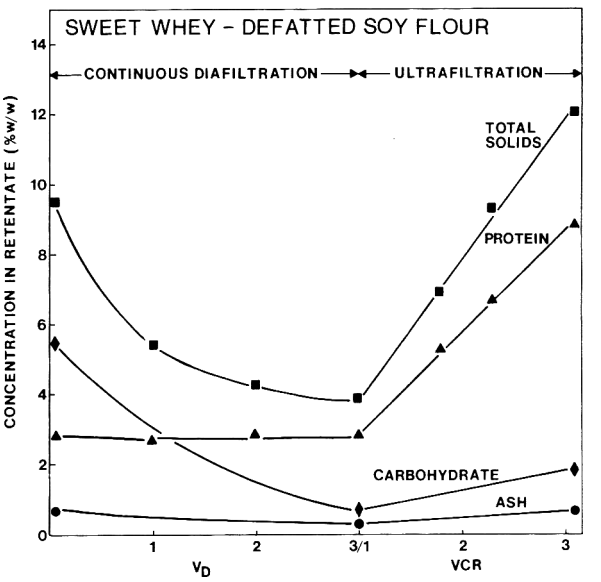


Fig. 4–Composition of retentate during ultrafiltration processing of sweet whey/defatted soy flour co-extracts.

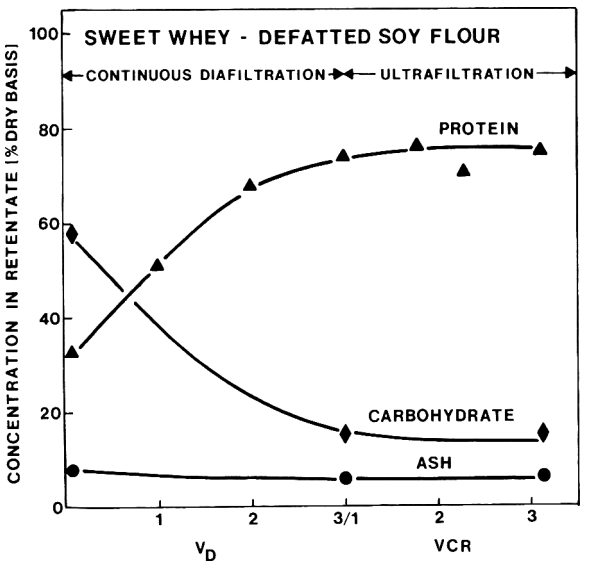


Fig. 5–Composition (% dry basis) of retentate solids during ultrafiltration processing of sweet whey/defatted soy flour coextract.

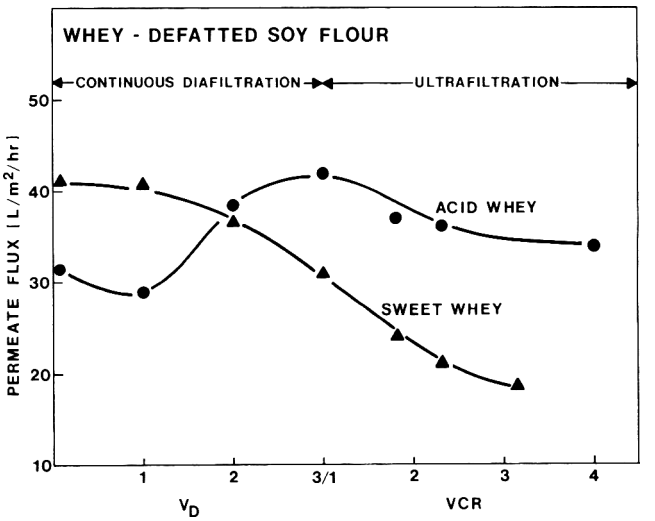


Fig. 6–Permeate flux behavior during hollow fiber [PM 50 (43)] processing of acid and sweet whey/defatted soy flour coextracts. Data are means of replicate runs. Operating conditions: 274 kPa (25 psig) inlet pressure, 205 kPa (15 psig) outlet pressure, 50° C, pH 7.0.

Table 6—Composition of whey/soy protein blends (% dry basis)

Type fo whey	Protein[a]	Ash	Fat	Carbohydrate[b]
Soy/acid whey blend	74.1	4.2	3	17.4
Soy/sweet whey blend	72.2	5.8	5	15.6

[a] Protein calculated as (Total N — NPN) x 6.25.
[b] Calculated as difference.

Table 7—Protein dispersibility index (PDI) of soy/whey protein blends[a]

pH	Soy/acid whey blend	Soy/sweet whey blend	Soy isolate[b]
3.0	89.3	75.8	70.0
4.0	10.1	9.9	5.5
4.5	10.5	11.2	6.0
5.5	16.8	16.2	30.0
6.0	43.9	48.9	55.0
7.0	92.5	80.7	75.0

[a] Data are means of two replicate runs.
[b] Promine D.

CONCLUSIONS

THESE EXPERIMENTS have shown that co-UF processing does offer some advantages in producing high protein dairy/vegetable blends. High protein blends can be produced without prior isolation or concentration of whey and soy protein. Blends with potentially good functional properties can be produced as a result of the mild operating conditions used in each stage of co-extraction and co-UF. The relative proportion of whey and soy protein can be controlled during the co-extraction process. A further advantage is the ability to control the final protein content of the blend by adjusting the extent of co-UF processing. The whey/soy protein blends can be used in the concentrated liquid form obtained from the UF process, or they can be further treated by vacuum evaporation and/or drying to yield a powder. These blends could be used as high protein supplements for the nutritional fortification of foods, as high protein, low lactose and low salt ingredients for speciality food items such as diet and health food beverages, and as functional food ingredients.

REFERENCES

Andres, C. 1980a. Soy isolate successfully replaces caseinate and/or NFDM in cakes. Food Proc. 41(9): 70.
Andres, C. 1980b. Protein update 1980. Products/research change to meet functional needs. Food Proc. 41(9): 38.
Anon. 1980. Dairy/non-dairy blends. Food Eng. 52(8): 110.
Anon. 1979. Dairy Blends. Food Eng. 51(8): 111.
AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.
Craig, T.W. 1975. Food ingredient alternatives in milk substitutes. American Dairy Review 37(2): 40.
De Boer, R. and Hiddink, J. 1980. Membrane processes in the dairy industry. State of the art. Desalination 35: 149.
Frazeur, D.R. and Huston, R.B. 1973. Protein and method for extracting same from soybeans employing reverse osmosis. U.S. Patent 3,728, 327.
Garbutt, J.T. 1979. Isolation of proteinaceous materials. U.S. Patent 4,163,010.
Goldsmith, R.L. 1981. Ultrafiltration production of whey protein concentrates. Dairy Field 164(8): 88.
Goodnight, K.C., Hartman, G.H. and Marquardt, R.F. 1976. Aqueous purified soy protein and beverage. U.S. Patent 3,995,071.
Lah, C.L. and Cheryan, M. 1980. Protein solubility characteristics of an ultrafiltered full-fat soybean product. J. Agric. Food Chem. 28: 911.
Merin, U. and Cheryan, M. 1980. Factors affecting the mechanism of flux decline during ultrafiltration of cottage cheese whey. J. Food Process. Preserv. 4: 183.
Muller, L.L., Hayes, J.F. and Griffin, A.T. 1973. Studies of whey processing by ultrafiltration. 1. Comparative performance of various ultrafiltration modules on whey from hydrochloric acid casein and cheddar cheese. Aust. J. Dairy Tech. 28(2): 70.
Nichols, D.J. and Cheyran, M. 1981a. Production of soy isolates by ultrafiltration: factors affecting yield and composition. J. Food Sci. 46: 367.
Nichols, D.J. and Cheryan, M. 1981b. Production of soy isolates by ultrafiltration: process engineering characteristics of the hollow fiber system. J. Food Process. Preserv. 5: 103.
O'Connor, D.E. 1971. Preparing light-colored protein isolate from sunflower meal by alkali extraction under inert gas blanket followed by membrane ultrafiltration. U.S. Patent 3,622,556.
Olsen, H.S. 1978. Continuous pilot plant production of bean protein by extraction, centrifugation, ultrafiltration and spray drying. Lebensm.-Wiss. u.-Technol. 11: 57.
Omosaiye, O. and Cheryan, M. 1979. Ultrafiltration of soybean water extracts: Processing characteristics and yields. J. Food Sci. 44: 1027.
Pompei, C. and Maletto, S. 1974. Production d'isolats de soja par ultrafiltration et diafiltration. Work Documents, IV Int. Congress of Food Sci. and Technol, Vol. 8, p. 70—72.

Ms received 7/21/81; revised 11/7/81; accepted 11/12/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 7—10, 1981.
This research was supported in part by the Agriculture Experiment Station, Univ. of Illinois. D.J. Nichols was the recipient of Fellowships from Standard Brands, Inc. and General Mills (administered by the Institute of Food Technologists) and of a Ralston Purina Company Food Science Fellowship Award.

Functional Properties of Lupin Seed (*Lupinus mutabilis*) Proteins and Protein Concentrates

S. K. SATHE, S. S. DESHPANDE, and D. K. SALUNKHE

ABSTRACT

Functional and electrophoretic properties of the seed flour and a protein concentrate prepared by alkaline extraction from lupin seeds (*Lupinus mutabilis*, cultivar H-6) were investigated. SDS-PAGE indicated presence of 13 and 12 subunits in the seed flour proteins and the protein concentrate, respectively. Lupin protein concentrate had good water and oil absorption and gelation properties. Solubility of lupin proteins was minimum at a pH of 4.0 but increased rapidly beyond pH 5.0. Foaming capacity of the protein concentrate could be improved by increasing concentration as well as by adding NaCl and was influenced by pH and incorporation of certain carbohydrates. Emulsion properties of lupin proteins were concentration and pH dependent. Moist heat improved the in vitro digestibility of the seed proteins. The seed flour as well as the protein concentrate did not have detectable trypsin, chymotrypsin, and α-amylase inhibitory activities.

INTRODUCTION

ALTHOUGH PRACTICALLY UNKNOWN outside South America's Andean region, lupin seeds (*Lupinus mutabilis*) are one of the highest protein containing (up to 50% protein) legumes, and contain 14–24% oil. Details of protein composition and potential for food processing, apparently, remain unexplored (NAS, 1979).

Composition and protein quality (Hove, 1974; Ballester et al., 1980), nutritive value (Mogghaddam et al., 1976; Ballester et al., 1980), hull composition and hull digestibility (Bailey et al., 1974), oligosaccharides (Macrae and Mogghaddam, 1978), oils (Grindley and Akour, 1955), and carotenoids (El-Difrawi and Hudson, 1979) of lupin seeds are studied. Recently, Ruiz and Hove (1976) reported studies on protein solubilization as affected by different processing conditions such as the particle size, pH, seed to solvent ratio, temperature, and time of extraction. These investigators found that defatting did not improve the protein solubilization and that more than 90% protein could be solubilized at pH above 8.0.

Functional properties of several different species of *Lupinus* have been reported in the literature. Malgarini and Hudson (1980) reported excellent emulsifying and solubility properties of a protein isolate prepared from *Lupinus albus* cv Buttercup. Sosulski et al. (1978) evaluated a protein isolate of *Lupinus angustifolius* as the protein component in imitation and blended milk products. The natural amber color of lupin (*Lupinus termis*) was reported to give a desirable color to macaroni when blended with wheat flours at 2–6% levels (Morad et al., 1980). Air-classification studies on pin-milled white lupin (*Lupinus angustifolius*) appeared to show no beneficial effects on enrichment and separation of a protein-rich fraction and most of the protein was found to be associated with the starch fraction (Sosulski and Youngs, 1979). The same authors also reported that lipids in lupin markedly reduced the foaming properties of the flour.

In the present investigation, we report preparation of a protein concentrate from *Lupinus mutabilis* (cultivar H-6) by an alkali solubilization method. The concentrate thus prepared was evaluated for oil and water absorption, foaming, gelation, and emulsion properties. The molecular weight distribution profiles and the in vitro digestibility of the whole seed proteins and the protein concentrate were evaluated. Since most legumes contain several enzyme inhibitors; trypsin, chymotrypsin, and α-amylase inhibitory activities were also assessed in both the whole seed flour and the protein concentrate. In view of the fact that appreciable amounts of lipids were associated with the protein concentrate prepared from the whole seed flour, the effects of defatting on certain functional properties of the protein concentrate were also investigated.

MATERIALS & METHODS

LUPIN SEEDS (*Lupinus mutabilis*, cultivar H-6) were a gift from Prof. R. Gross (Lima, Peru). Unless mentioned otherwise, all chemicals used were of reagent grade.

Preparation of flour

The dry seeds were ground in a Fitz Mill (The W.J. Fitzpatrick Co., Chicago, IL) to 42 mesh flour.

Preparation of protein concentrate

The method employed for the preparation of protein concentrate is presented in Fig. 1. The whole seed flour was extracted twice with dilute alkali (0.02N NaOH), centrifuged, combined supernatants dialyzed, and freeze-dehydrated.

Defatted protein concentrate

Since the protein concentrate prepared by above method was found to contain an appreciable amount of lipids, a defatted protein concentrate was prepared. Defatting was accomplished by extraction of the protein concentrate with hexane (1:10, w/v) for 12 hr at room temperature (21°C) with intermittant shaking. The slurry was filtered through Whatman #1 filter paper under vacuum and the residue air-dried for 48 hr at room temperature (21°C).

Physicochemical analyses

Proximate analyses for moisture, protein (N X 6.25), fat, and ash were carried out in triplicate according to AOAC (1975) methods and the means reported on a dry weight basis.

Total sugars were extracted with 80% ethanol according to the method of Hymowitz et al. (1972) and determined on aliquots by the method of Dubois et al. (1956).

A 1-g sample was extracted with 10 ml of distilled water at room temperature (21°C) for 2 hr, centrifuged at 5,000 X *g* for 15 min, and the reducing sugars were estimated on aliquots by the method of Sumner (1924).

Protein solubility

To determine the protein solubility profile of the protein concentrate, 10 mg of the concentrate was dissolved in 10 ml of 1N NaOH, pH adjusted to the desired value with 1N HCl, centrifuged (5,000 X *g*, 15 min), and the protein content of supernatants determined by Lowry's method (Lowry et al., 1951). Analyses were performed in triplicate and the means reported.

–Continued on next page

Author Deshpande is with the Dept. of Nutrition & Food Sciences, Utah State Univ., Logan, UT 84322. Author Sathe, formerly with Utah State, is now with the Dept. of Nutrition & Food Science, Univ. of Arizona, Tucson, AZ 85721. Author Salunkhe, formerly with Utah State, is currently Vice Chancellor, Mahatma Phule Agricultural Univ., Rahuri 413 722, Maharashtra State, India.

Polyacrylamide gel electrophoresis (PAGE)

Apparent molecular weights of protein subunits were estimated by the method of Weber and Osborn (1969). Thyroglobulin (330,000), ferritin (18,500 and 220,000), phosphorylase b (94,000), albumin (67,000), catalase (60,000), ovalbumin (43,000), lactate dehydrogenase (36,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400) were used as molecular weight markers (Pharmacia Fine Chemicals, Piscataway, NJ). Ten mg of sample were dissolved in 2 ml of 0.01M sodium phosphate buffer (pH 7.0) containing 2% of each of sodium dodecyl sulfate (SDS) and β-mercaptoethanol, incubated for 12 hr at 50°C, centrifuged (10,000 X *g*, 15 min, 4°C), and the supernatant was employed for electrophoresis. Gels (0.5 X 12.5 cm, 10% monomer concentration) were loaded with 20–30 μl of sample and electrophoresis conducted for 10 hr with 6 mA current per gel. Staining and destaining were accomplished respectively by naphthol blue black (CI 20470) and 10% acetic acid containing methanol (10:1, v/v). Phosphate buffer (0.02M, pH 7.2) served as electrode buffer (for both cathode and anode).

Gelation

The method of Coffmann and Garcia (1977) was employed with slight modifications. Appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, and 16% (w/v) were prepared in 5 ml distilled water. The test tubes containing these suspensions were then heated for 1 hr in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were then further cooled for 2 hr at 4°C. The least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

Water and oil absorption

For oil (Crisco Vegetable Cooking oil, Procter and Gamble, Cincinnati, OH) and water absorption determinations, the method of Beuchat (1977) was followed. A 1-g sample was mixed with 10 ml distilled water or oil for 30 sec in a mixer (Vari-Whirl, mixing control – "Fast"). The samples were then allowed to stand at room temperature (21°C) for 30 min, centrifuged at 5,000 X *g* for 30 min, and the volume of the supernatant noted in a 10 ml graduated cylinder. Density of water was assumed to be 1 g/ml and that of oil was determined to be 0.88 g/ml. Means of triplicate determinations were reported on a dry weight basis.

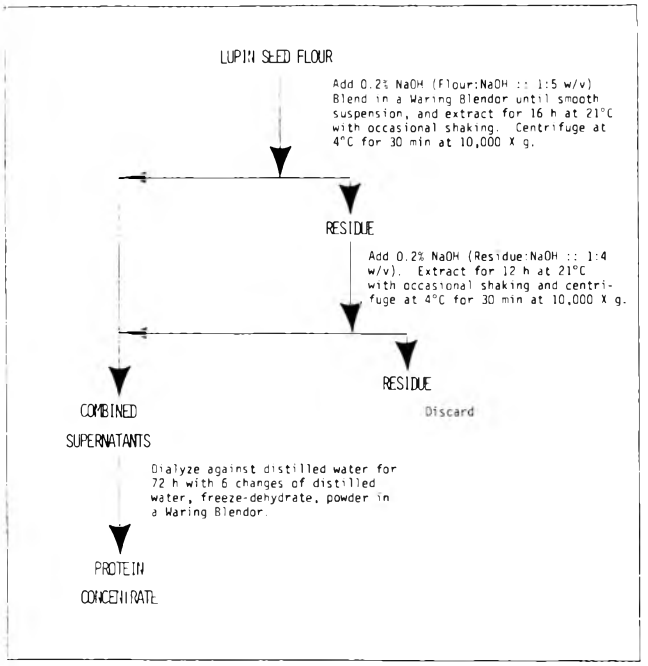


Fig. 1—Schematic diagram for the preparation of lupin seed protein concentrate.

Emulsion properties

Emulsions were prepared according to the method of Beuchat (1977). The details have been described earlier (Sathe and Salunkhe, 1981a).

To study the emulsion capacity and stability, 2% (w/v) emulsions were prepared. Stability of the emulsions was evaluated for 10, 20, 35, 60, and 120 hr at room temperature (21°C) by noting the separation of water in graduated cylinders.

Effects of concentration were evaluated at concentrations of 2, 4, 6, 8, and 10% (w/v).

Effects of pH on emulsion capacity were evaluated on 2% (w/v) slurries by adjusting the pH to a desired value with 1N HCl or 1N NaOH prior to preparing the emulsions.

All experiments were conducted in duplicate at room temperature (21°C) and the means reported.

Foaming properties

Foaming capacity and stability were studied according to the method of Coffmann and Garcia (1977). A 2-g sample was whipped with 100 ml distilled water for 5 min in a Waring Blendor at speed setting "HI" and was poured into a 250 ml graduated cylinder. The total volume at time intervals of 0.0, 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, 14.0, 25.0, and 36 hr was noted. Volume increase (%) was calculated according to the following equation.

$$\text{Volume increase (\%)} = \frac{\text{Volume after whipping (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

Effects of concentration on foaming were evaluated by whipping 2, 4, 6, 8, and 10% (w/v) slurries as described above.

To study the effects of salt (NaCl) concentration on foaming, 2% (w/v) slurries were employed. Salt concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0% (w/v) were investigated.

To study the effects of pH on foaming properties, 2% (w/v) slurries were employed. The pH was adjusted to a desired value with either 1N HCl or 1N NaOH prior to whipping. The foaming capacity and stability were investigated as above.

Effects of certain carbohydrates on foaming properties were investigated. Galactose, sucrose, soluble potato starch (all from J.T. Baker Chemical Co., Phillipsburgh, NJ), amylopectin (ICN Pharmaceuticals, Inc., OH), amylose (potato, Type III, Sigma Chemical Co., St. Louis), gum arabic (Fisher Scientific Co., NJ), and pectin (low methoxyl, Sunkist Growers, Inc., CA) were employed at a concentration of 0.25 g/g protein concentrate.

All experiments were conducted at room temperature (21°C) in duplicate and the means reported.

Enzyme inhibitory activities

Trypsin and chymotrypsin inhibitory activites were evaluated as described earlier (Sathe and Salunkhe, 1981b).

α-Amylase inhibitory activity was evaluated as follows. The seed flour or the protein concentrate (100 mg) was extracted in 3 ml of distilled water at 4°C for 12 hr, centrifuged at 5,000 X *g* for 20 min, and the supernatants were tested for α-amylase inhibitory activity. Enzyme α-amylase (Type I-A, from porcine pancreas) was from Sigma Chemical Co., St. Louis, MO; and soluble potato starch (sub strate) was from J.T. Baker Chemical Co. (Phillipsburgh, NJ).

The enzyme assay (control) was as follows: Soluble potato starch (100 mg) in 9 ml sodium phosphate buffer (0.2M, pH 7.0) was incubated at 37°C with 1 ml enzyme solution containing 150 units activity for 30 min and the reaction was stopped by heating in a boiling water bath for 5 min. The liberated reducing sugars were estimated by the method of Sumner (1924). For the enzyme inhibitor assay, soluble starch (100 mg) in 8 ml sodium phosphate buffer (0.2M, pH 7.0) was incubated with the enzyme inhibitor solution (1 ml enzyme solution containing 150 units activity which was preincubated for 5 min at 37°C with 1 ml of the sample supernatant) at 37°C for 30 min. The reaction was stopped by heating in a boiling water bath for 5 min and the liberated reducing sugars estimated by the method of Sumner (1924). The reducing sugar content of of the original sample was substracted prior to the calculation of the liberated reducing sugars.

All assays were conducted in triplicate.

In vitro protein digestibility

Fifty ml of aqueous suspension of sample (6.25 mg protein/ml) in distilled water were adjusted to pH 8.0 with 0.1N HCl and/or 0.1N NaOH. The slurry was then incubated for 15 min at 37°C (in a water bath). The multienzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase/ml (peptidase and trypsin from Sigma Chemical Co., St. Louis, MO; chymotrypsin from P.L. Biochemicals, Milwaukee, WI), was maintained in an ice bath and adjusted to pH 8.0 with 0.1N HCl and/or 0.1N NaOH. Five ml of this multienzyme solution was added to the sample suspension with constant shaking at 37°C. The pH of the suspension after incubation for 10 min at 37°C was recorded and the in vitro digestibility was calculated according to the regression equation of Hsu et al. (1977);

$$Y = 210.464 - 18.103\ X$$

where, Y = In vitro digestibility (%), and X = pH of the sample suspension after 10 min digestion with multienzyme solution.

RESULTS & DISCUSSION

Composition

Results of the proximate composition of the seed flour and the protein concentrate are presented in Table 1. The seed flour contained 44.43% protein, 19.12% fat, 13.40% total sugars, and 4.92% of ash as the major components. The protein concentrate had 78.80% protein and 17.93% fat as the major constituents. Wide differences in compositional characteristics of lupin seeds belonging to different species are reported in the literature. Gross and Baer (1977) reported 39.8 and 41.4% protein and 20.9 and 20.1% fat content (on as is basis), respectively, for H-6 and H-1 cultivars of *Lupinus mutabilis* which are in agreement with our results. The same authors reported lower protein and fat contents (35.5–38.1% and 11.7–12.2%, respectively on as is basis) for cultivars of *Lupinus albus*. Sosulski et al. (1976) and Sosulski and Youngs (1979) reported a protein content of 45.4% and 47.4% (on a dry weight basis), respectively, for cultivar of *Lupinus angustifolius*. These authors also reported fat content of 7.6% and 7.8% for dehulled seeds of lupin. Such wide variation in compositional characteristics of lupin may primarily be due to genetic differences in different species.

Protein recovery

Since Ruiz and Hove (1976) observed that defatting did not improve the lupin protein solubilization, whole seed flour was used in the present investigation to prepare the protein concentrate. The protein concentrate (72.80% protein, on a dry weight basis) represented 40% of the starting material (whole seed flour) and 69.09% original protein recovery. These results were higher than those reported by Ruiz and Hove (1976) who recovered 19.8% of starting material and 52% protein recovery from seeds with single extraction at pH 8.5 for 30 min (solvent to flour ratio of 10:1. These investigators reported 25.4% recovery of starting material when extraction was performed twice (10:1 and 5:1, solvent to flour ratios). The higher protein recovery in the present investigation may have been due to longer extraction time (16 hr followed by 12 hr extraction, Fig. 1), and higher extraction pH (10.0) employed in the present investigation.

Protein solubility

The protein solubility profile (Fig. 2) of the protein concentrate indicated that the minimum protein solubility was at a pH of about 4.0 and increased with increasing pH. These observations are in agreement with those of Ruiz and Hove (1976) who observed isoelectric pH of 4.5 for dehulled lupin (*Lupinus angustifolius*) seed proteins. Sosulski et al. (1976) observed least protein solubility for the defatted lupin flour among the ten legume flours investigated. These authors suggested that the nitrogen solubility index of defatted lupin flour could be increased from 20.8 to 63.9% by increasing the pH from 5.5 to 6.5. In an earlier investigation on dispersibility of legume flour proteins, Fan and Sosulski (1974) reported that alkali (0.2% NaOH) is an efficient solvent in solubilizing the flour nitrogen. The alkali extracted proteins had lower solubility at pH 2–3, but at higher pH levels, greater dispersibility was observed. A marked increase in solubility at pH 6–8 was reported by these authors. In the present investigation, we also observed a lower solubility of alkali extacted proteins at pH 3–4 which increased rapidly as the pH was raised beyond 5.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoretic analyses of the seed flour proteins (Fig. 3A) and protein concentrate (Fig. 3B) indicated presence of 13 and 12 subunits respectively. The whole seed proteins were char-

Table 1–Proximate composition of lupin seed flour and protein concentrate[a]

Component	Flour	Protein conc
Moisture (%)	6.86	1.36
Protein (N X 6.25) (%)	44.43	72.80
Crude fat (%)	19.12	17.93
Ash (%)	4.92	0.71
Total sugars (%)	13.40	2.30
Reducing sugars (%)	3.17	0.26

[a] Mean of triplicate determinations on dry weight basis.

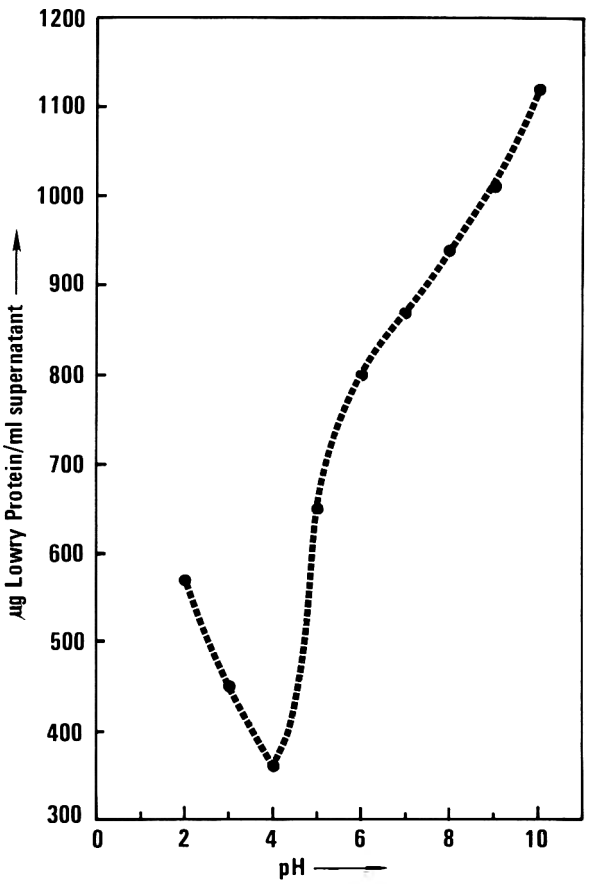


Fig. 2–Protein solubility profile of lupin seed protein concentrate.

acterized by the predominance of six subunits with apparent molecular weights of 98,000; 85,000; 59,000; 51,000; 43,500; and 22,000 while five subunits with apparent molecular weights of 136,000; 90,000; 67,000; 59,000; and 25,000 characterized protein concentrate. The predominance of subunits was judged on the basis of band width and intensity. It appears that the major protein subunits of lupin seed proteins are in a range of 20,000–150,000 daltons.

Gelation

Least gelation concentrations for the seed flour and the protein concentrate were 14 and 8% (w/v), respectively.

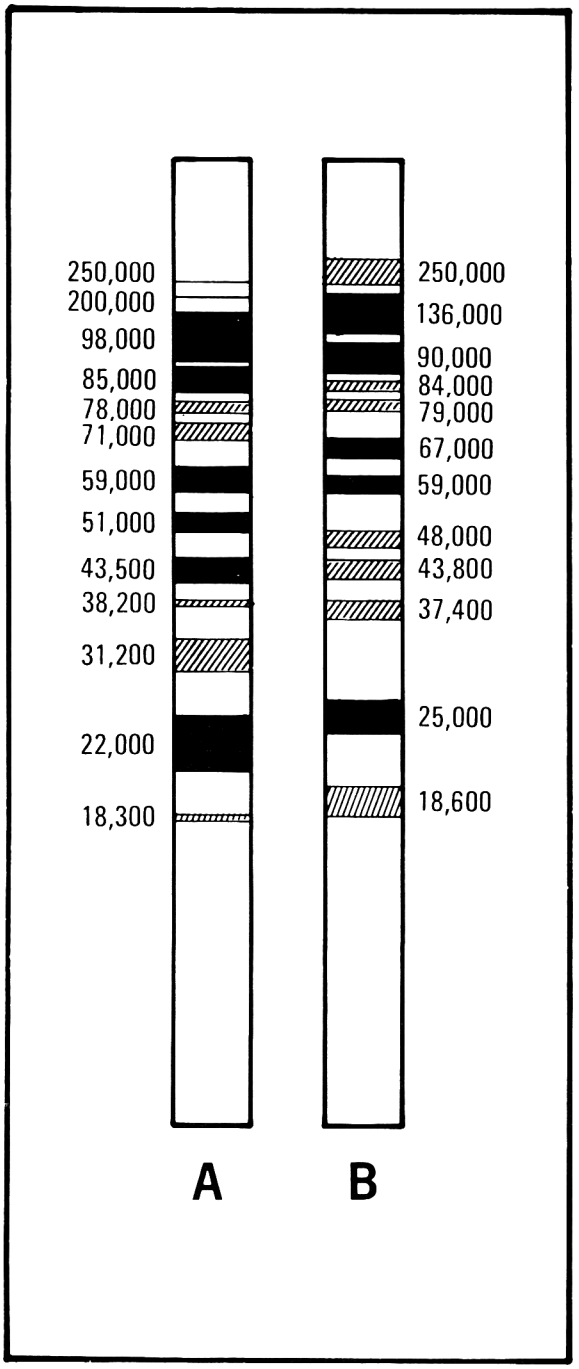


Fig. 3–Schematic diagram for the molecular weight distribution profiles of lupin seed proteins by SDS-PAGE. A–Lupin seed flour proteins; B–Lupin seed protein concentrate.

The least gelation concentration of 14% for the seed flour in the present investigation is higher than that for the Great Northern bean flour (10%, w/v) observed in our earlier investigation. The least gelation concentration for protein concentrate (8%, w/v) was, however, the same as that for the Great Northern bean protein concentrate (8%, w/v) in our earlier studies (Sathe and Salunkhe, 1981a). Sosulski et al. (1976) also observed the Great Northern bean gel to be firmer while that of lupin flour was relatively fluid among the ten legume flours investigated. Such variation in the gelling properties of different legume flours may be ascribed to the relative ratios of different constituents–proteins, carbohydrates, and lipids–suggesting that interactions between such components may also have a significant role in functional properties.

Water and oil absorption

Results of water and oil absorption are presented in Table 2. Water absorption by the seed flour and the protein concentrate was, respectively, 1.20 and 1.37 g/g while corresponding figures for oil absorption were 1.67 and 2.86 g/g, respectively. Water absorption by the seed flour and the protein concentrate in the present investigation were higher than that for the Great Northern bean flour (1 g/g) (Sathe and Salunkhe, 1981a) but was lower than those of soybean flour (2.4 g/g), soybean concentrate (3.6 g/g), sunflower flour (1.8 g/g), and sunflower protein concentrate (3.9 g/g) reported by Sosulski and Fleming (1977). Hydration levels for soybean flours, protein concentrates, and protein isolates have been noted to be 2:1, 2.5:1, and 3–4:1, respectively (Rakosky, 1974). The lower water absorption for lupin seed flour and the protein concentrate than for soy flours, protein concentrates, and protein isolates could be due to the presence of fat and the low availability of polar amino acids as the latter have been shown to be primary sites for water interaction of proteins (Kuntz, 1971). Oil absorption by the seed flour (1.67 g/g) and the protein concentrate (2.86 g/g) were also lower than those for Great Northern bean albumins, globulins, and protein concentrate (3.29, 3.23, and 4.12 g/g, respectively) (Sathe and Salunkhe, 1981a). Oil absorption by the protein concentrate was higher than those for soy flour, soy protein concentrate, sunflower flour, and sunflower protein concentrate (a range of 84.4–256.7% oil absorption) reported by Lin et al. (1974).

Defatting improved the water and oil absorption of lupin seed protein concentrate by 18 and 103%, respectively, compared to those of undefatted protein concentrate (Table 2). The increased water absorption of the defatted protein concentrate may have been due to exposure of water binding sites on side chain groups of proteins previously blocked in a lipophilic environment. Water binding by proteins is influenced by its physicochemical environment (Chou and Morr, 1979). Such increased water absorption due to potential increase of the number of water binding sites by changing the physicochemical environment of proteins due to succinylation of glandless cottonseed flour (Childs and Park, 1976), and succinylation and

Table 2–Water and oil absorption capacity of lupin seed flour and protein concentrate[a]

	Water absorbed g/g	Oil absorbed g/g
Bean flour	1.20	1.67
Protein concentrate	1.37	2.86
Defatted protein concentrate	1.55	3.89

[a] Mean of triplicate determinations.

acetylation of sunflower protein (Canella et al., 1979) is also reported in the literature. Increased oil absorption of the defatted protein concentrate may suggest the lipophilic nature of lupin seed proteins. The presence of several non polar side chains may bind the hydrocarbon chains of fats, thereby resulting in higher absorption of oil. Lin et al. (1974) observed sunflower proteins to be more lipophilic than soy proteins, and concluded that sunflower proteins contained more non polar side chains than soy proteins which retained oil by associative binding.

Emulsion properties

Emulsion capacity of lupin seed flour and the protein concentrate was, respectively, 55.1 and 89.9 g/g (Table 3). Lin et al. (1974) reported emulsion capacity of wheat flour, soy flour, sunflower flour, and protein concentrates and isolates from soy and sunflower flour to be in the range 10.1–25.6% with the exception of soybean flour (95.1% oil emulsified). Canella et al. (1979) reported 54.1% emulsion capacity for sunflower flour. Zakaria and McFeeters (1978) observed emulsion capacity of 62% for soy protein isolate. The emulsion capacity of 88.9 g/g for lupin seed protein concentrate in the present investigation was higher/comparable to the literature values reported for several oil seed flours and protein concentrates/isolates (Crenwelge et al., 1974; Cante et al., 1979; Kinsella, 1979; Sosulski and Youngs, 1979). The high emulsion capacity of lupin seed protein concentrate may be useful for food applications. Protein concentrate emulsion broke down after 60 hr standing at 21°C and hence the emulsion stability was considered to be poor.

Effects of pH and concentration on emulsion capacity of lupin seed protein concentrate are summarized in Table 4. A decrease in an emulsion capacity with increased concentration of protein observed in the present investigation is in agreement with the observations of Lin et al. (1974) on emulsion capacities of sunflower and soybean flours and protein concentrates/isolates. Emulsion capacity was pH dependent and an acid pH improved the emulsion capacity more than did the alkaline pH. At pH 2.0, emulsion capacity increased 3.55 times compared to the emulsion capacity of 2% (w/v) suspension in distilled water without pH adjustment. Dependence of emulsion capacity on pH was expected as it is known that emulsion capacity of soluble proteins depends upon the hydrophilic-lipophilic balance (Sosulski, 1977) which is affected by pH. Similar observations on pH dependence of emulsifying ability of proteins have been reported by several investigators (Swift and Sulzbacher, 1963; Lin et al., 1974; Crenwelge et al., 1974; Hutton and Campbell, 1977; Canella et al., 1977; Lah and Cheryan, 1980).

Foaming properties

Results of foaming capacity and stability of lupin seed flour and the protein concentrate are presented in Table 5. After 36 hr standing at room temperature (21°C) the foams did not collapse completely (104 and 114 ml volume for the flour and the protein concentrate, respectively) indicating good foam stability. Defatting increased the foaming capacity of the protein concentrate by 8%. However, the foams were less stable than those of undefatted protein concentrate and the flour, and collapsed within 3 hr. Foaming capacity of both the flour and the protein concentrate was lower than those reported for soybean and sunflower protein concentrates/isolates by Sosulski and Fleming (1977) and mung bean protein isolate by Coffmann and Garcia (1977) which may have been due to the differences in proteins and the concentrations employed.

–Continued on next page

Table 3–Emulsion capacity and stability of lupin seed proteins[a]

Sample	Oil emulsified g/g	Initial volume of emulsion ml	Volume (ml) of water separated at room temperature (21°C) after time (hr)					
			0	10	20	35	60	120
Bean flour	55.1	192	0	56	60	60	62	62
Protein concentrate	88.9	290	0	0	0	0	0	25

[a] Mean of duplicate determination.

Table 4–Effects of pH and concentration on emulsion capacity of lupin protein concentrate[a]

pH[b]	Emulsion capacity g/g	Concentration % w/v	Emulsion capacity g/g
2	315.5	2	88.9
4	222.2	4	82.2
6	80.0	6	77.3
8	155.5	8	66.7
10	137.8	10	56.9

[a] Mean of duplicate determinations.
[b] A 2% (w/v) slurry was employed to study the effects of pH on emulsion capacity.

Table 5–Foaming capacity and stability of lupin seed proteins[a]

Sample	Volume after whipping ml	% Volume increase	Volume (ml) at room temperature (21°C) after time (hr)								
			0.25	0.5	1.0	1.5	2.0	3.0	14.0	24.0	36.0
Bean flour	132	32	128	126	124	122	122	120	112	106	104
Protein concentrate	150	50	144	142	142	140	132	130	122	120	114
Defatted protein concentrate	158	58	147	143	139	135	120	100	100	100	100

[a] Mean of duplicate determinations on 2% (w/v) slurries.

Foaming was concentration dependent and increased with increasing concentration of protein concentrate in the aqueous dispersion registering 92% increase in volume at 10% (w/v) concentration (Table 6). These results are in agreement with our earlier observations on foaming properties of the Great Northern bean proteins (Sathe and Salunkhe, 1981a).

Addition of salt (NaCl) improved foaming capacity of the protein concentrate (Table 7). Improvement was maximum at a salt concentration of 0.6% (w/v) in the slurry. This improvement in foaming may have been due to increased protein solubility (Sosulski, 1977). Increased foaming of yeast proteins on adding 3% NaCl has also been reported (Schachtel, 1981).

Foaming properties of lupin seed protein concentrate were pH dependent (Table 8). The foaming capacity of the protein concentrate was highest at pH 2.0 and least in the iso-electric region (pH 4.0). However, maximum foam stability was observed at pH 4.0 and it progressively decreased at alkaline pH. Such pH dependence of foaming characteristics was also reported for soy and sunflower proteins (Lin et al., 1974), succinylated and acetylated sunflower proteins (Canella et al., 1979), and peanut protein (Cherry et al., 1979). The high stability of foams in the acid pH range observed in the present investigation may have been due to the formation of stable molecular layers in the air-water interface, which impart texture, stability, and elasticity to the foams. Such molecular stabilizing effect in acidic pH is also reported by Richert (1979).

In food systems, foams are often very complex, including several phases such as a mixture of gases, subdivided solids, subdivided liquids, and multicomponent solutions of water, polymers, and surfactants (Richert, 1979). We investigated the effects of certain carbohydrates on foaming properties of lupin protein concentrate (Table 9). Incorporation of potato starch, amylopectin, sucrose, and amylose at a concentration of 0.25 g/g protein concentrate, increased the foaming capacity of the protein concentrate by 4, 10, 12, and 22%, respectively. Galactose, gum arabic, and pectin had depressing effects on foaming capacity, however. All the carbohydrates investigated decreased the foam stability of the protein concentrate. Foams containing gum arabic and pectin were least stable and collapsed in 24 hr. In most foams, the liquid phase exists

Table 6—Effect of concentration on foaming capacity of lupin seed proteins[a]

Concentration (%, w/v)	Bean flour		Protein concentrate	
	Final foam volume (ml)	% volume increase	Final foam volume (ml)	% volume increase
2	132	32	150	50
4	160	60	170	70
6	180	80	182	82
8	184	84	186	86
10	186	86	192	92

[a] Mean of duplicate determinations.

Table 7—Effect of salt on foaming capacity of lupin seed protein concentrate[a]

Salt concentration %	Final volume ml	% Volume increase
0.0	150	50
0.2	152	52
0.4	158	58
0.6	174	74
0.8	166	66
1.0	160	60
1.5	156	56
2.0	152	52

[a] Mean of duplicate determinations on 2% (w/v) slurries.

Table 8—Effect of pH on foaming capacity and stability of lupin seed protein concentrate[a]

pH[b]	Volume after whipping ml	% Increase	Volume (ml) at room temperature (21°C) after time (hr)								
			0.25	0.5	0.75	1.0	1.5	2.0	6.0	24.0	48.0
2	158	58	156	154	152	149	145	128	100	100	100
4	128	28	121	120	118	116	114	114	110	108	103
6	140	40	132	128	126	121	116	110	104	100	100
8	132	32	126	118	116	114	110	108	100	100	100
10	140	40	106	100	100	100	100	100	100	100	100

[a] Mean of duplicate determinations.
[b] A 2% (w/v) slurry was employed to study the effect of pH on foaming capacity and stability.

Table 9—Effect of carbohydrates on foaming capacity and stability of lupin seed protein concentrate[a]

Carbohydrate[b]	Volume after whipping ml	% Increase	Volume (ml) at room temperature (21°C) after time (hr)							
			0.25	0.5	1.0	1.5	2.0	14.0	24.0	36.0
Control	150	50	144	142	142	140	132	122	120	114
Galactose	132	32	110	108	108	108	108	108	106	106
Sucrose	162	62	134	132	128	126	124	120	116	110
Amylose	172	72	160	154	146	142	140	128	120	108
Amylopectin	160	60	146	140	134	130	128	124	108	105
Potato starch	154	54	148	140	134	132	130	116	106	102
Gum arabic	132	32	122	120	116	112	110	104	100	100
Pectin	138	38	126	126	120	118	116	104	100	100

[a] Mean of duplicate determinations.
[b] A 2% (w/v) slurry was employed to study the effect of carbohydrates (0.25 g/g protein concentrate) on foaming properties of lupin seed protein concentrate.

as lamellae between adjacent bubbles. Instability of foams is indicated by drainage of liquid from the lamellae and by an increase and then rupture in the size of bubbles (Richert, 1979). This is dependent on surface viscosity, capillary forces, film thickness, and orientation of surface active solutes such as proteins and carbohydrates. The adverse effects of carbohydrates on foam stability of lupin proteins observed in the present investigation may have resulted from thinning of films due to random distribution of carbohydrate solids and also by increased coalescence of gas bubbles dispersed in the liquid. Richert (1979) also reported the whey protein foam performance to be carbohydrate dependent (concentration). Our observations were in agreement with his findings.

In vitro protein digestibility

In vitro protein digestibility of the protein concentrate was higher (75.05) compared to that of the flour (71.07%). Moist heat (heating in a boiling water bath for 30 min) improved the in vitro protein digestibility of the seed proteins (Table 10). Improved digestibility on heating may have been due to denaturation of proteins facilitating susceptibility towards enzymic attack. Improved in vitro protein digestibility on heating observed in the present investigation is in agreement with reported improvement in protein digestibility of beans of *Phaseolus vulgaris* (Romero and Ryan, 1978; Chang and Satterlee, 1980; Iyer et al., 1980) and of winged beans [*Psophocarpus tetragonolobus* (L.) DC] (Ekpenyong and Borchers, 1980).

Enzyme inhibitory activities

Lupin seed flour and the protein concentrate did not have detectable trypsin, chymotrypsin, and α-amylase inhibitory activity. Antinutritional factors are characteristically present in legumes (Liener, 1979) which partially account for their underutilization. The absence of enzyme (trypsin, chymotrypsin, and α-amylase) inhibitory activities (Table 11) in lupin seeds may offer nutritional advantage.

CONCLUSIONS

A PROTEIN CONCENTRATE (72.80% protein on a dry weight basis) from lupin seeds (*Lupinus mutabilis* cultivar H-6) was prepared. Protein recovery was 69.09% of original protein content of seeds. Solubility of the protein concentrate was minimum at a pH of about 4.0 and increased rapidly beyond pH 5.0. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) indicated the presence of 13 and 12 subunits in seed flour proteins and protein concentrate, respectively. Least gelation concentrations for the seed flour and the protein concentrate were 14 and 8% (w/v), respectively. Water and oil absorption capacities of the seed flour and the protein concentrate were 1.20 and 1.37 g/g and 1.67 and 2.86 g/g, respectively. Defatting improved the water and oil absorption capacities of the protein concentrate by 18 and 103%, respectively. The protein concentrate had good emulsion capacity but the stability of the emulsions was poor. Emulsion capacity decreased with increased concentration and acidic pH improved the emulsion capacity more than did alkaline pH. Foaming capacity of the protein concentrate could be improved by increasing concentration as well as by adding NaCl. Maximum increase was registered at 10% (w/v) concentration and at 0.6% salt concentration in the slurry (92% and 74% volume increase, respectively). Foaming capacity was maximum at pH 2.0 whereas highest foam stability was recorded at pH 4.0. Incorporation of potato starch, amylose amylopectin, and sucrose increased the foaming capacity of the protein concentrate whereas galactose, gum arabic, and pectin had depressing effects. All the carbohydrates investigated decreased the foam stability of the protein concentrate. Moist heat improved the in vitro digestibility of the seed proteins. Unlike most other legumes, lupin seeds did not have detectable trypsin, chymotrypsin, and α-amylase inhibitory activities which could be nutritionally advantageous.

Table 10—In vitro protein digestibility of lupin seed proteins[a]

Sample	In vitro protein digestibility (%)
Bean flour (control)	71.07
Bean flour (30 min moist heat)	77.58
Protein concentrate (control)	75.05
Protein concentrate (30 min moist heat)	80.12

[a] Mean of duplicate determinations.

Table 11—Anti-enzyme activities in lupin seeds

	Bean flour[a]	Protein conc[a]
Trypsin inhibition	–	–
Chymotrypsin inhibition	–	–
α-Amylase inhibition	–	–

[a] – = Inhibitory activity was absent.

REFERENCES

AOAC. 1975. "Official Methods of Analysis." Association of Official Analytical Chemists, Washington, DC.

Bailey, R.W., Mills, S.E., and Hove, E.L. 1974. Composition of sweet and bitter lupin seed hulls with observations on the apparent digestibility of sweet lupin seed hulls by young rats. J. Sci. Food Agric. 25: 955.

Ballester, D., Yanez, E., Garcia, R., Erazo, S., Lopez, F., Haardt, E., Cornejo, S., Lopez, A., Pokniak, J., and Chichester, C.O. 1980. Chemical composition, nutritive value, and toxicological evaluation of two species of sweet lupin (Lupinus albus and Lupinus luteus). J. Agric. Food Chem. 28: 402.

Beuchat, L.R. 1977. Functional and electrophoretic characteristics of succinylated peanut flour proteins. J. Agric. Food Chem. 25: 258.

Canella, M., Castriotta, G., and Bernardi, A. 1979. Functional and physicochemical properties of succinylated and acetylated sunflower proteins. Lebens. Wiss. u-Technol. 12: 95.

Cante, C.J., Franzen, R.W., and Saleeb, F.Z. 1979. Proteins as emulsifiers: Methods for assessing the role. J. Amer. Oil Chem. Soc. 56: 71A.

Chang, K.C. and Satterlee, L.D. 1980. Isolation and characterization of the major protein from Great Northern beans (Phaseolus vulgaris). Paper presented at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 9—11.

Cherry, J.P., McWatters, K.H., and Beuchat, L.R. 1979. Oilseed protein properties related to functionality in emulsions and foams. In "Functionality and Protein Structure," Ed. Pour-El, A., p. 1. ACS Sym. Series, Washington, DC.

Childs, E.A. and Park, K.K. 1976. Functional properties of acylated glandless cottonseed flour. J. Food Sci. 41: 713.

Chou, D.H. and Morr, C.V. 1979. Protein-water interactions and functional properties. J. Amer. Oil Chem. Soc. 56: 53A.

Coffmann, C.W. and Garcia, V.V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. J. Food Technol. (U.K.) 12: 473.

Crenwelge, D.D., Dill, C.W., Tybor, P.T., and Landmann, W.A. 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci. 39: 175.

Dubois, M., Gilles, K., Hamilton, J.K., Rebrers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Ekpenyong, T.E. and Borchers, R.L. 1980. Effect of cooking on the chemical composition of winged beans (Psophocarpus tetragonolobus). J. Food Sci. 45: 1559.

El-Difrawi, E.A. and Hudson, BJ.F. 1979. Identification and estimation of carotenoids in the seeds of four Lupinus species. J. Sci. Food Agric. 30: 1168.

Fan, T.Y. and Sosulski, F.W. 1974. Dispersibility and isolation of proteins from legume flours. Can. Inst. Food Sci. Technol. J. 7: 256.

Grindley, D.N. an Akour, A.A. 1955. The seed oils of Bombax sessile and Lupinus termis. J. Sci. Food Agric. 6: 11.

Gross, R. and Baer, E.V. 1977. Posibilidades del Lupinus mutabilis y Lupinus albus en los paises andinos. Arch. Latinoam. Nutr. 27: 451.

Hove, E.L. 1974. Composition and protein quality of sweet lupin seed. J. Sci. Food Agric. 25: 851.

—Continued on page 497

Oligosaccharides in Eleven Legumes and Their Air-Classified Protein and Starch Fractions

F.W. SOSULSKI, L. ELKOWICZ, and R.D. REICHERT

ABSTRACT

Oligosaccharide compositions of flours from dehulled seeds of eleven legumes were determined by gas-liquid chromatography and mass spectroscopy. While soybean contained 11.7% sugars, concentrations in lupine, cowpea, chickpea and lentil were about 8%; lima, navy and northern beans, field pea, mung bean and fababean contained about 5% of sugars. Sucrose represented 20–55% of the total sugars; stachyose was the principal α-galactoside in most species. In addition, high amounts of manninotriose were found in chickpea and lentil flours whereas verbascose was a major component in field pea, mung bean and fababean flours. The nine species and biotypes which contained starch were pin milled and air-classified into protein-rich and starch-rich fractions. The protein fractions were 40–90% higher than the flours in α-galactosides, especially raffinose, manninotriose, stachyose and verbascose. The starch fractions were depleted in α-galactosides, the concentrations varied from 1.2–2.8% of the fraction.

INTRODUCTION

DRY, MATURE LEGUMES are widely reported to promote the formation of intestinal gas following ingestion. A limited number of experiments with human subjects has demonstrated that legume species, biotypes and cultivars exhibit wide variations in flatus production. In general terms, the comparative gas-forming properties of dry legumes, in decreasing order, are reported to be the field bean biotypes, chickpea, lima bean, soybean, pigeon pea, mung bean, smooth peas and lentil (Calloway et al., 1971; Fleming, 1981; Hulse, 1975; Rackis et al., 1970a).

A number of investigators have demonstrated that the oligosaccharides, raffinose and stachyose, are the principal causes of flatulence in human and animal studies (Calloway and Murphy, 1968; Fleming, 1981; Rackis et al., 1970b, Reddy et al., 1980). Normally, the low molecular weight sugars such as sucrose are absorbed along the lining of the small intestine. However, the human digestive system lacks the enzyme, α-galactosidase, and the raffinose family of oligosaccharides pass into the large intestine where they are fermented anaerobically to produce gas.

Stachyose and, to a lesser extent, raffinose are the principal α-galactosides in soybean (Eldridge et al., 1979), chickpea (Åman, 1979; Lineback and Ke, 1975), field bean (*Phaseolus vulgaris*) (Naivikul and D'Appolonia, 1978) and lupine (Macrae and Zand-Moghaddam, 1978). Species such as fababean, field pea, lentil and mung bean contain a substantial quantity of the higher molecular weight α-galactoside, verbascose, and proportionately less of stachyose (Cerning-Beroard and Filiatre, 1976; Lineback and Ke, 1975; Naivikul and D'Appolonia, 1978; Vose et al., 1976). Reddy et al. (1980) concluded that the oligosaccharides in black gram (*Phaseolus mungo*), primarily verbascose, induced a high level of flatus in rats. In a similar investigation, Fleming (1981) determined that stachyose concentrations in seven legumes were highly correlated with hydrogen production in rats. Raffinose gave a lower positive correlation whereas verbascose contents showed a negative relation with hydrogen production.

Recently, Schweizer et al. (1978) and Åman (1979) have reported significant quantities of galactopinitol, galactinol and manninotriose among the oligosaccharides extracted from several legume seeds. Specifically, chickpea and lentil contained higher amounts than soybean. Fababean, field pea, common bean and mung bean showed only traces of these sugars. The roles of these α-galactosides in flatus formation has not been elucidated.

Aqueous or alcoholic extraction of soybean flours during protein concentration will reduce oligosaccharide amounts quite markedly (Eldridge et al., 1979). Flatus volumes after ingestion of alcohol-extracted field beans (Calloway et al., 1971) and soybean (Rackis et al., 1970a) were reduced substantially. Protein concentrates can be prepared from starchy legumes by dry processing techniques. Vose et al. (1976) and Eskin et al. (1980) have reported that protein concentrates obtained by air classification of field pea and fababean flours contained greater concentrations of oligosaccharides than the original flours.

To obtain more information on potential problems with flatulence in air classified protein fractions, a study was undertaken to determine the composition of oligosaccharides in flours of eleven legumes. The nine legumes which contained starch were pin milled and air classified into fine (protein) and coarse (starch) fractions to assess the distribution of oligosaccharides between the two fractions.

MATERIALS & METHODS

Materials

Seeds of mung bean (*Vigna radiata*), small fababean (*Vicia faba minor*), baby lima bean (*Phaseolus lunatus*), navy or pea bean and Great Northern bean (*Phaseolus vulgaris*), field pea (*Pisum savitum arvense*) and lentil (*Lens culinaris*) were grown on experimental plots at the University of Saskatchewan. Chickpea (*Cicer arietium*) and cowpea (*Vigna unguiculata*) were obtained from commercial sources. These legumes were dehulled, pin-milled in an Alpine Pin Mill model 250 CW, and then fractionated into light and dense particles on an Alpine Air Classifier Type 132 MP using a cut point of about 800 mesh (15 μ diameter). As proposed by Vose et al. (1976), the dense starch fractions were remilled and air classified to remove additional light proteinaceous material in order to produce a more refined starch fraction. Analyses for oligosaccharides were conducted on the pin-milled flours, the initial protein fractions and the final starch fractions. The intermediate proteinaceous material, which was not analyzed, represented about 10–15% of the original flour.

Commercial soybean flour (Staley F-200) and protein concentrate (Promosoy 100) were included in the study for comparative purposes. Lupine seed, which contained essentially no starch and showed little protein segregation during air classification (Sosulski and Youngs, 1979), was evaluated only as a flour for comparative purposes. Lupine (*Lupinus albus*) flours of the Kievsky mutant (Department of Food Science, University of Reading) and the cultivar Reuscher (Department of Plant Science, University of Manitoba) were prepared for analysis by dehulling, grinding and hexane extraction of lipids, followed by solvent removal from the residue and grinding in a pin mill.

Authors Sosulski and Elkowicz are affiliated with the Dept. of Crop Science, Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada. S7N 0W0. Author Reichert is affiliated with the Prairie Regional Laboratory, National Research Council.

Extract preparation

Samples (5.0g) were refluxed with 25 ml of 80% methanol for 1 hr and centrifuged. Residues were mixed for 1 hr with 50 ml of 80% methanol using a magnetic stirrer, and centrifuged again. Supernatant solutions were pooled and diluted to 100 ml with 80% methanol.

Sugar analyses

Based on a modification of the Vose et al. (1976) procedure, the sugars were quantitated by transferring 1 ml of methanol extract into a test tube containing 1 ml of internal standard solution (phenyl α-D-glucoside, 1 mg/ml methanol). Four drops of saturated lead acetate were used to precipitate noncarbohydrate material. Following centrifugation to remove the precipitate, the excess lead was removed by addition of one drop of saturated monopotassium phosphate. The samples were dried by rotovaporation at 50°C and the sugars derivatized by the addition of 3 ml of pyridine, 1 ml of HMDS (hexamethyl disilazane) and 0.1 ml TFA (trifluoracetic acid) obtained from Sigma Chemical Co. Samples (1 μl) were injected onto a Hewlett Packard 5710A gas-liquid chromatograph (GLC) equipped with a flame ionization detector and twin stainless steel columns (0.46 m x 3 mm i.d.) packed with 3% Dexsil 300 on 80–100 mesh Chromosorb W treated with HMDS (AW-DMCS) from Chromatographic Specialties Ltd., Brockville, Ontario. Injection port and detector temperatures were 350° and 400°C, respectively. Oven temprature was programmed from 130° to 370°C at 8°/min. The helium carrier gas flow rate was 40 cc per min.

The response factors for glucose, sucrose, galactinol, raffinose and stachyose were obtained using a standard mixture of the sugars (1 mg sugar/ml 80% methanol). The response factors for the other α-galactosides were obtained by extrapolation as for verbascose in Fig. 1 where, in the regression equation, x represents the number of sugar residues. For these standards, the correlation of response factors with number of sugar residues was R = −0.989. Retention times and temperatures of the sugars are shown in Table 1. The repeatability of the results decreased with longer retention times.

Total sugars in the methanol extracts were measured by the phenol-sulfuric acid colorimetric procedure of Dubois et al. (1956) using raffinose as the standard (Cerning-Beroard, 1975).

Gas chromatography-mass spectrometry (GC-MS) of the TMS-derivatives of the sugars was achieved by electron impact ionization using a Finnigan Model 3300 instrument interfaced with an Incos Model 2300 data acquisition system. The glass column (1.83 m x 2 mm i.d.) was packed with 1.5% Dexsil 300 on Chromosorb W. The helium carrier gas flow rate was 28 cc/min. The temperature was programmed from 130° to 350°C at 8°/min. Injector temperature was 300°C, separation oven was at 350°C. Mass spectra were taken at an electron energy of 70 eV and a scan time of 3.0 sec from mass 40 to mass 950.

Standards for galactopinitol and manninotriose were not available. The MS fragments of galactopinitol were essentially identical to those reported by Schweizer et al. (1978). The Åman (1979) procedure for mild acid hydrolysis of stachyose was followed to produce manninotriose which gave the same retention time as component 6 in the GLC chromatograms of the TMS derivatives of oligosaccharides. The results were confirmed using acetylated derivatives and fragmentation by chemical ionization.

The 70 eV mass spectrum for the TMS-derivative of galactose had its base peak at m/e 204 and major peaks at m/e 191 and 217. In addition galactopinitol gave major peaks at m/e 393 and 315 while manninotriose showed peaks at m/e 361, 451, 317, 273 and 271.

RESULTS & DISCUSSION

Composition of legume flours

Except for soybean, the legume flours had only 0.1–0.3% of monosaccharides and inositol derivatives (Table 2). Because the GLC column temperature was increased at 8°/min, it was not possible to distinguish between the various simple sugars and cyclitols which appeared in Fig. 2 and 3.

Compared to the low amounts of simple sugars, sucrose represented 20–55% of the total sugars determined by GLC, the highest amounts being obtained in soybean flour. The high sucrose concentration in soybean was a major factor contributing to the total sugar content of 11.7% in this defatted flour. This total greatly exceeded the range 4.5–8.6% for the other legume flours. Analyses of the total sugars by the phenol-sulfuric acid method gave values slightly higher than the GLC total for most legumes.

The α-galactosides constituted only 40% of total sugars in soybean whereas the proportion ranged up to 73% in the other legume flours. The concentrations of stachyose, raffinose and galactopinitol isomers in soybean flour were somewhat lower than literature values (Eldridge et al., 1979; Macrae and Zand-Moghaddam, 1978; Schweizer et al. 1978). The present soybean sample showed no traces of verbascose which was present in all of the other flours.

Stachyose was the principal sugar component in the lupine and cowpea flours which also contained significant quantities of raffinose, verbascose and galactinol (Table 2). The high amounts of total α-galactosides (5.4–5.9%) were within the ranges reported for lupines (Macrae and Zand-Moghaddam, 1978) and cowpea cultivars (Akpapunam and Markakis, 1979). One of the lupine samples also contained 0.6% of lupanine. *–Continued on next page*

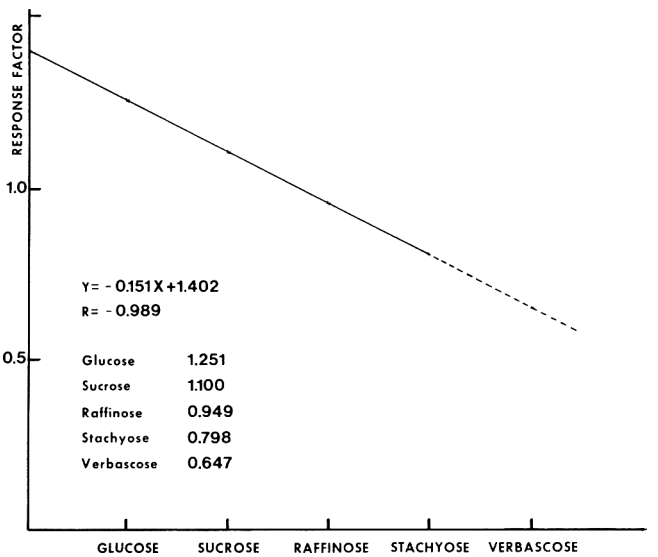


Fig. 1–Response factors for GLC analysis of sugars including the extrapolation for verbascose.

Table 1–Retention temperatures and retention times of the sugar derivatives on a 0.46 m x 3 mm i.d. twin column with 3% Dexsil 300 on Chromosorb W treated with HMDS (AW-DMCS), and the repeatability of the results

Sugars	Retention temp °C	Retention time min	Repeatability of analyses: Std dev	Repeatability of analyses: Coeff. of variation
α-glucose	160	3.7	–	–
β-glucose	170	4.9	–	–
phenyl α-D-glucoside	200	8.6	–	–
Sucrose	220	11.5	0.03	1.3
Galactopinitol isomers	230	12.6	–	–
Galactinol	255	15.7	–	–
Raffinose	265	17.6	0.02	2.7
Manninotriose	286	19.5	–	–
Stachyose	310	22.7	0.06	3.9
Unknown	322	24.0	–	–
Verbascose	345	26.7	0.22	13.4

Chickpea and lentil flours exhibited a wide distribution of α-galactosides with manninotriose and stachyose being the major components (Table 2). Low concentrations of galactopinitols, galactinol, raffinose, an unknown component (8) and verbascose were recorded on the chromatograms of the flours as shown for chickpea in Fig. 2. Galactopinitol usually appeared as three constitutional isomers (Schweizer et al., 1978) following the sucrose peak. An unknown component containing galactose eluted after stachyose on the GLC chromatograms of both flours. Positive identification of the complete oligosaccharide by GC-MS was difficult because of its high molecular weight.

The compositions of total sugars and α-galactosides in lima, navy and northern beans were low in comparison with the chickpea and lentil (Table 2). Stachyose was the major α-galactoside with raffinose, galactinol and verbascose being minor components. Naivikul and D'Appolonia (1978) obtained similar results with navy and pinto beans but Schweizer et al. (1978) found much higher values for stachyose and raffinose in two *Phaseolus vulgaris* cultivars.

Verbascose was the predominant α-galactoside in field pea, mung bean and fababean (Table 2). Field pea contained more stachyose and raffinose than mung or fababean. Mung bean exhibited low concentrations of the other α-

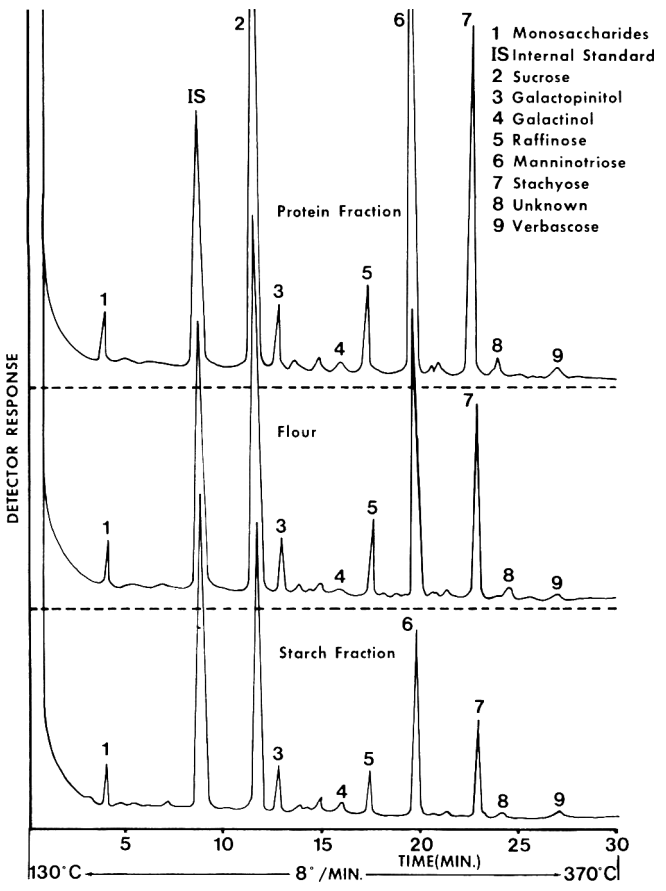


Fig. 2—GLC chromatograms of TMS derivatives of oligosaccharides in chickpea flour, protein fraction and starch fraction.

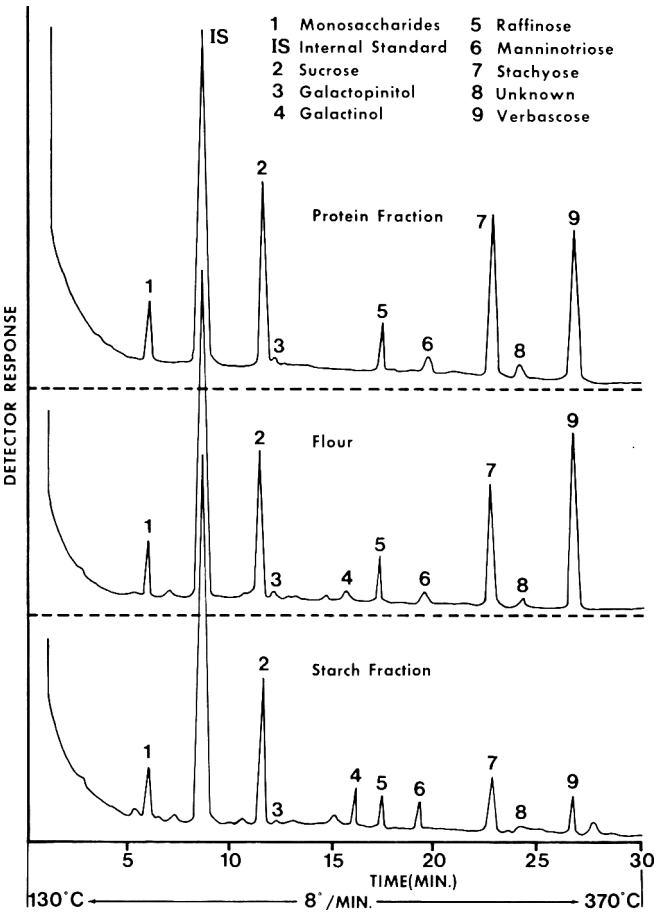


Fig. 3—GLC chromatograms of TMS derivatives of oligosaccharides in mung bean flour, protein fraction and starch fraction.

Table 2—Composition of sugars and inositols in hull-free flours of eleven legumes (% of dry matter)

Legume	Monosaccharides & inositols[a]	Sucrose	Galactopinitol isomers	Galactinol	Raffinose	Manninotriose	Stachyose	Unknown	Verbascose	Total GLC	Total colorimetric	Total α-galactosides
Soybean	0.74	6.35	0.59	tr	1.15	tr	2.85	–	–	11.68	12.20	4.59
Lupine[b]	0.12	2.63	–	0.23	0.82	0.20	4.11	0.06	0.48	8.65	9.95	5.90
Cowpea	0.10	2.64	–	0.12	0.41	–	4.44	–	0.48	8.19	9.04	5.45
Chickpea	0.21	2.69	0.34	0.39	0.45	2.33	1.72	0.18	0.10	8.37	7.75	5.47
Lentil	0.27	3.36	0.34	tr	0.31	1.41	1.47	0.12	0.47	7.75	7.66	4.12
Lima bean	0.26	18.5	tr	0.10	0.46	0.12	2.76	–	0.31	5.86	5.82	3.75
Navy bean	0.10	2.62	–	0.15	0.37	–	2.36	–	0.05	5.65	5.84	2.93
Northern bean	–	3.01	–	0.15	0.26	0.03	2.16	–	0.03	5.64	6.27	2.63
Field pea	–	1.85	–	0.17	0.60	–	1.71	–	2.30	6.63	7.49	4.78
Mung bean	0.22	0.96	tr	0.19	0.23	0.12	0.95	tr	1.83	4.50	4.78	3.32
Fababean	–	2.00	0.17	0.22	0.22	–	0.67	–	1.45	4.73	4.78	2.73

[a] Including glucose, fructose, myo- and scyllo-inositols, pinitol.
[b] Average of two cultivars

galactosides (Fig. 3) present in chickpea and lentil. In contrast, fababean flour contained only galactopinitol and galactinol among the minor constituents (Fig. 4).

Composition of protein fractions

The sample of soybean protein concentrate, obtained by wet processing, contained less than one-tenth the concentration of α-galactosides found in the soybean flour and the total sugar content was only 0.6–0.7% (Table 3). On the other hand, the air classified protein fractions had from 6.7–12.9% of sugars based on the colorimetric procedure. The contents of monosaccharides in the air classified protein fractions were similar to the flours but sucrose concentrations were higher for several species. On the other hand, the total α-galactoside contents of these fractions were 45–90% higher than in the flours.

Cowpea protein fraction contained 6.8% stachyose and 1.3% of verbascose for a high α-galactoside composition (Table 3). Based on its high level of manninotriose, chickpea protein fraction contained 9.2% of total α-galactosides. Lentil concentrate was high in manninotriose, stachyose and verbascose but lima, navy and northern bean protein fractions were mainly enriched in stachyose. While field pea flour was intermeidate in α-galactoside content (Table 2), the protein fraction was very high in verbascose as well as stachyose (Table 3). Mung bean and fababean protein fractions were also enriched in these α-galactosides. It appears that, for these legume species, the major α-galactosides shifted with the protein fraction during air classification.

Composition of starch fractions

The concentrations of monosaccharides, sucrose, galactopinitol and galactinol in the starch fractions were similar to those found in the original flours (Table 4). Apparently, there was little differential segregation of these sugars due to the air classification process. On the other hand, the concentrations of raffinose, manninotriose, stachyose and verbascose were substantially depleted in the starch fractions.

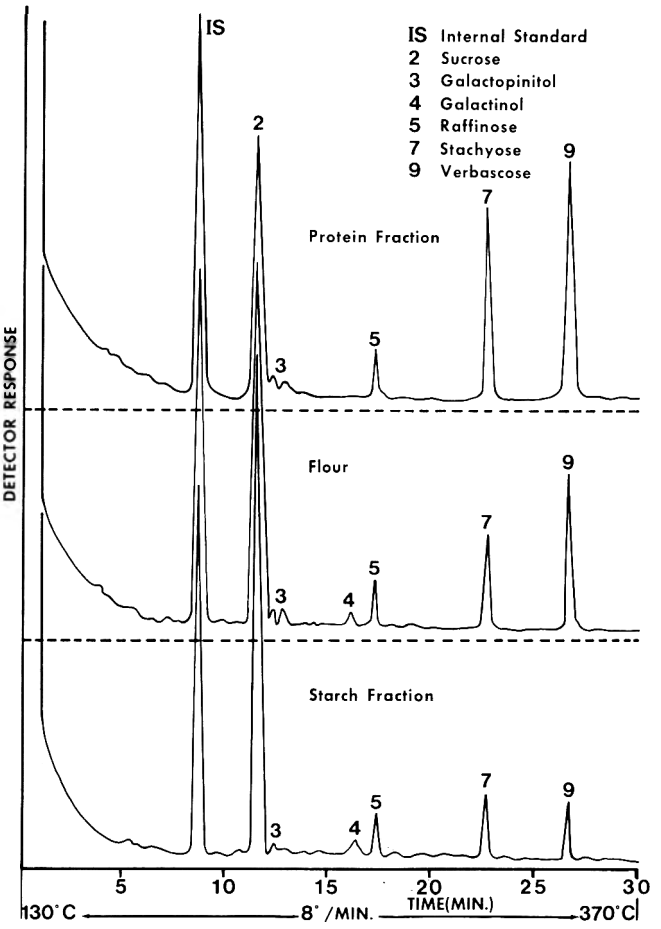


Fig. 4—GLC chromatograms of TMS derivatives of oligosaccharides in fababean flour, protein fraction and starch fraction.

Table 3—Composition of sugars and inositols in protein fractions from ten legume flours (% of dry matter)

Legume	Monosaccharides and inositols	Sucrose	Galactopinitol	Galactinol	Raffinose	Manninotriose	Stachyose	Unknown	Verbascose	Total GLC	Total Colorimetric	Total α-galactoside
Soybean	–	0.28	–	–	0.09	–	0.25	–	–	0.62	0.68	0.34
Cowpea	0.09	2.78	–	tr	0.52	–	6.85	–	1.27	11.51	11.24	8.64
Chickpea	0.24	4.03	0.44	0.23	0.70	4.01	2.98	0.45	0.35	13.43	12.93	9.16
Lentil	0.27	3.18	0.28	0.27	0.50	2.18	2.87	0.32	1.06	10.93	10.53	7.48
Lima bean	0.21	2.39	tr	0.18	0.60	0.09	5.26	–	0.36	9.09	9.56	6.49
Navy bean	0.12	3.38	tr	0.16	0.48	tr	3.84	–	0.25	8.23	8.02	4.73
Northern bean	–	4.50	tr	0.21	0.36	0.11	3.56	–	0.12	8.86	8.95	4.50
Field pea	–	2.83	–	tr	1.24	–	3.76	–	4.64	12.47	12.94	9.64
Mung bean	0.30	1.20	tr	–	0.32	0.21	1.49	–	3.13	6.65	6.73	5.15
Fababean	–	1.35	0.32	–	0.33	–	1.37	–	3.96	7.33	6.95	5.98

Table 4—Composition of sugars and inositols in starch fractions from nine legume flours (% of dry matter)

Legume	Monosaccharides and inositols	Sucrose	Galactopinitol	Galactinol	Raffinose	Manninotriose	Stachyose	Verbascose	Total GLC	Total Colorimetric	Total α-galactoside
Cowpea	0.04	2.38	–	0.11	0.29	–	2.29	–	5.11	5.77	2.69
Chickpea	0.21	1.85	0.32	0.14	0.28	1.32	0.79	–	4.91	4.73	2.85
Lentil	0.29	3.07	0.32	–	0.17	0.78	0.33	–	4.96	4.67	1.60
Lima bean	0.27	1.52	tr	0.13	0.34	tr	1.55	–	3.81	3.78	2.02
Navy bean	–	1.85	–	–	0.21	–	1.00	–	2.54	2.59	1.21
Northern bean	–	2.31	–	0.21	0.19	0.10	0.92	0.01	3.74	3.75	1.43
Field pea	–	1.81	–	tr	0.42	–	0.78	0.59	3.60	3.43	1.79
Mung bean	0.21	0.80	tr	0.29	0.25	0.08	0.45	0.60	2.68	2.62	1.67
Fababean	–	2.72	0.08	0.20	0.25	–	0.48	0.44	4.17	4.18	1.45

tions. Total sugar amounts ranged from 2.6–5.8% by the colorimetric procedure and total α-galactosides ranged from 1.2–2.8% of the starch fractions. Generally, these starchy fractions would have very limited flatulence-inducing properties, although the α-galactoside concentrations still exceeded that of wet processed material such as the soybean concentrate (Table 3).

REFERENCES

Akpapunam, M.A. and Markakis, P. 1979. Oligosaccharides of 13 American cultivars of cowpea (Vigna sinensis). J. Food Sci. 44: 1320.

Åman, P. 1979. Carbohydrates in raw and germinated seeds from mung bean and chick pea. J. Sci. Food Agr. 30: 869.

Calloway, D.H. and Murphy, E.L. 1968. The use of expired air to measure intestinal gas formation. Ann. N.Y. Acad. Sci. 150: 82.

Calloway, D.H., Hickey, C.A., and Murphy, E.L. 1971. Reduction of intestinal gas-forming properties of legumes by traditional and experimental food processing methods. J. Food Sci. 36: 251.

Cerning-Beroard, J. 1975. A note on sugar determination by the anthrone method. Cereal Chem. 52: 857.

Cerning-Beroard, J. and Filiatre, A. 1976. A comparison of the carbohydrate composition of legume seeds: horsebeans, peas and and lupines. Cereal Chem. 53: 968.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Eldridge, A.C., Black, L.T., and Wolf, W.J. 1979. Carbohydrate composition of soybean flours, protein concentrates, and isolates. J. Agr. Food Chem. 27: 799.

Eskin, N.A.M., Johnson, S., Vaisey-Genser, M., and McDonald, B.E. 1980. A study of oligosaccharides in a select group of legumes. Can. Inst. Food Sci. Technol. J. 13: 40.

Fleming, S.E. 1981. A study of relationships between flatus potential and carbohydrate distribution in legume seeds. J. Food Sci. 46: 794.

Hulse, J.H. 1975. Problems of nutritional quality of pigeonpea and chickpea and prospects of research. Presented at International Workshop on Grain Legumes. ICRISAT, Hyderabad, India, p. 189.

Lineback, D.R. and Ke, C.H. 1975. Starches and low-molecular weight carbohydrates from chick pea and horse bean flours. Cereal Chem. 52: 334.

Macrae, R. and Zand-Moghaddam, A. 1978. The determination of the component oligosaccharides of lupin seeds by high pressure liquid chromatography. J. Sci. Food Agr. 29: 1083.

Navikul, O. and D'Appolonia, B.L. 1978. Comparison of legume and wheat flour carbohydrates. I. Sugar analysis. Cereal Chem. 55: 913.

Rackis, J.J., Honig, D.H., Sessa, D.J., and Steggerda, F.R. 1970a. Flavor and flatulence factors in soybean protein products. J. Agr. Food Chem. 18: 2805.

Rackis, J.J., Sessa, D.J., Steggerda, F.R., Shimizu, T., Anderson, J., and Pearl, S.L. 1970b. Soybean factors relating to gas production by intestinal bacteria. J. Food Sci. 35: 634.

Reddy, N.R., Salunkhe, D.K., and Sharma, R.P. 1980. Flatulence in rats following ingestion of cooked and germinated black gram and a fermented product of black gram and rice blend. J. Food Sci. 45: 1161.

Schweizer, T.F., Horman, I., and Würsch, P. 1978. Low molecular weight carbohydrates from leguminous seeds; a new disaccharide: galactopinitol. J. Sci. Food Agr. 29: 148.

Sosulski, F. and Youngs, C.G. 1979. Yield and functional properties of air-classified protein and starch fractions from eight legume flours. J. Am. Oil Chem. Soc. 56: 292.

Vose, J.R., Basterrechea, M.J., Gorin, P.A.J., Finlayson, A.J., and Youngs, C.G. 1976. Air classification of field peas and horsebean flours: chemical studies of starch and protein fractions. Cereal Chem. 53: 928.

Ms received 7/28/81; revised 11/5/81; accepted 11/9/81.

LUPIN SEED PROTEINS . . . From page 497

Hsu, H.W., Vavak, D.L., Satterlee, L.D., and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Hutton, C.W. and Campbell, A.M. 1977. Functional properties of a soy concentrate and a soy isolate in simple systems and in a food system: Emulsion properties, thickening function, and fat absorption. J. Food Sci. 42: 457.

Hymowitz, T., Collins, F.I., Panczner, J., and Walker, W.M. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. Agron. J. 64: 613.

Iyer, V., Salunkhe, D.K., Sathe, S.K., and Rockland, L.B. 1980. Quick-cooking beans (Phaseolus vulgaris L.). 1. Investigations on quality. Qual. Plant. Plant Fds. Hum. Nutr. 30: 27.

Kinsella, J.E. 1979. Functional properties of soy proteins. J. Amer. Oil Chem. Soc. 56: 242.

Kuntz, I.D. 1971. Hydration of macromolecules. 3. Hydration of polypeptides. J. Amer. Chem. Soc. 93: 514.

Lah, C.L. and Cheryan, M. 1980. Emulsifying properties of a full-fat soy protein product produced by ultrafiltration. Lebens. Wiss. u-Technol. 13: 259.

Liener, I.E. 1979. The nutritional significance of plant protease inhibitors. Proc. Nutr. Soc. 38: 109.

Lin, M.J.Y., Humbert, E.S., and Sosulski, F.W. 1974. Certain functional properties of sunflower meal products. J. Food Sci. 39: 368.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol Chem. 193: 265.

Macrae, R. and Mogghaddam, A.Z. 1978. The determination of the component oligosaccharides of lupin seeds by high pressure liquid chromatography. J. Sci. Food Agric. 29: 1083.

Malgarini, G. and Hudson, B.J.F. 1980. Valutazione delle caratteristiche chimiche e di alcune proprieta funzionali di prodotti derivati dal lupino (Lupinus albus). Riv. Ital. Sost. Grasse 57: 378.

Mogghaddam, A.Z., Fleetwood, J.G., and Hudson, B.J.F. 1976. Nutritional value of seeds of the lupinus species. J. Sci. Food Agric. 27: 787.

Morad, M.M., El-Magoli, S.B., and Afifi, S.A. 1980. Macaroni supplemented with lupin and defatted soybean flours. J. Food Sci. 45: 404.

NAS 1979. Tropical Legumes: Resources for the Future. Report of an Ad Hoc Committee on Technology Innovation, Board on Science and Technology for International Development, Commission on International Relations, and National Research Council. National Academy of Sciences, Washington, DC.

Rakosky, J. Jr. 1974. Soy grits, flour, concentrates, and isolates in meat products. J. Amer. Oil Chem. Soc. 51: 123A.

Richert, S.H. 1979. Physical-chemical properties of whey protein foams. J. Agric. Food Chem. 27: 665.

Romero, J. and Ryan, D.S. 1978. Susceptibility of major storage proteins of the bean, Phaseolus vulgaris L., to in vitro enzymatic hydrolysis. J. Agric. Food Chem. 26: 784.

Ruiz, L.P., Jr. and Hove, E.L. 1976. Conditions affecting production of a protein isolate from lupin seed kernels. J. Sci. Food Agric. 27: 667.

Sathe, Ş.K. and Salunkhe, D.K. 1981a. Functional properties of the Great Northern bean (Phaseolus vulgaris L.) proteins: Emulsion, foaming, viscosity, and gelation properties. J. Food Sci. 46: 71.

Sathe, S.K. and Salunkhe, D.K. 1981b. Studies on trypsin and chymotrypsin inhibitory activities, hemagglutinating activity, and sugars in the Great Northern beans (Phaseolus vulgaris L.). J. Food Sci. 46: 626.

Schachtel, A.P. 1981. Effects of preparative processes on the composition and functional properties of protein preparations from Candida utilis. J. Food Sci. 46: 377.

Sosulski, F.W. 1977. Concentrated seed proteins. In "Food Colloids," Ed. Graham, H.D., p. 152. The Avi Publishing Co., Inc., Westport, CT.

Sosulski, F.W., Chakraborty, P., and Humbert, E.S. 1978. Legume-based imitation and blended milk mproducts. Can. Inst. Food Sci. Technol. J. 11: 117.

Sosulski, F.W. and Fleming, S.E. 1977. Chemical, functional, and nutritional properties of sunflower protein products. J. Amer. Oil Chem. Soc. 54: 110A.

Sosulski, F.W., Garratt, M.D., and Slinkard, A.E. 1976. Functional properties of ten legume flours. Can. Inst. Food Sci. Technol. J. 9: 66.

Sosulski, F.W. and Youngs, C.G. 1979. Yield and functional properties of air-classified protein and starch fractions from eight legume flours. J. Amer. Oil Chem. Soc. 56: 292.

Sumner, J.B. 1924. The estimation of sugar in diabetic urine, using dinitrosalicylic acid. J. Biol. Chem. 62: 287.

Swift, C.E. and Sulzbacher, W.L. 1963. Comminuted meat emulsions: Factors affecting meat proteins as emulsion stabilizers. Food Technol. 17: 224.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis.. J. Biol. Chem. 244: 4406.

Zakaria, F. and McFeeters, R.F. 1978. Improvement of the emulsification properties of soy protein by limited pepsin hydrolysis. Lebens. Wiss. u-Technol. 11: 42.

Ms received 5/21/81; revised 9/28/81; accepted 9/29/81.

Journal Paper No. 2701 of the Utah Agricultural Experiment Station and a contribution of Western Regional Project W-150.

The authors thank Prof. R. Gross, Jr. (Tizon y Bueno 276 - Lima 11 - Peru) for a generous gift of lupin seeds.

Functional Properties of Winged Bean [*Psophocarpus tetragonolobus* (L.) DC] Proteins

S. K. SATHE, S. S. DESHPANDE, and D. K. SALUNKHE

ABSTRACT

A protein concentrate (71.45% protein on a dry weight basis) was prepared from winged bean [*Psophocarpus tetragonolobus* (L.) DC] seeds. Solubility of the protein concentrate was minimal at a pH of 4.0. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the bean flour proteins and protein concentrate indicated 9 subunits each, with apparent molecular weight ranges of 27,000–380,000 and 14,200–143,000 daltons, respectively. Least gelation concentrations and water and oil absorption capacities of the seed flour and the protein concentrate were determined. Emulsion and foaming properties of the protein concentrate were investigated. Effect of moist heat on seed protein digestibility was assessed in vitro. Protein concentrate had lower tannins and trypsin, chymotrypsin, and α-amylase inhibitory activities compared to bean flour.

INTRODUCTION

WINGED BEAN [*Psophocarpus tetragonolobus* (L.) DC], a tropical legume, is a promising source of oil and proteins and is listed as one of the under-exploited legumes by NAS (1979). It is unique among leguminous crops in that several parts of the plant (leaves, pods, seeds, and tubers) are edible. The winged bean proteins have an amino acid composition similar to that of soybean proteins, with methionine and cystine being the limiting amino acids (Cerny et al., 1971; Ekpenyong, 1978; Gillespie and Blagrove, 1978a). The major storage proteins of winged bean are predominantly water soluble (approximately 60% soluble at pH 6.6) and unlike soybean proteins, have a characteristic sedimentation coefficient of 7S (Gillespie and Blagrove, 1978b; Garcia, 1981). Winged bean also has less lipoxygenase activity than soybeans (Van Den et al., 1981; de Lumen et al., 1981) and thus may be less susceptible to development of off flavors during handling, storage, and processing.

Earlier investigations from our laboratories (Sathe and Salunkhe, 1981a) indicated that dilute alkali soaking of winged beans could significantly reduce their trypsin inhibitory activity and tannins, and eliminate their hemagglutinating activity. The present investigation was undertaken to prepare and evaluate certain functional properties of winged bean protein concentrate.

MATERIALS & METHODS

WINGED BEAN [*Psophocarpus tetragonolobus* (L.) DC] seeds were donated by Dr. J.M. Spata, Ralston Purina Co., 900 Checkerboard Sq., St. Louis, MO. Unless mentioned otherwise, all chemicals used were of reagent grade.

Preparation of bean flour

The whole dry bean seeds were ground in a UDY Cyclone Mill (Tecator, Inc., Boulder, CO) to obtain a 60 mesh bean flour.

Author Deshpande is with the Dept. of Nutrition & Food Sciences, Utah State Univ., Logan, UT 84322. Author Sathe, formerly with Utah State Univ., is now with the Dept. of Nutrition & Food Science, Univ. of Arizona, Tucson, AZ 85721. Author Salunkhe, formerly with Utah State Univ., is now Vice Chancellor, Mahatma Phule Agrl. Univ., RAHURI 413 722, Maharashtra State, India.

Preparation of protein concentrate

The scheme employed for the preparation of protein concentrate is presented in Fig. 1. Whole beans were extracted twice with dilute alkali (0.2% NaOH) and centrifuged. The combined supernatants were dialyzed and freeze-dehydrated to obtain a protein concentrate.

Physicochemical analyses

Proximate analyses for moisture, protein (N X 6.25), fat, and ash were carried out according to AOAC (1975) methods.

Total sugars were extracted by the procedure of Hymowitz et al. (1972) and determined on aliquots according to the method of Dubois et al. (1956). Reducing sugars were estimated by the method of Sumner (1924).

Each sample (0.5g) was extracted with 10 ml absolute methanol for 24 hr at 4°C and its tannin content was determined by the modified vanillin-hydrochloric acid method of Price et al. (1978) using appropriate blanks. Catechin (Sigma Chemical Co., St. Louis, MO) was used as a reference standard.

All analyses were conducted in triplicate and results expressed on a dry weight basis.

Polyacrylamide gel electrophoresis

Apparent molecular weights of protein subunits were estimated by the method of Weber and Osborn (1969). The details have been described earlier (Sathe and Salunkhe, 1981a).

Protein solubility

To determine the protein solubility profile of the protein concentrate, 10 mg of the concentrate was dissolved in 10 ml of 1N NaOH, pH adjusted to a desired value with 1N HCl, centrifuged (5,000 X *g*, 15 min), and the protein content of supernatants deter-

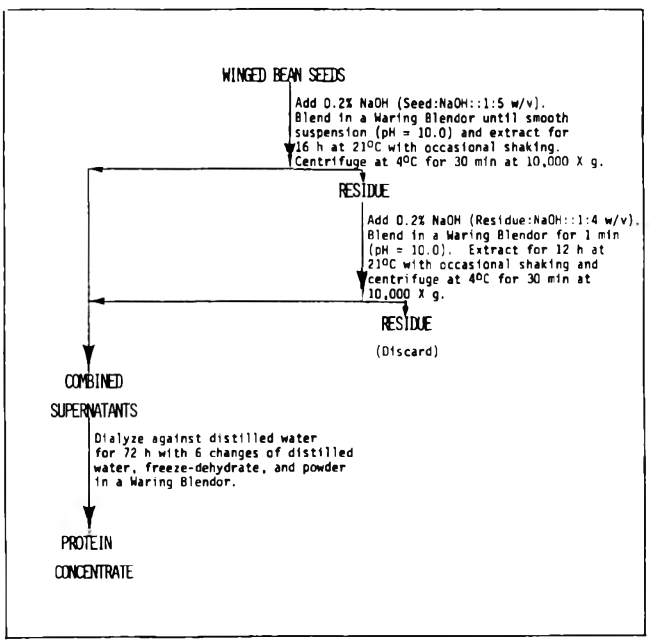


Fig. 1—Schematic diagram for the preparation of winged bean protein concentrate.

mined by Lowry's method (Lowry et al., 1951). Analyses were performed in triplicate and the means reported.

Water and oil absorption

For water and oil (Crisco Vegetable Cooking oil, Procter and Gamble, Cincinnati, OH) absorption determinations, the method of Beuchat (1977) was followed. A 1-g sample was mixed with 10 ml distilled water or oil for 30 sec in a mixer (Vari-Whirl, mixing control - "Fast"). The samples were then allowed to stand at room temperature (21°C) for 30 min, centrifuged at 5,000 X *g* for 30 min, and the volume of the supernatant noted in a 10 ml graduated cylinder. Density of water was assumed to be 1 g/ml and that of oil was determined to be 0.88 g/ml. Means of triplicate determinations were reported on a dry weight basis.

Gelation

The method of Coffmann and Garcia (1977) was employed with slight modifications. Appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20% (w/v) were prepared in 5 ml distilled water. The test tubes containing these suspensions were then heated for 1 hr in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were then further cooled for 2 hr at 4°C. The least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

Emulsion properties

Emulsions were prepared according to the method of Beuchat (1977). The sample (2g) was blended in a Waring Blendor with 100 ml of distilled water for 30 sec at "HI" speed. Oil was added in 5 ml portions continuing blending. The drop in consistency (from a maximum) judged by a decrease in resistance to blending (subjectively) was considered to be the point of discontinuation of oil addition. The amount of oil added upto this point was interpreted as the emulsifying capacity of the sample. The emulsion so prepared was then allowed to stand in a graduated cylinder and the volume of water separated with time was noted in each case, to study the emulsion stability. All experiments were conducted at room temperature (21°C).

Effects of concentration on emulsion capacity were evaluated at concentrations of 2, 4, 6, 8, and 10% (w/v).

Effects of pH on emulsion capacity were evaluated on 2% (w/v) slurries by adjusting the pH to a desired value with 1N HCl or 1N NaOH prior to preparing the emulsions.

Foaming properties

Foaming capacity and stability were studied according to the method of Coffmann and Garcia (1977). A 2-g sample was whipped with 100 ml distilled water for 5 min in a Waring Blendor at "HI" speed and was poured in a 250 ml graduated cylinder. The total volume at time intervals of 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 was noted. Volume increase (%) was calculated according to the following equation.

$$\text{Volume increase (\%)} = \frac{\text{Volume after whipping (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

Effects of concentration on foaming capacity were evaluated by whipping 2, 4, 6, 8, 10% (w/v) slurries as described above.

Salt (NaCl) concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0% (w/v) on 2% (w/v) slurries were employed to study the effects of salt on foaming capacity.

Effects of pH on foaming properties were studied on 2% (w/v) slurries by adjusting the pH to a desired value before whipping. Foam stability ratings at different pH levels were determined at 4°C for 12 hr.

Effects of certain carbohydrates on foaming properties of protein concentrate were investigated. Galactose, sucrose, soluble potato starch (all from J.T. Baker Chemical Co., Phillipsburgh, NJ), amylopectin (ICN Pharmaceuticals, Inc., OH), amylose (potato, Type III, Sigma Chemical Co., St. Louis, MO), gum arabic (Fisher Scientific Co., NJ), and pectin (low methoxyl, Sunkist Growers, Inc., CA) were employed at a concentration of 0.25 g/g protein concentrate.

All experiments were conducted at room temperature (21°C) in duplicate and the means reported.

Table 1—Proximate composition of winged bean flour and protein concentrate[a]

Component (%)	Flour	Protein conc
Moisture	10.18	3.44
Protein (N X 6.25)	39.95	71.45
Fat	17.11	9.03
Ash	4.56	1.85
Reducing sugars	2.04	0.47
Total sugars	9.63	2.96

[a] Mean of triplicate determination. Results are expressed on a dry weight basis.

In Vitro protein digestibility

Fifty ml of an aqueous suspension of sample (6.25 mg protein/ml) in distilled water was adjusted to pH 8.0 with 0.1N HCl and/or 0.1N NaOH. The slurry was then incubated for 15 min at 37°C in a water bath. The multienzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase/ml (peptidase and trypsin from Sigma Chemical Co., St. Louis, MO; chymotrypsin from P.L. Biochemicals, Milwaukee, WI) was maintained in an ice bath and adjusted to pH 8.0 with 0.1N HCl and/or 0.1N NaOH. Five ml of this multienzyme solution was added to the sample suspension with constant shaking at 37°C. The pH of the suspension after incubation for 10 min at 37°C was recorded and the in vitro digestibility was calculated according to the regression equation of Hsu et al. (1977):

$$Y = 210.464 - 18.103\,X$$

where, Y = In vitro digestibility (%), and X = pH of the sample suspension after 10 min digestion with multienzyme solution.

Enzyme inhibitors

Trypsin, chymotrypsin, and α-amylase enzyme inhibitory activities of the bean flour and protein concentrate were determined as described by Decker (1977). Following were the sources of chemicals for the assays: Trypsin, α-amylase (Type I-A, from porcine pancreas), p-tosyl-L-arginine methyl ester (TAME) from Sigma Chemical Co., St. Louis, MO; α-chymotrypsin from P.L. Biochemicals, Milwaukee, WI; and benzoyl-L-tyrosine ethyl ester (BTEE) from Aldrich Chemical Co., Milwaukee, WI.

Statistical analyses

Analyses of variance were conducted using STATPAC/BASIC and STATPAC/FCTCVR programs on a Burroughs 6800 computer. Least significant differences for multiple mean comparison tests were computed according to Ostle and Mensing (1975).

RESULTS & DISCUSSION

Composition

Results of proximate composition of winged bean seeds and protein concentrate are presented in Table 1. The proximate composition of winged bean seeds was comparable to those reported by Ekpenyong (1978) and Garcia and Palmer (1980). The seed flour contained 39.95% protein, 17.11% fat, 9.63% total sugars, and 4.56% ash (on a dry weight basis) as the major components. The protein and fat contents of the protein concentrate were 71.45% and 9.03%, respectively. The high fat content of the protein concentrate suggests that an appreciable portion of the seed lipids is protein bound. The protein concentrate was chocolate brown in color.

Protein recovery

Yield of the protein concentrate was 38% of the starting material (whole seeds), representing a 67.96% protein recovery. These results were comparable to those of Escueta and Tisalona (1981), who reported recoveries of 70% and 60% for Batangas long and Batangas short varieties of winged bean at 1:20 (w/v) meal to solvent (water) ratio.

These authors observed an increased protein recovery with an increasing meal to solvent ratio, with maximum recovery at 1:20 (w/v) dilution. The recovery of protein, however, levelled off after 1:30 (w/v) meal to solvent ratio, which was attributed to decreased filtration efficiency. The protein recovery of 67.96% obtained in the present investigation at low dilution [1:5 followed by 1:4 w/v, meal to solvent (NaOH) ratio] may have been due to cultivar differences, higher extraction pH (10.0), and longer extraction time (16 hr followed by 12 hr extraction, Fig. 1).

Protein solubility

The protein solubility profile (Fig. 2) of the protein concentrate indicated minimum protein solubility at a pH of 4.0, the apparent isoelectric pH of the alkali-extracted winged bean proteins. The solubility of proteins increased rapidly up to about pH 7.0 and then slowly up to pH 12.0. These results are in agreement with those of Garcia (1981) and Escueta and Tisalona (1981). Gillespie and Blagrove (1978a) reported the solubility of winged bean proteins to be 80% from pH 5.0–9.0 at low ionic strengths (less than 0.1). They suggested that the solubility of winged bean proteins was not only affected by the inherent solubility of the proteins but was also related to the disruption of internal cell membranes.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) analyses of the seed flour proteins (Fig. 3A) and protein concentrate (Fig. 3B) indicated the presence of nine subunits each. The three major subunits of whole seed proteins, as judged on the basis of band width and intensity, had apparent molecular weights of 190,000, 82,500, and 39,500 daltons. The protein concentrate was characterized by the presence of seven major subunits having apparent molecular weights of 143,000, 82,500, 56,000, 35,000, 22,500, 16,500, and 14,200 daltons. The predominance of lower molecular weight protein subunits in the protein concentrate may have been partly due to partial hydrolysis of proteins during extraction under alkaline conditions. Garcia (1981) reported that the molecular weights of the water-soluble proteins of winged bean (cultivar TPT-2) were in the range of 22,500–97,000 daltons as estimated by SDS-PAGE. He observed six protein subunits when the beans were extracted with water for 1 hr at pH 6.6. The higher number (9) of subunits and the apparent molecular weight range of 27,000–380,000 daltons for seed flour proteins observed in the present investigation may have been due to cultivar differences. Such variability in the SDS gel patterns due to cultivar differences was previously reported by Gillespie and Blagrove (1975) for cultivars of *Lupinus angustifolius*.

Gelation

Least gelation concentrations for seed flour and protein concentrate were 18 and 14% (w/v), respectively, which

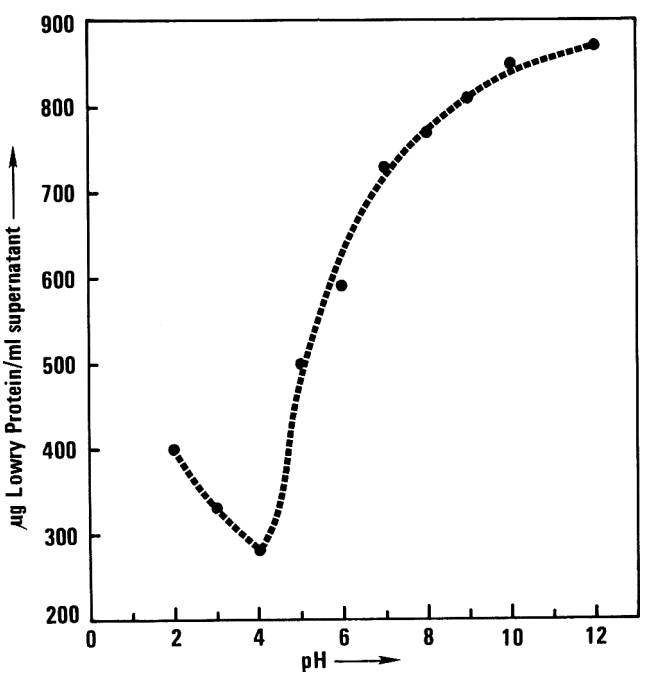


Fig. 2—Protein solubility profile of winged bean protein concentrate.

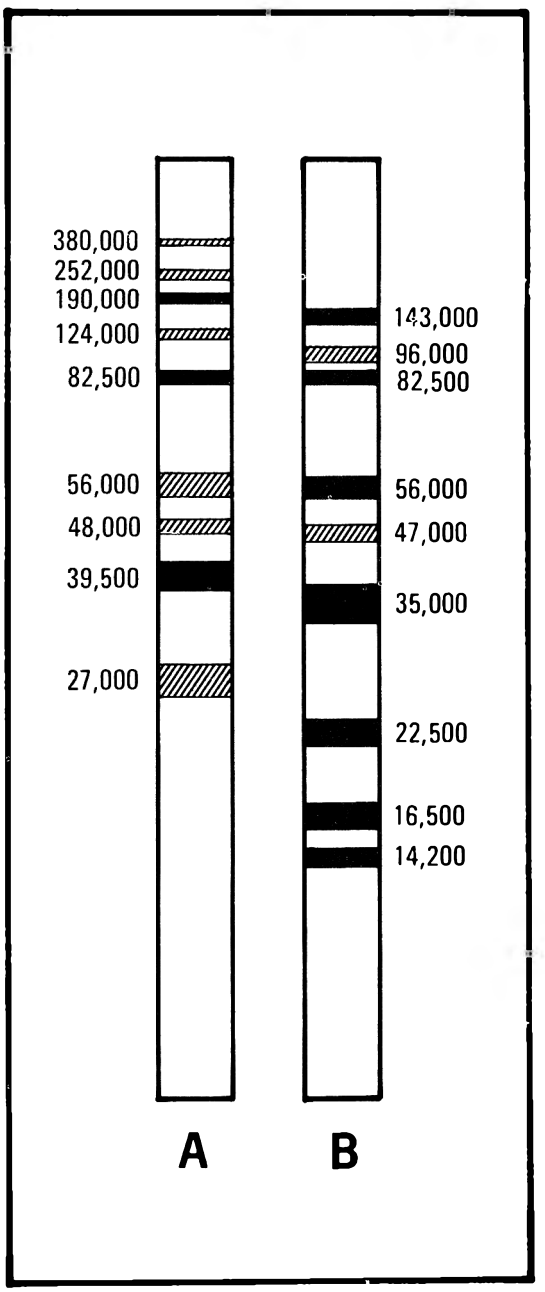


Fig. 3—Schematic diagram for the molecular weight distribution profiles of winged bean proteins by SDS-PAGE. (A) Winged bean seed flour proteins; (B) Winged bean protein concentrate.

were higher than those reported for Great Northern bean (*Phaseolus vulgaris* L.) flour (10%, w/v) and protein concentrate (8%, w/v) (Sathe and Salunkhe, 1981b), and mung bean (*Vigna radiata* L.) protein isolate (10%, w/v) (Coffmann and Garcia, 1977). The low gelling ability of winged bean flour and protein concentrate appears to be a function of seed coat fractions as well as the nature and type of proteins. Seed coat contributes 15.9% of the whole seed by weight of winged bean seeds (Garcia and Palmer, 1980) as compared to 8–12% by weight in several species of *Phaseolus* genera (Singh et al., 1968). Gelation is often an aggregation of denatured molecules and contrary to coagulation where the aggregation is random, gelation involves the formation of a continuous network which exhibits a certain degree of order (Hermansson, 1979). Apparently, the seed coat fractions interfere in the formation of such a continuous network of molecules to form gels. Higher least gelation concentration for winged bean protein concentrate compared to that for the Great Northern bean protein concentrate and mung bean protein isolate may partially be due to the fact that winged bean proteins are predominantly globular in nature (Gillespie and Blagrove, 1978a, b). According to Schmidt (1981), considerably higher protein concentration is usually required for the gelation of globular proteins. Our observations on gelation of winged bean proteins are in agreement with his findings.

Water and oil absorption

Data on water and oil absorption are summarized in Table 2. Water absorption by the seed flour and protein concentrate were respectively, 2.28 and 3.52 g/g, while the corresponding figures for oil absorption were 2.03 and 4.01 g/g, respectively. Water absorption by seed flour in the present investigation was comparable to that of soybean flour (2.4 g/g) and sunflower flour (1.8 g/g) as reported by Sosulski and Fleming (1977). Water absorption of protein concentrate (3.52 g/g) in the present investigation was also similar to that of soybean concentrate (3.6 g/g) (Sosulski and Fleming, 1977). Lin et al. (1974) reported 130% water absorption for soy flour and 227.3 and 196.1%, respectively, for two commercial soy protein concentrates, Isopro and Promosoy. Oil absorptions by the seed flour (2.03 g/g) and protein concentrate (4.01 g/g) in the present investigation were higher compared to soy flour (84.4%) and two commercial soy protein concentrates, Isopro (133.0%) and Promosoy (92.0%) as reported by Lin et al. (1974). The high oil absorption capacity of the winged bean proteins compared to soy proteins suggests that the former may have more apolar amino acids in their composition.

Table 2—Water and oil absorption, emulsion, and foaming capacities of winged bean proteins[a]

Sample	Water absorbed g/g	Oil absorbed g/g	Oil emulsified[b] g/g	Foaming capacity[b] % vol. increase
Bean flour	2.28	2.03	71.10	16
Protein conc	3.52**	4.01**	222.20**	36**

[a] Mean of duplicate determination
[b] A 2% (w/v) slurry was employed.
** Significantly different at p = 0.01.

Table 3—Foam and emulsion stability of winged bean proteins[a]

	Foam stability[b]			Emulsion stability[c]	
Time (hr)	Bean flour	Protein conc	Time (hr)	Bean flour	Protein conc
0.0	116	136	0	0	0
0.5	114	120	10	0	0
1.0	114	120	20	50	0
1.5	114	116	60	50	0
2.0	112	114	100	50	0
3.0	110	110	450	54	0
4.0	110	100	1400	ND[d]	2

[a] Mean of duplicate determination
[b] Volume (ml) at room temp (21°C) of a 2% (w/v) slurry
[c] Volume (ml) of water separated at room temp (21°C)
[d] ND = Not determined.

Emulsion properties

Emulsion capacities of seed flour and protein concentrate were, respectively, 71.10 g/g and 222.20 g/g (Table 2). Lin et al. (1974) reported emulsion capacities of wheat flour, soy flour, sunflower flour, and protein concentrates and isolates from soy and sunflower flours to be in the range of 10.1 to 25.6% with the exception of sunflower flour (95.1% oil emulsified). The emulsion capacity of 222.2 g/g for winged bean protein concentrate in the present investigation was higher compared to the literature values reported for several oilseed and dry bean flours and protein concentrates/isolates (Crenwelge et al., 1974; Cante et al., 1979; Kinsella, 1979; Sosulski and Youngs, 1979). The high emulsion capacity of winged bean protein concentrate may be helpful in stabilizing colloidal food systems.

Emulsions prepared from protein concentrate also had exceptional stability (Table 3). First phase separation occurred after 1400 hr standing at room temperature (21°C). Canella et al. (1979) reported improved emulsion stability of sunflower protein concentrate on succinylation and acetylation. The emulsion stability of the untreated sunflower protein concentrate increased from 46% to 56% on 10% degree of succinylation and up to 60% on acetylation. Lah and Cheryan (1980) evaluated the emulsion properties of a soy isolate (Promine-D) and a full-fat soy protein product produced by ultrafiltration. They reported emulsion stabilities of 86.5% and 83%, respectively, for these two products at a pH of 6.7. It has been indicated that emulsion stability can be greatly increased when highly cohesive films are formed by the adsorption of rigid globular molecules such as lysozyme, which are more resistant to mechanical deformation (both dilatation and shear), than are films containing the flexible β-casein molecules (Graham and Phillips, 1980). Such mechanical properties of the adsorbed interfacial films of protein are supposed to be responsible for resistance to coalescence. The high emulsion stability of protein concentrate may be due to the major proteins of winged beans being globular in nature (Gillespie and Blagrove, 1978b).

Effects of pH and concentration on the emulsion capacity of the protein concentrate are summarized in Table 4. As expected, the emulsion capacity of the protein concentrate decreased with increasing concentration. These results are in agreement with those of Lin et al. (1974) on emulsion capacities of sunflower and soybean flours and protein concentrates/isolates. The dependence of emulsion capacity on concentration of proteins has been explained on the basis of adsorption kinetics (Phillips, 1981). At a low protein concentration, the rate of adsorption is diffusion controlled, but at high protein concentration, there is an activation barrier to adsorption. Under the latter conditions, the ability of the protein molecules to create space in the existing film and to penetrate and rearrange on the surface is rate determining. The dependence of emulsion capacity on protein concentration is also related to the interfacial tension between water and fat globules (Kinsella, 1979). Because of their molecular size, the globular proteins diffuse relatively slowly, but once at the interface, they initially spread easily, though subsequent penetration

of newly arriving molecules into the film may slow further spreading. This results in a decreased emulsion capacity of proteins.

Emulsion capacity was also pH dependent (Table 4). Alkaline pH improved the emulsion capacity more than did acid pH. Similar pH dependence of the emulsifying ability of proteins has been reported by several investigators (Lin et al., 1974; Franzen and Kinsella, 1976; Hutton and Campbell, 1977; Canella et al., 1979; Lah and Cheryan, 1980). It is well known that emulsion capacity of soluble proteins depends upon the hydrophilic-lipophilic balance which is affected by pH (Sosulski, 1977). The net charge at the interface may impede or facilitate emulsifying activity of proteins. According to Kamat et al. (1978) proteins near their isoelectric points (IpH) should perform well because protein adsorption and viscoelasticity at an oil-water interface is maximum near or at IpH. Also, the protein is soluble and not strongly repelled. Hence, maximum emulsifying properties are expected near IpH. However, Kamat et al. (1978) and Franzen and Kinsella (1976) reported that soy protein stabilized emulsions upon heating were most unstable in the isoelectric pH range. They concluded that as net charge was near minimum in this pH range, the protein may have aggregated and destabilized the interfacial membrane. Our observations on increased emulsion capacity of winged bean proteins in the alkaline pH range are in agreement with the findings of Franzen and Kinsella (1976).

Foaming properties

Data on foaming capacity (Table 2) and stability (Table 3) of seed flour and protein concentrate suggest that both seed flour and protein concentrate had lower foaming capacities (16 and 36% volume increase, respectively) as compared to soy flour, concentrate, and isolate (70, 170, and 235% volume increase, respectively) (Lin et al., 1974). The stability of the foam prepared from protein concentrate was also low (complete collapse within 4 hr at 21°C). Foamability is related to the rate of decrease of the surface tension of the air/water interface caused by adsorption of protein molecules. Graham and Phillips (1976) observed that flexible protein molecules such as β-casein, which can rapidly reduce surface tension give good foamability, whereas a highly ordered globular molecule, such as lysozyme, which is relatively difficult to surface denature, gives low foamability. The major proteins of winged bean are also globular in nature (Gillespie and Blagrove, 1978a, b) which may be difficult to surface denature, hence, resulting in lower foaming properties. However low foamabilty may not be associated with all globular proteins. Susheelamma and Rao (1974) reported excellent foaming abilities of a surface active principle in black gram (*Phaseolus mungo*) of the nature of a globulin. Probably, the black gram globulin is easier to surface denature than most other globular proteins, thus, resulting in excellent foaming properties.

Foaming was concentration dependent and increased with increasing concentration of protein concentrate in aqueous dispersions (Table 4). There was a rapid increase in volume up to 6% (w/v) solids concentration with a maximum at 10% (w/v) solids concentration. Improved foaming properties of unmodified and succinylated soy protein isolate (Franzen and Kinsella, 1976) and of glandless cottonseed flour (Cherry and McWatters, 1981) with increasing concentration of flour/isolate are also reported suggesting that increasing the protein concentration in the aqueous dispersions may result in higher foam volumes.

Foaming properties of protein concentrate were pH dependent (Table 4). Maximum increase in volume was observed at pH 2.0, with a progressive lowering as the pH increased. To study foam stability as a function of pH, the foams were stored at 4°C for 12 hr. Foams at pH 4.0 had higher stability (final volume 111 ml) compared to at all the other pH values which reached original volume (100 ml) during this time. Such pH-dependence of foaming characteristics has also been reported for soy and sunflower proteins (Lin et al., 1974), succinylated and acetylated sunflower proteins (Canella et al., 1979), and peanut protein (Sekul et al., 1978). High foam stability in the isoelectric region has been attributed to the formation of stable molecular layers in the air-water interface of the foams (Canella et al., 1979). Protein adsorption and viscoelasticity at an air-water interface is maximum near or at isoelectric pH as the protein is soluble and not strongly repelled. Also, the protein has low net charge near isoelectric pH which may contribute to the formation of stable molecular layers in the air-water interface, which impart texture, stability, and elasticity to the foam. Similarly, the possibility of greater surface denaturation of proteins near isoelectric pH may facilitate the association of the polypeptides, which then can form a continuous cohesive film around the air vacuoles imparting greater foam stability near isoelectric pH (Kinsella, 1979).

Addition of salt (NaCl) improved foaming capacity of the protein concentrate (Table 5). Maximum improvement in foaming was observed at a salt concentration of 0.8% (w/v) in the slurry. Such improvement in foaming due to addition of salt is attributed to increased protein solubility through partial dentaturation (Sosulski, 1977). Kinsella (1976) reported formation of high capacity-low stability foams when salt was added to soy protein suspensions. Addition of salt may improve protein solubility at the interface of the colloidal suspensions during foam formation, thus, improving foaming capacity.—*Continued on next page*

Table 4—Effects of pH and concentration on foaming and emulsion properties of winged bean protein concentrate[a]

pH[b]	Foaming capacity % vol. increase	Emulsion capacity g oil/g sample	Conc. %	Foaming capacity % vol. increase	Emulsion capacity g oil/g sample
2	96	200.0	2	36	222.2
4	60	93.3	4	80	112.0
6	52	173.2	6	88	81.5
8	52	222.2	8	90	63.3
10	50	311.1	10	92	53.3
LSD[c]	(3.98)	(6.26)	LSD[c]	(2.82)	(6.99)

[a] Mean of duplicate determinations
[b] A 2% (w/v) slurry was employed at different pH levels.
[c] Least significant difference at p = 0.05. Ratios of two means exceeding this value are significant.

Table 5—Effects of salt on foamability of winged bean protein concentrate[a]

Salt conc % w/v	Total volume ml	Volume increase %
0.0	136	36
0.2	186	86
0.4	190	90
0.6	194	94
0.8	204	104
1.0	200	100
1.5	200	100
2.0	194	94
LSD[b]	(2.21)	

[a] Mean of duplicate determinations. A 2% (w/v) aqueous suspension was employed.
[b] Least significant difference at p = 0.05. Ratios of two means exceeding this value are significant.

Effects of carbohydrates on foaming properties of the protein concentrate are summarized in Table 6. Incorporation of carbohydrates other than galactose at a level of 0.25 g/g protein concentrate, increased the foaming capacity over that of the control. Addition of polymeric carbohydrates (amylose, amylopectin, potato starch, gum arabic, and pectin) also improved foam stability. Foams containing pectin were most stable and did not collapse completely even after 12 hr standing at 21°C. High foam stability associated with pectin may be due to hydration coupled with the polymeric form. Such increased hydration may increase film thickness and viscosity, thereby reducing air leakage and enhancing foam stability.

In Vitro protein digestibility

In vitro protein digestibility of raw bean flour and protein concentrate was 69.45 and 73.52%, respectively (Table 7). Moist heat (heating in a boiling water bath for 15 and 30 min) improved in vitro protein digestibility of both the seed flour and protein concentrate. Improvement in in vitro protein digestibility of beans of *Phaseolus vulgaris* (Romero and Ryan, 1978; Chang and Satterlee, 1980) and of winged beans [*Psophocarpus tetragonolobus* (L.) DC] (Ekpenyong, 1978) by heating has been reported by several investigators . Such improvement in in vitro digestibility could be at least partially attributed to protein denaturation which improves protein susceptibility to enzymatic attack.

Enzyme inhibitors and tannins

Data on trypsin, chymotrypsin, and α-amylase inhibitory activities of bean flour and protein concentrate are summarized in Table 7. Protein concentrate had 32.17 and 49.66% lower residual trypsin and chymotrypsin activities, respectively, as compared to bean flour. α-Amylase inhibitory activity could not be detected in the protein concentrate. The partial or complete elimination of antienzyme activities observed in the protein concentrate might have been primarily due to the alkali treatment (pH 10.0) employed for solubilizing the proteins. That treatment might have inactivated the inhibitor(s) to a certain extent and/or dialysed the inhibitor(s) out, as these inhibitors are generally low molecular weight proteins (less than 10,000 daltons, Stein, 1976).

The protein concentrate also had a 25.54% lower tannin content than the bean flour (Table 7). In our earlier investigations (Sathe and Salunkhe, 1981a), soaking of whole beans in dilute alkali (2% w/v, NaOH and KOH, respectively) significantly reduced the tannin content (approximately 87% reduction) of beans. That reduction was attributed to leaching losses during soaking of beans. Although the protein concentrate had a lower tannin content than did whole beans, tannin reduction (25.54%) was much less, indicating that alkali solubilization of proteins also extracted a large amount of tannins from the bean flour.

Table 6—Effects of carbohydrates on foaming properties of winged bean protein concentrate[a]

Carbohydrate[b]	Volume after whipping ml	% Increase	Volume (ml) at room temperature (21°C) after time (hr)						
			0.5	1.0	1.5	2.0	3.0	4.0	12.0
Control	136	36	120	120	116	114	110	100	100
Galactose	136	36	122	116	110	100	100	100	100
Sucrose	140	40	130	120	112	110	104	100	100
Amylose	150	50	144	137	132	130	116	108	100
Amylopectin	142	42	132	124	120	118	115	110	100
Potato starch	138	38	130	120	118	114	106	102	100
Gum arabic	144	44	136	128	120	118	116	110	100
Pectin	148	48	144	142	140	140	136	132	104
LSD[c]	(3.82)								

[a] Mean of duplicate determinations
[b] A 2% (w/v) slurry was employed to study the effects of carbohydrates (0.25 g/g protein conc) on foaming properties.
[c] Least significant difference at p = 0.05. Ratios of two means exceeding this value are significant.

Table 7—In Vitro protein digestibility, antienzymes, and tannins of winged bean seed flour and protein concentrate

Sample	*In vitro* protein digestibility[a] %	Trypsin[b,c] inhibitory activity X 10^{-3} per g protein	Chymotrypsin[b,c] inhibitory activity X 10^{-3} per g protein	α-Amylase[b,c] inhibitory activity X 10^{-3} per g protein	Tannins[b] mg/100 g catechin equivalent
Bean flour					
Raw (control)	69.45	283.08	663.34	129.4	235.0
15 min moist heat	73.06	ND	ND	ND	ND
30 min moist heat	74.42	ND	ND	ND	ND
Protein concentrate					
Raw (control)	73.52	192.02**	333.93**	Not detected	175.0**
15 min moist heat	79.85	ND	ND	ND	ND
30 min moist heat	81.75	ND[e]	ND	ND	ND
LSD[d]	(2.73)				

[a] Mean of duplicate determinations
[b] Mean of triplicate determinations
[c] One unit of inhibitor activity is that which reduces the activity of the corresponding enzyme by one unit under assay conditions.
[d] Least significant difference at p = 0.05. Ratios of two means within the same group (bean flour/protein concentrate) or between the group exceeding this value are significant.
[e] ND = Not determined ** = Significant at p = 0.01

CONCLUSIONS

A PROTEIN CONCENTRATE (71.45% protein on a dry weight basis) was prepared from winged bean [*Psophocarpus tetragonolobus* (L.) DC] seeds. Protein recovery was 67.96% of the original protein content. Solubility of the protein concentrate was minimum at a pH of 4.0. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the bean flour proteins and the protein concentrate indicated 9 subunits each with apparent molecular weight ranges of 27,000–380,000 and 14,200–143,000 daltons, respectively. Least gelation concentrations for the seed flour and protein concentrate were 18 and 14% (w/v), respectively. Water and oil absorption capacities of the seed flour and protein concentrate were 2.28 and 3.52 g/g and 2.03 and 4.01 g/g, respectively. Emulsion properties of the protein concentrate were concentration and pH-dependent. Protein concentrate emulsion had exceptional stability (1400 hr). Winged bean proteins had moderate foaming properties. However, foaming capacity of the protein concentrate could be improved by increasing its concentration as well as by adding NaCl. Foaming properties of the protein concentrate were pH-dependent. Maximum foaming was observed at pH 2.0, whereas foams at pH 4.0 were most stable. Polymeric carbohydrates (amylose, amylopectin, potato starch, gum arabic, and pectin) improved foaming properties of the protein concentrate. Moist heat improved in vitro protein digestibility of seed proteins. Protein concentrate had lower trypsin and chymotrypsin inhibitory activities and tannins compared to bean flour. α-Amylase inhibitor activity could not be detected in the protein concentrate.

REFERENCES

AOAC. 1975. "Official Methods of Analysis." Association of Official Analytical Chemists, Washington, DC.

Beuchat, L.R. 1977. Functional and electrophoretic characteristics of succinylated peanut flour proteins. J. Agric. Food Chem. 25: 258.

Canella, M., Castriotta, G., and Bernardi, A. 1979. Functional and physicochemical properties of succinylated and acetylated sunflower protein. Lebensm. Wiss. u-Technol. 12: 95.

Cante, C.J., Franzen, R.W., and Saleeb, F.Z. 1979. Proteins as emulsifiers: Methods for assessing the role. J. Amer. Oil Chem. Soc. 56: 71A.

Cerny, K., Korydylas, M., Pospisil, F., Svabensky, O., and Zajir, B. 1971. Nutritive value of the winged bean (Psophocarpus palustris Desv.). Brit. J. Nutr. 26: 293.

Chang, K.C. and Satterlee, L.D. 1980. Isolation and characterization of the major protein from Great Northern beans (Phaseolus vulgaris). Paper presented at the 40th Annual Meeting of the Institute of Food Technologists. New Orleans, LA, June 9—11.

Cherry, J.P. and McWatters, K.H. 1981. Whippability and aeration. In "Protein Functionality in Foods," Ed. Cherry, J.P., ACS Symp. Ser. 147, p. 149. Amer. Chem. Soc., Washington, DC.

Coffmann, C.W. and Garcia, V.V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. J. Food Technol. (U.K.) 12: 473.

Crenwelge, D.D., Dill, C.W., Tybor, P.T., and Landmann, W.A. 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci. 39: 175.

de Lumen, B.O., Reyes, P.S., and Belo, P.S. Jr. 1981. Studies on winged bean (Psophocarpus tetragonolobus) lipoxygenase. Paper presented in the 2nd International Symposium on Winged Bean, Colombo, Sri Lanka, Jan. 19—23.

Decker, L.A. 1977. "Worthington Enzyme Manual." Worthington Biochemical Corp., Freehold, NJ.

Dubois, M., Gilles, K., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Ekpenyong, T.E. 1978. Biochemical and nutritional evaluation of the winged bean, Psophocarpus tetragonolobus (L.) DC. Ph.D. dissertation, Univ. of Nebraska, Lincoln, NE.

Escueta, E.E. and Tisalona, H.M. 1981. Solubility profile of winged bean protein. Paper presented in the 2nd International Symposium on Winged Bean, Colombo, Sri Lanka, Jan. 19—23.

Franzen, K.L. and Kinsella, J.E. 1976. Functional properties of succinylated and acetylated soy protein. J. Agric. Food Chem. 24: 788.

Garcia, V.V. 1981. The water soluble proteins of winged beans, Psophocarpus tetragonolobus (L.) DC. Paper presented in the 2nd International Symposium on Winged Bean, Colombo, Sri Lanka, Jan. 19—23.

Garcia, V.V. and Palmer, J.K. 1980. Proximate analysis of five varieties of winged beans, Psophocarpus tetragonolobus (L.) DC. J.Food Technol. (U.K.) 15: 469.

Gillespie, J.M. and Blagrove, R.J. 1975. Variability in the proportion and type of subunits in lupin storage globulins. Aust. J. Plant Physiol. 2: 29.

Gillespie, J.M. and Blagrove, R.J. 1978a. Isolation and composition of the seed globulins of winged bean, Psophocarpus tetragonolobus (L.) DC. Aust. J. Plant Physiol. 5: 357.

Gillespie, J.M. and Blagrove, R.J. 1978b. The proteins of winged bean seed In The Winged Bean, Papers presented in the 1st International Symposium on Developing the Potentials of the Winged Bean, January 1978, Manila, Philippines, p. 358.

Graham, D.E. and Phillips, M.C. 1976. The conformation of proteins at the air-water interface and their role in stabilizing foam. In "Foams," Ed. Akers, R.J., p. 237. Academic Press, London.

Hermansson, A.-M. 1979. Aggregation and denaturation involved in gel formation. In "Functionality and Protein Structure," Ed. Pour-El, p. 81. ACS Symp. Ser. 92, Amer. Chem. Soc., Washington, DC.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Hutton, C.W. and Campbell, A.M. 1977. Functional properties of a soy concentrate and a soy isolate in simple systems and in a food system: Emulsion properties, thickening function, and fat absorption. J. Food Sci. 42: 457.

Hymowitz, T., Collins, F.I., Panczner, J., and Walker, W.M. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. Agron. J. 64: 613.

Kamat, V.B., Graham, G.E., and Davis, M.A.F. 1978. Vegetable protein-lipid interactions. Cereal Chem. 55: 295.

Kinsella, J.E. 1976. Functional properties of proteins in foods. CRC Crit. Rev. Food Sci. Nutr. 7: 219.

Kinsella, J.E. 1979. Functional properties of soy proteins. J. Amer. Oil Chem. Soc. 56: 242.

Lah, C.L. and Cheryan, M. 1980. Emulsifying properties of a full-fat soy protein product produced by ultrafiltration. Lebens. Wiss. u-Technol. 13: 259.

Lin, M.J.Y., Humbert, E.S., and Sosulski, F. 1974. Certain functional properties of sunflower meal products. J. Food Sci. 39: 368.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265.

NAS. 1979. Tropical Legumes: Resources for the Future. Report of an Ad Hoc Committee on Technology Innovation, Board on Science and Technology for International Development, Commission on International Relations, and National Research Council. National Academy of Sciences, Washington, DC.

Ostle, B. and Mensing, R.W. 1975. "Statistics in Research." 3rd ed. The Iowa State Univ. Press, Ames, IA.

Phillips, M.C. 1981. Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. Food Technol. 35(1): 50.

Price, M.L., Van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26: 1214.

Romero, J. and Ryan, D.S. 1978. Susceptibility of major storage proteins of the bean, Phaseolus vulgaris L., to in vitro enzymatic hydrolysis. J. Agric. Food Chem. 26: 784.

Sathe, S.K. and Salunkhe, D.K. 1981a. Investigations on winged bean [Psophocarpus tetragonolobus (L.) DC] proteins and antinutritional factors. J. Food Sci. 46(5): 1389.

Sathe, S.K. and Salunkhe, D.K. 1981b. Functional properties of the Great Northern bean (Phaseolus vulgaris L.) proteins: Emulsion, foaming, viscosity, and gelation properties. J. Food Sci. 46: 71.

Schmidt, R.H. 1981. Gelation and coagulation. In "Protein Functionality in Foods," Ed. Cherry, J.P. p. 131. ACS Symp. Ser. 147, Amer. Chem. Soc., Washington, DC.

Sekul, A.A., Vinnett, C.H., and Ory, R.L. 1978. Some functional properties of peanut proteins partially hydrolyzed with papain. J. Agric. Food Chem. 26: 855.

Singh, S., Singh, H.D., and Sikka, K.C. 1968. Distribution of nutrients in the anatomical parts of common Indian pulses. Cereal Chem. 45: 13.

Sosulski, F.W. 1977. Concentrated seed proteins. In "Food Colloids," Ed. Graham, H.D., p. 152. The Avi Publishing Co., Inc., Westport, CT.

Sosulski, F.W. and Fleming, S.E. 1977. Chemical, functional, and nutritional properties of sunflower protein products. J. Amer. Oil Chem. Soc. 54: 100A.

Sosulski, F. and Youngs, C.G. 1979. Yield and functional properties of air-classified protein and starch fractions from eight legume flours. J. Amer. Oil Chem. Soc. 56: 292.

Stein, M. 1976. Natural toxicants in selected leguminous seeds with special reference to their metabolism and behaviour on cooking and processing. Qual. Plant. Pl. Foods Hum. Nutr. 26: 227.

Sumner, J.B. 1924. The estimation of sugar in diabetic urine, using dinitrosalicylic acid. J. Biol. Chem. 62: 287.

Susheelamma, N.S. and Rao, M.V.L. 1974. Surface-active principle in black gram (Phaseolus mungo) and its role in the texture of leavened foods containing the legume. J. Sci. Food Agric. 25: 665.

Van Den, T., Raymundo, L.C., and Mendoza, E.M.T. 1981. Lipoxygenase activity in germinating winged bean seed and its role in beany flavor formation. Paper presented in the 2nd International Symposium on Winged Bean, Colombo, Sri Lanka, Jan. 19—23.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406.

Ms received 7/24/81; revised 9/25/81; accepted 9/28/81.

Journal Paper No. 2722 of the Utah Agricultural Experiment Station and a contribution of Western Regional Project W-150.

The authors gratefully acknowledge the gift of winged bean seeds from Dr. J.M. Spata, Ralston Purina Co., 900 Checkerboard Sq., St. Louis, MO.

Studies on Desi and Kabuli Chickpea (Cicer arietinum L.) Cultivars. The Levels of Amylase Inhibitors, Levels of Oligosaccharides and In Vitro Starch Digestibility

U. SINGH, M. S. KHERDEKAR, and R. JAMBUNATHAN

ABSTRACT

Amylase inhibitor activity (AIA) of chickpea extracts was investigated using pancreatic and salivary amylases. The extracts showed higher inhibitor activity towards pancreatic amylase than salivary amylase. Mean values indicated slightly higher inhibitory activity in desi than kabuli cultivars, though clear-cut differences were not observed among the cultivars. While in vitro starch digestibility of meal samples indicated no large differences among desi and kabuli types of chickpea, the mean values of digestibility of isolated starches of kabuli types was higher than those of desi types. The mean values of stachyose were higher in desi cultivars. When desi and kabuli types were considered together, stachyose and raffinose contents were not found significantly related to the concentrations of total soluble sugars while stachyose showed a significant correlation with raffinose.

INTRODUCTION

ALTHOUGH NUTRITIONAL significance of α-amylase inhibitors of cereal grains has been studied (Granum and Eskeland, 1981), amylase inhibitors of grain legumes have not received much attention. The growth inhibiting properties of raw beans have been reported to be due to the presence of heat labile factor which inhibited the in vitro activity of pancreatic amylase (Jaffe and Vega, 1968). A large variation in the inhibitor activity of pancreatic amylase among the several species of food legumes has been reported (Jaffe et al., 1973).

The food legumes are also regarded as notorious inducers of flatulence when they are consumed in large quantity. It has been reported that the two oligosaccharides, raffinose and stachyose, are the causative factors for flatulence and uncomfortable feeling often experienced upon ingestion of soybean products (Steggerda and Rackis, 1967). In particular, the hydrogen component of intestinal gas is formed by the fermentation of low molecular weight galactosido-oligosaccharides raffinose and stachyose which are not digested by human digestive enzymes (Hellendoorn, 1969). Udyashanker Rao and Belavady (1978) reported the presence of considerable amount of stachyose and raffinose in whole-chickpea seeds. Earlier studies revealed marked differences in the in vitro digestibility of carbohydrates of different legumes (Srinivasa Rao, 1969).

Previous studies of some of the antinutritional factors in desi and kabuli types of chickpea showed a considerable variation in the levels of protease inhibitors, in vitro protein digestibility and polyphenolic compounds (Singh and Jambunathan, 1981). In this paper, the levels of amylase inhibitors and oligosaccharides-stachyose and raffinose and the results of in vitro starch digestibility of some desi and kabuli cultivars are reported.

Authors Singh, Kherdekar, and Jambunathan are affiliated with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), ICRISAT Patancheru P.O. 502 324, Andhra Pradesh, India.

MATERIALS & METHODS

Materials

Seed samples of eight desi and seven kabuli cultivars grown at Hissar, India (29°N), during the post-rainy season of 1977-78 were decorticated and ground in a Udy cyclone mill to pass a 0.4 mm screen as described earlier (Singh and Jambunathan, 1981).

Methods of Analysis

Soluble sugars and starch content. Sugars were extracted using 80% ethanol in a Soxhlet apparatus and estimated by the phenol-sulphuric acid method (Dubois et al., 1956). Starch content in the dry residue was determined by enzymatic hydrolysis (Singh et al., 1980).

Amylase inhibitor activity. The inhibitor activity of pancreatic amylase (obtained from Sigma Chem. Co., USA) was carried out according to the method of Jaffe et al. (1973). The salivary amylase inhibitor activity was determined according to the procedure of Granum (1978). Human saliva was collected and diluted about five-fold in 0.02M phosphate buffer, pH 6.9. After standing overnight at 5°C, the mixture was centrifuged at 10,000 x *g* for 15 min. Amylase inhibitor was extracted by shaking a finely ground and defatted chickpea sample with 0.02M phosphate buffer, pH 6.9 (1:10, w/v) for 2 hr at room temperature. The suspension was then centrifuged at 10,000 x *g* for 15 min at room temperature. The supernatant was then heated for 10 min at 70°C, centrifuged again at 10,000 x *g* for 15 min at room temperature, and the supernatant so obtained was tested for amylase inhibitor activity.

Estimation of stachyose and raffinose. The oligosaccharides were extracted with 80% ethanol for 6 hr in a Soxhlet apparatus. The separation of these oligosaccharides was accomplished on a Whatman No. 1 chromatographic paper by descending chromatography using the solvents butanol-pyridine-water (5:1:4, v/v). The chromatogram was run for about 72 hr. The paper was removed, dried with hot air and marginal strips were cut off and sprayed with a solution of ammonical silver nitrate (Leslie, 1968). Th strips were heated in an oven at 110°C until the dark spots indicating the position of the sugars appeared. With the aid of lines rules on the central unsprayed portion of the chromatogram and using the developed spots on the marginal strips as indicators, sections of paper corresponding to raffinose and stachyose positions were cut from the central portion. The sugars from the strips were eluted with water and their concentrations estimated colorimetrically by the phenol-sulphuric acid method as mentioned earlier.

Isolation of starch and in vitro digestiblity. In vitro digestibility of meal starch and of the isolated starch was determined using pancreatic amylase. Starch was isolated according to the procedure of Garwood et al. (1976). A suitable amount of defatted meal (50 mg) or the isolated starch (25 mg) was dispersed in 1.0 ml of 0.2M phosphate buffer, pH 6.9. Pancreatic amylase (20 mg) was dissolved in 50 ml of the same buffer and 0.5 ml was added to the sample suspension and incubated at 37°C for 2 hr. After the incubation period, 2 ml of 3–5 dinitrosalicyclic acid reagent was quickly added and the mixture was heated for 5 min in a boiling water bath. After cooling, the solution was made to 25 ml with distilled water, and filtered prior to measurement of the absorbance at 550 nm. A blank was run simultaneously by incubating the sample first and 3–5 dinitrosalicyclic acid was added before the addition of the enzyme solution. Maltose was used as the standard and the values were expressed as mg of maltose released per gram of sample.

Table 1—Amylase inhibitor activity (AIA) and in vitro starch digestibility of eight desi and seven kabuli cultivars of chickpea

Cultivar	Starch (%)	Amylase inhibitor activity[e]		*In vitro* starch digestibility		
		Salivary amylase	Pancreatic amylase	a	b	c
Desi						
Range	48.4–53.4	3.7–8.4	7.8–10.5	39.8–50.5	85.4–99.5	108.3–123.0
Mean ± SE	50.8 ± 1.6	5.9 ± 0.2	9.0 ± 0.3	45.2 ± 2.0	89.7 ± 4.6	114.7 ± 5.4
Kabuli						
Range	49.6–54.8	3.1–7.3	5.6–10.0	40.5–51.7	86.6–100.2	120.4–148.5
Mean ± SE[d]	51.5 ± 1.5	4.3 ± 0.2	7.4 ± 0.3	47.1 ± 2.3	91.5 ± 5.1	135.0 ± 5.7

[a] mg maltose released/g meal
[b] mg maltose released/g meal starch
[c] mg maltose released/g isolated starch
[d] Standard error of estimation
[e] Units inhibited/g meal

Table 2—Correlations between starch content and in vitro starch digestibility of 15 cultivars of chickpea

	Correlation coefficients			
	In vitro starch digestibility (IVSD)			Starch content (%)
	a	b	c	
Amylase inhibitor activity[d]	−0.587*	−0.304	0.235	−0.151
IVSD: a	–	0.642**	−0.016	0.203
b	–	–	0.435	0.154
c	–	–	–	0.182

[a] mg maltose released/g meal
[b] mg maltose released/g meal starch
[c] mag maltose released/g isolated starch
[d] pancreatic anylase
*Significant at 5% level
**Significant at 1% level

RESULTS & DISCUSSION

Pancreatic and salivary amylase inhibitor activities

The amylase inhibitor activity of chickpea cultivars, when examined using pancreatic amylase (enzyme units inhibited/g meal), ranged between 7.8 and 10.5 in desi and 5.6 and 10.0 in kabuli cultivars (Table 1) indicating considerable variations among these cultivars. A similar variation but of lower magnitude was observed with salivary amylase. A comparison under similar assay conditions indicated that the amylase inhibitor activity was more towards pancreatic amylase than salivary amylase and this was found to be the case in both desi and kabuli cultivars. Jaffe et al. (1973) have reported that the partially purified kidney bean inhibited the salivary amylase more than the pancreatic amylase. This shows that amylase inhibitors from different legume seeds may behave differently towards the enzyme.

Pancreatic amylase inhibitor is present in most of the legumes, but the highest inhibitor activity has been reported in kidney bean (Jaffe et al., 1973). The inhibitor activity in chickpea cultivars appeared to be considerably lower than in other important food legumes. However, in well-cooked *Phaseolus vulgaris,* the inhibitor was reported to be completely inactivated at 100°C (Hernandex and Jaffe, 1968). We also observed that amylase inhibitors of a few chickpea cultivars became completely inactive when extracts were boiled for 10 min. But the findings reported here suggest that in case of unheated chickpea meal, some inhibition of starch digestion by amylase inhibitors may be expected.

In vitro starch digestibility

The starch digestibility was studied using pancreatic amylase. An increase in digestibility was observed with increasing periods of incubation up to 2 hr and thereafter no measurable changes were noticed. Therefore, for comparing the digestibility of cultivars, an incubation period of 2 hr was followed. The results expressed as mg maltose released/g meal and mg maltose released/g meal starch are reported in Table 1. No large variations in the starch digestibility of meal was observed among the cultivars studied and apparently no large differences in the digestibility of meal starches were noticed between desi and kabuli types. However, the mean value for digestibility of isolated starch was slightly higher for kabuli than for desi types (Table 1). On the other hand, digestibility of isolated starch from kabuli types was found to be higher than desi types. Moreover, the digestibility of isolated starch was apparently higher than that of the meal starch. There appeared to be no relationship between the digestibility of meal starch and isolated starch of chickpea (Table 2). Perhaps, some interfering substances are present in meal samples and in higher concentration in desi than in kabuli ones. In order to confirm this hypothesis, determination of in vivo digestibility of starch of these cultivars is required.

A statistically significant negative correlation was obtained between the amylase inhibitor activity and digestibility of meal (Table 2) indicating that the digestibility of starch is adversely affected by the levels of amylase starch is adversely affected by the levels of amylase inhibitor. But there was no significant correlation between amylase inhibitor activity and digestibility of isolated starch. It is known that oligosaccharides such as raffinose, stachyose, and verbascose are present in considerable amount in several grain legumes (Nigam and Giri, 1961). However, due to the nonsignificant differences in these oligosaccharides among the legumes, the observed differences in the α-amylolysis of different legumes could not be explained on the basis of the presence of these oligosaccharides (Srinivasa Rao, 1969). Also, our results revealed no relationship between the in vitro starch digestibility and the stachyose and raffinose contents of chickpea.

Stachyose and raffinose content

Data on the concentrations of total soluble sugars and oligosaccharides in chickpea cultivars are given in Table 3. While the percentages of soluble sugars in these cultivars did not differ considerably, fairly large variations in stachyose and raffinose contents were observed. When the results of desi and kabuli were considered together, it was noticed that on average, stachyose accounted for 26.7% and raffinose accounted for 10.2% of the total soluble sugars.

These results are comparable to those of earlier workers who reported that in chickpea, stachose and raffinose account for 27.3 and 7.7% of total soluble sugars, respec-

Table 3—Soluble sugars, stachyose and raffinose contents in eight desi and seven kabuli cultivars of chickpea

Cultivar	Soluble sugars (%)	Stachyose		Raffinose	
		a	b	a	b
Desi:					
Range	4.15–5.08	1.06–1.85	25.01–42.82	0.36–0.66	8.91–13.65
Mean ± SE	4.47 ± 0.12	1.34 ± 0.03	29.97 ± 1.58	0.50 ± 0.01	11.07 ± 0.50
Kabuli:					
Range	4.68–5.67	0.82–1.38	17.94–28.87	0.36–0.62	7.25–12.95
Mean ± SE[c]	5.06 ± 0.15	1.16 ± 0.04	23.41 ± 1.03	0.46 ± 0.02	9.27 ± 0.48

[a] g/100g sample
[b] g/100g soluble sugars
[c] Standard error of estimation

tively (Lineback and Ke, 1975). Earlier workers (Udashanker Rao and Belavady, 1978) reported that chickpea also contained one more oligosaccharide-verbascose. But in the present study, this oligosaccharide was not determined because the standard verbascose required for determination, was not available commercially.

In order to know if any relationship exists between the oligosaccharides and total soluble sugars, correlation coefficients among these variables were worked out (Table 4). Total soluble sugars were not significantly correlated with either of these two oligosaccharides expressed either as g/100g sample or as g/100g soluble sugars. The present study gave enough indication that the concentration of these oligosaccharides is independent of the levels of total soluble sugars in these cultivars. On the other hand, stachyose and raffinose were positively and significantly correlated with each other when the results were expressed either as g/100g sample or as g/100g soluble sugars.

The ingestion of large quantities of legumes is known to cause flatulence in experimental animals and humans due to the presence of these oligosaccharides. Germinated or cooked chickpea or mungbean did not greatly alter their flatus-inducing capacity as compared to the raw forms (Shurpalaker, 1973). In view of the observed variations in the levels of oligosaccharides among the chickpea cultivars and their implication in human nutrition, attempts should be made to screen and then select cultivars having lower amounts of these oligosaccharides.

Table 4—Correlation coefficients between total soluble sugars, stachyose and raffinose in 15 cultivars of chickpea

Oligosaccharides	Raffinose		Stachyose		Soluble sugars (%)
	a	b	a	b	
Raffinose					
a	–	0.923**	0.765**	0.696**	0.091
b	–	–	0.692**	0.781**	–0.289
Stachyose					
a	–	–	–	0.917**	0.154
b	–	–	–	–	–0.244

[a] g/100g sample
[b] g/100g soluble sugars
**Significant at 1% level

REFERENCES

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Garwood, D.L., Shannon, J.C. and Green, R.G. 1976. Starches of endosperms possessing different alleles at the amylose extender of locus in Zea mays L. Cereal Chem. 53: 355.

Granum, P.E. 1978. Purification and characterization of an α-amylase inhibitor from rye (Secale cereal) flour. J. Food Biochem. 2: 103.

Granum, P.E. and Eskeland, B. 1981. Nutritional significance of α-amylase inhibitors from wheat. Nut. Rep. Int. 23: 156.

Hellendoorn, E.W. 1969. Intestinal effects following ingestion of beans. Food Technol. 23: 87.

Hernandex, A. and Jaffe, W.G. 1968. Inhibitor de la amilasa pancreatica en caraotas (Phaseolus vulgaris). Acta cient Venez. 19: 183.

Jaffe, W.G. and Vega, C.L. 1968. Heat-labile growth inhibiting factors in beans (Phaseolus vulgaris). J. Nutr. 94: 203.

Jaffe, W.G., Raquol Moreno and Valentina Wallis. 1973. Amylase inhibitors in legume seeds. Nutr. Rep. Int. 7: 169.

Leslie, H. 1968. Analysis of mixtures of sugars by paper and cellulose column chromatography. Methods Biochem. Anal. 1: 205.

Lineback, D.R. and Ke, C.H. 1975. Starches and low molecular weight carbohydrates from chickpea and horse bean flours. Cereal Chem. 52: 334.

Nigam, V.N. and Giri, K.V. 1961. Sugar in pulses. Can J. Biochem. Physiol. 39: 1847.

Shurpalaker, K.S., Sundaravalli, O.E., and Desazi, B.L.M. 1973. Effect of cooking and germination on the flatus inducing capacity of some legumes. In "Nutritional Aspects of Common Beans and Other Legume Seeds as Animal and Human Foods." Proceedings of a meeting held in Ribeireio Preto, S.P., Brazil, November 6—9, 1973.

Singh, U. and Jambunathan, R. 1981. Studies on desi and kabuli chickpea (Cicer arietinum L.) cultivars. The levels of protease inhibitors, levels of polyphenolic compounds and in vitro protein digestibility. J. Food Sci. 46: 1364.

Singh, U., Jambunathan, R., and Narayanan, A. 1980. Biochemical changes in developing seeds of pigeonpea (Cajanus cajan). Phytochemistry 19: 1291.

Srinivasa Rao, P. 1969. Studies on the digestibility of carbohydrates in pulses. Indian J. Med. Res. 57: 2151.

Steggerda, F.R. and Rackis, J.J. 1967. Is the oligosaccharide fraction in soybean product responsible for flatulence. Paper No. 21, Abstracts of Papers, 52nd Annual Meeting, American Association of Cereal Chemists, April 2—6. Cereal Science Today 12: 103.

Udyashanker Rao, P. and Belavady, B. 1978. Oligosaccharides in pulses: Varietal differences and effects of cooking and germination. J. Agri. Food Chem. 26: 316.

Ms received 8/3/81; revised 10/6/81; accepted 10/10/81.

Submitted as J.A. No. 184 by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT).

Authors thank Dr. Jagdish Kumar for the supply of seed material and Mr. N. Subrahmanyam for technical assistance during this investigation.

Phytic Acid Determination in Soybeans

D. B. THOMPSON and J. W. ERDMAN, JR.

ABSTRACT

Several established methods of phytic acid determination in soybeans were evaluated. Iron analysis methods, which rely on a 4:6 molar ratio of Fe:P, were eliminated because this ratio was not dependable. Three assay methods relying on phosphorus analysis were then compared. The anion-exchange method was considered most accurate but not convenient for routine analysis. Analysis of the ferric phytate precipitate and a new method, analysis of the supernatant before and after ferric chloride precipitation, were judged against the anion-exchange method under different extraction conditions. Based upon good agreement with anion-exchange column results and acceptable reproducibility, the best methods were (1) precipitate analysis of phosphorus after extraction with 3% TCA + 10% sodium sulfate, or (2) the supernatant difference method after extraction with 1.2% HCl.

INTRODUCTION

PLANT PROTEINS play an important role in the diet of humans. Traditionally consumed in the orient, soybean foods are finding an increasing market in Western countries. In addition, human use of soy products such as concentrates, isolates, and texturized products is increasing.

Many of the antinutritional factors associated with raw soybeans can be eliminated or minimized by proper heat treatment. However, poor mineral bioavailability is not directly improved by heating (Erdman, 1979). Soybean zinc is 20-100% available (O'Dell, 1979; Forbes et al., 1979; Erdman et al., 1980) to experimental animals depending upon the type of soy product measured and the animal model utilized. Numerous investigators, beginning with O'Dell and Savage (1960), have suggested that phytic acid may be responsible for reduced zinc availability.

In order to effectively evaluate the relationship between phytic acid and mineral availability in soybeans, one must be able to accurately measure both. Several different methods of analysis of phytic acid can be found in the literature (See Fig. 1 and Table 1). These methods have been developed to measure phytic acid content of a variety of materials other than soybeans.

Presently, most assays for phytic acid employ ferric chloride to precipitate ferric phytate. Fig. 1 shows that the precipitate may be digested directly and analyzed for either phosphous (1) or iron (2). Alternatively, sodium hydroxide may be used to convert the precipitate to sodium phytate and ferric hydroxide. It is convenient to analyze for iron after taking up the ferric hydroxide in acid (7).

Determination of residual iron in the supernatant (3) after precipitation is also a rapid method. Determination of residual phosphorus in the supernatant has not been mentioned in the literature as of this writing. [Recently another group (Tangkongchitr et al., 1981) employed this determination as part of a total phosphorus balance in the analysis of phytate phosphorus during bread baking.]

Anion exchange separation of phytate from the extract (10) was performed by Harland and Oberlease (1977) on wheat bran. This accurate but time-consuming method is inconvenient for the routine determination of large numbers of samples.

The environment in which phytate is found in various materials differs. The mineral environment as well as the protein environment will specifically determine the natural surroundings of phytate in the seed. Phytate found in a different mineral and protein environment will not necessarily behave in precisely the same way for a given assay procedure. The application to soybeans of procedures developed for such diverse materials as feces, potatoes, peas, corn and soil has gone unchallenged despite the obvious differences between these materials and soybeans. For confidence in the accuracy and reliability of a phytic acid value for soybeans, a phytic acid assay specific for soybeans in needed. The purpose of this study was to evaluate the applicability of several assays to soybeans under various extraction conditions. Several established assay procedures were used, as well as one new procedure.

EXPERIMENTAL

SOYBEANS utilized throughout these studies were the Bonus '75 variety grown in Champaign County, IL. Beans were stored at 1°C until processing. Whole soybeans were heated at 93°C for 20 min in a Variable Air Dryer (Proctor and Schwartz, Inc., Philadelphia, PA) to harden the hulls. Hulls were loosened with the aid of a spinning drum-plate apparatus. Spacing between the drum and plate was

Table 1—Representative methods of phytic acid analysis

Method[a]	Material tested	Reference
	rat feces	Ellis et al. (1977)
	corn	Earley & deTurk (1944)
(1)	wheat	Nahapetian & Bassiri (1975)
	cereal, grains & oilseeds	deBoland et al. (1975)
	not specific	Oberleas (1971)
	peas	Crean & Haisman (1963)
(2)	not specific	Forbes, 1978 (per. comm.)
	corn	Earley (1944)
	potato	Samotus & Schwimmer (1962)
(3)	peas	Holt (1955)
	not specific	Heubner & Stadler (1914)
	barley, oats, soy, wheat	Lolas et al. (1976)
(4)	bread	Tangkongchitr et al. (1981)
(5)	not specific	Oberleas (1971)
(6)	not specific	McCance & Widdowson (1935)
	not specific	Pons et al. (1953)
	pinto beans	Makower (1970)
	wheat fractions	Wheeler & Ferrel (1971)
(7)	feces	Young (1936)
	whole soy extracts	Omosaiye (1979)
	bread	deLange et al. (1961)
(8)	sodium phytate	Reeves et al. (1979)
(9)	peanut	Noor (1980)
(10)	vegetative plant mtl.	Marrese et al. (1961)
	wheat bran	Harland & Oberleas (1977)

[a]Method number corresponds to numbers in parentheses in Fig. 1.

Authors Erdman and Thompson are with the Dept. of Food Science, 567 Bevier Hall, Univ. of Illinois, Urbana, IL 61801.

adjusted to split the cotyledon and loosen the hulls. Hulls and split cotyledons were partitioned using a forced air conveyor belt system. Remaining hulls were removed by hand. Soy meal was prepared using a Grain Mill attachment to a model K5-A Hobart Mixer (Hobart Corporation, Troy, OH). Soy meal was passed through a 60 mesh screen and stored in plastic pouches at 4°C until analysis. Moisture was determined to be 8.3% by drying overnight at 105°C. All subsequent analyses of this soy meal was on an "as is" basis.

Iron was analyzed with a model 306 Perkin Elmer spectrophotometer with digital ppm read-out (with a linear range of 1–3 ppm). Phytic acid was calculated from iron on the assumption that the iron:phosphorus molar ratio of the precipitate was 4:6. Analysis of phosphorus in the digested ferric phytate precipitate was done by the method of Bartlett (1959), a modification of the method of Fiske and Subbarow (1925).

Phytic acid analysis procedures

Precipitate analysis methods. Phytate assays employing analysis of iron were done by a modification of the method of Earley and deTurk (1944) made by Forbes (personal communication), and further modified by the present investigators.

A 2-g sample of soy meal was placed in a flask, into which 100.0 ml of 1.2% HCl + 10% Na_2SO_4 was added. The flask was stoppered and shaken for 2 hr on a mechanical shaker. The extract was vacuum filtered through #4 Whatman paper. 10.0 ml of the filtrate were pipeted into a 50 ml centrifuge tube.

Ten ml deionized water were added, followed by 12 ml of $FeCl_3$ solution (2.0g $FeCl_3 \cdot 6H_2O$ + 16.3 ml conc HCl/L). The contents were stirred, heated 75 min in boiling water, and cooled covered for 1 hr at room temperature. The tube was centrifuged at 1000 x *g* for 15 min in a model SBV International centrifuge (International Equipment Co., Boston, MA). The supernatant was decanted and discarded, and the pellet was thoroughly washed three times with a solution of 0.6% HCl and 2.5% Na_2SO_4. After each wash the contents were centrifuged at 1000 x *g* for 10 min and the supernatant discarded. Ten ml concentrated HNO_3 were added to the resulting pellet and the contents transferred quantitatively to a 400 ml beaker with several small portions of deionized water. Four drops of concentrated H_2SO_4 were added and the contents were heated approximately 30 min on a hot plate until only the H_2SO_4 remained. Approximately 4–5 ml of 30% H_2O_2 were added and the mixture was returned to the hot plate at a low heat until bubbling ceased. The residue was dissolved in 15 ml 3N HCl and heated for 10–15 min. The resulting solution was made up to 100.0 ml volume, diluted 1:5, and then analyzed for iron, phosphorus, or both.

Experimental modification of conditions used in the precipitate analysis method are described in Results & Discussion.

Supernatant difference method. This procedure was the same as the precipitate analysis method through the first centrifugation step. After centrifugation, the supernatant was filtered through No. 1 Whatman paper. The paper was rinsed with several small volumes of deionized water, whereupon the filtrate was made up to 50.0 ml. 2.00 ml filtered supernatant were analyzed for total phosphorus by the method of Bartlett (1959).

A 1.00 ml sample of the initial extract was made up to 100.0 ml and 2.00 ml of this were analyzed. The amount of phytic acid was obtained by difference of the phosphorus values for the extract and for the post-precipitation supernatant.

Anion-exchange separation and analysis. Column separation was performed by a modification of the procedure of Harland and Oberleas (1977), who separated the phytate anion from wheat bran using an anion-exchange resin and subsequently analyzed the phytate phosphorus colorimetrically. Modification of sample size from 5 to 2 grams and resin amount from 0.5 to 0.15 grams was done to allow complete elution of the extract without blockage of the column. Elution of phytate was done with 45 ml 0.7M NaCl rather than 15 ml to allow complete phytate elution. Harland and Oberleas (1977) did not indicate in their paper the amount of extract that they applied to the column. In our work 50.0 ml of the 100.0 ml of extract was diluted to 190 ml and applied. Thus about 0.14 meq phytate anion was applied to the column.

A 2-g sample of soy meal was extracted with 100.0 ml of 1.2% HCl for 2 hr with mechanical shaking. The extract was vacuum filtered as described previously. 50.0 ml of the extract were diluted to 190 ml total with deionized water. The diluted solution was applied to the resin.

Bio-Rad AGI-X8 200-400 mesh resin in the chloride form (Biorad Laboratories, Richmond, CA) was used. 0.15 grams of resin was slurried in 2–3 ml deionized water and applied to a 0.7 x 15 cm glass barrel column (Biorad Laboratories, Richmond, CA). The resin was eluted with 10 ml 0.7M NaCl to ensure the chloride form of the resin. The resin was then rinsed four times with 10 ml of de-

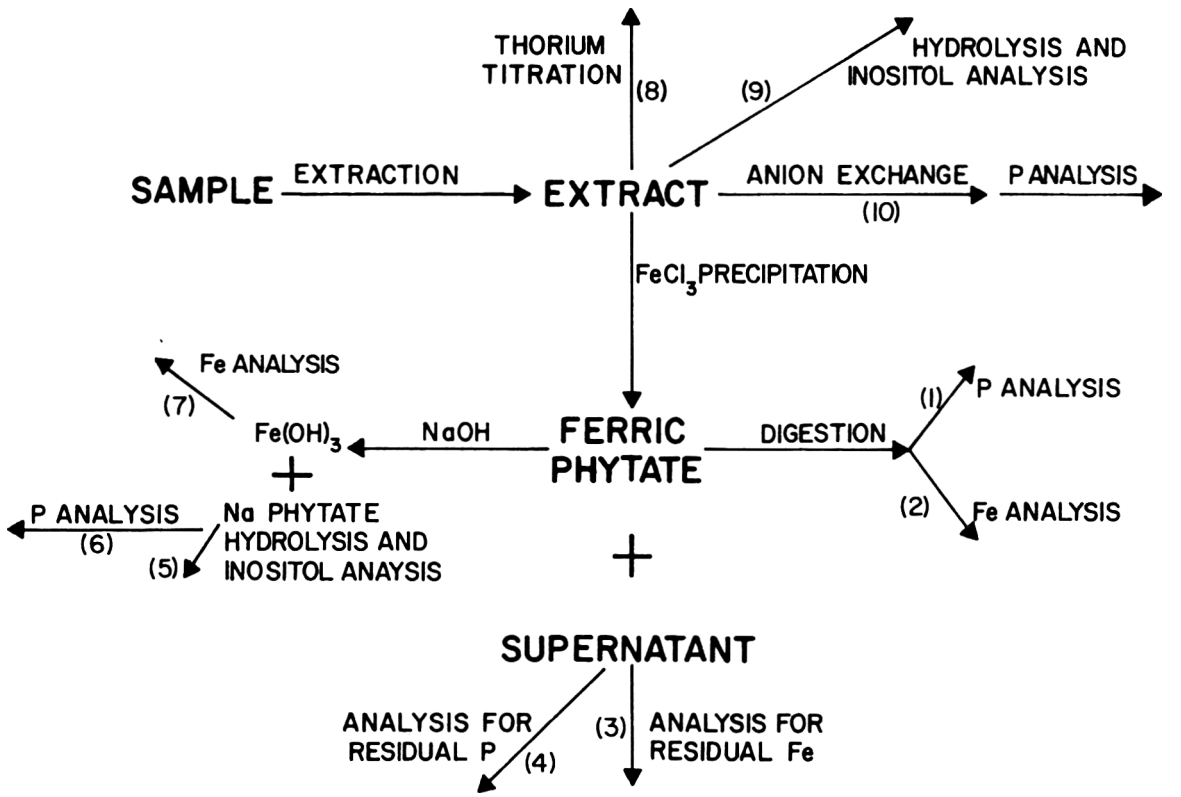


Fig. 1—Selected approaches to phytic acid analysis.

ionized water. The 190 ml of diluted extract was applied to the column and eluted by gravity. The column was then rinsed with 15 ml deionized water. 15 ml of 0.05M NaCl was applied to elute any inorganic phosphate. Phytate anion was then eluted with 45 ml of 0.7 M NaCl and analyzed based upon phosphorus content of the eluate.

The above procedure was modified slight when 3% TCA + 10% Na_2SO_4 were used for the extraction of soy meal. All volumes, dilutions, and treatments were the same except that 2.0 grams of resin was applied to the column.

Comparison of the Three Methods

Three samples were extracted with 100.0 ml of either 1.2% HCl, or 3% TCA + 10% sodium sulfate. From each 100 ml, a 50.0 ml portion was used for the anion-exchange procedure; three 10.0 ml portions were used for $FeCl_3$ precipitation for both the supernatant analysis and the precipitate analysis; and a 1.00 ml portion was used to determine total extract phosphorus for the supernatant difference method. Although anion-exchange procedures could not be successfully performed with the 1.2% HCl + 10% Na_2SO_4 extraction, the precipitate analysis and supernatant difference methods were compared.

RESULTS & DISCUSSION

Precipitate analysis methods

Analysis of the ferric phytate precipitate for iron was performed on 13 different days on soybean meal with 1.2% HCl + 10% Na_2SO_4 as the extraction solution. A phytic acid content of 1.40 ± 0.13% (Mean ± S.D.) was sound, with a range of 1.12–1.60%. Analysis of the ferric phytate precipitate for phosphorus was performed on the same number of days with the same extraction and wash conditions. The value obtained was 1.38 ± 0.07%, with a range of 1.29–1.53%. These results indicate that for a large number of tests on a single sample these methods will produce a similar mean, but for any given test, the results of the phosphorus assay will be more reliable.

The ferric phytate precipitate forms in an aqueous ferric chloride medium. Washing steps are crucial if one is to analyze the iron in the precipitate. The effect of washing on the iron:phosphorus ratio is shown in Fig. 2. The iron:phosphorus ratio decreased with each wash. Further investigation revealed that the iron:phosphorus ratio varied with other conditions as well. The presence of sulfate ion or chloride ion in the extraction, or extraction with TCA, instead of HCl, altered the ratio.

The above information regarding the iron:phosphorus ratio of the precipitate has led to an hypothesis regarding the structure of the ferric phytate precipitate, as illustrated in Fig. 3. It is possible to have eight ferric ions interact with a phytate anion if one assumes a repeating sheet-like structure for the precipitate. Each ferric ion would be shared between two phytates. Such a model would account for a 4:6 iron:phosphorus ratio for the ferric phytate system, the ratio first suggested by Earley (1944).

It would be exceedingly difficult for the phytate anions to become arranged in exactly the proper configuration to allow maximum ferric ion bridging between phytates to occur. According to Earley (1944), the fourth mole of iron is incorporated into the precipitate with difficulty. This model allows speculation regarding the cause of a decreased iron:phosphorus ratio with washing of the precipitate. In such a complex system in which the large polyvalent anions are linked through metal ions, it seems very unlikely that a perfectly repeating structure would ever form; consequently all ferric ion would not be equally firmly held. Some would be an integral part of the precipitate and some would be more peripherally bound. Washing would tend to remove the more peripherally bound ferric ions, but would have little effect upon the phytate phosphorus since each phytate anion would be bound to the precipitate by numerous links. (Further discussion of the precipitate structure will be found in Thompson and Erdman, manuscript in preparation.)

Given the fluctuating nature of the iron:phosphorus ratio, analysis of the precipitate for iron, which depends on the 4:6 ratio, was abandoned. The analysis of phosphorus in the ferric phytate precipitate was chosen for further study. The data in Table 2 show that precipitate analysis results were highest in the presence of sodium sulfate. That the higher values with sodium sulfate were not simply the result of the higher ionic strength of the extraction and precipitation medium is demonstrated by the TCA + NaCl extraction results (1.17%) as compared to the TCA + Na_2SO_4 extraction results (1.42%).

Supernatant difference method

Other methods which would not rely on the 4:6 iron:phosphorus ratio of the precipitate were considered.

–Continued on next page

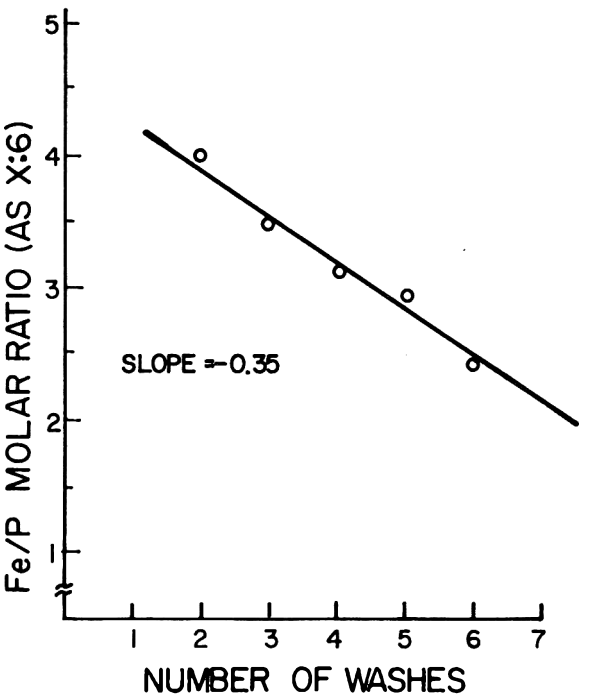


Fig. 2–Effect of washing the ferric phytate precipitate upon the iron to phosphorus molar ratio.

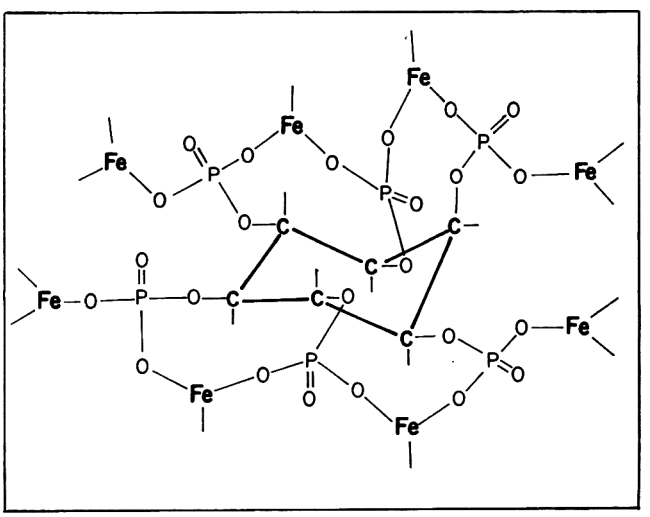


Fig. 3–Proposed structure for ferric phytate.

Measurement of total phosphorus in the extract and in the supernatant (i.e., in the post-ferric chloride precipitation supernatant) by difference gives phytate phosphorus with minimal handling of the precipitate. The results of this method are summarized in Table 2. Three percent TCA extraction gave a value of 1.31% phytic acid. The phytic acid value with 1.2% HCl was 1.42%. With 10% sodium sulfate present both TCA and HCl gave the higher phytic acid values of 1.57% and 1.48%, respectively. There are at least two possible explanations for this variation in phytic acid values. Under conditions of high inorganic phosphorus (e.g., rat feces), non-phytate phosphorus has been shown by Ellis et al. (1977) to be incorporated into the precipitate. Thus phytic acid values would be falsely high to varying extents, depending upon levels of non-phytate phosphorus and conditions. However, in soy systems this effect should be slight (Ellis et al., 1977).

The second possible cause of variation is incomplete precipitation of phytate under certain conditions. This possibility may explain the lower value for the 3% TCA extract without any salt added. For the supernatant difference method to be employed, some means is needed to evaluate the results from the various extraction conditions, which gave values ranging from 1.31% to 1.57%.

Table 2—Effect of extraction solution upon analysis of phytic acid

Extraction solution	% Phytic acid[a]	
	Precipitate analysis	Supernatant difference
3% TCA	1.18[c]	1.31[c]
3% TCA + NaCl[b]	1.17[c]	1.46[c]
3% TCA + 10% Na_2SO_4	1.42 ± 0.04 (5)[d]	1.57 ± 0.06 (6)
1.2% HCl	1.10 ± 0.06 (6)	1.42 ± 0.02 (5)
1.2% HCl + 10% Na_2SO_4	1.38 ± 0.07 (13)	1.48 ± 0.06 (6)

[a] By phosphorus analysis
[b] NaCl added to produce the same ionic strength as 10% Na_2SO_4
[c] Mean of two determinations
[d] Mean ± S.D. (N)

Table 3—Separation of phytate by anion-exchange chromatography[a]

Eluate	Total phosphorus[b,c]	
	1.2% HCl Extraction	3% TCA + 10% Na_2SO_4 Extraction
Extract	715	710
H_2O	1	Trace
0.05M NaCl	Trace	Trace
0.7M NaCl	4040	4000
1.4M NaCl	10	60

[a] Method is adapted from Harland and Oberleas (1977)
[b] µg P/g soy meal, as is basis.
[c] Average from samples extracted in triplicate.

Table 4—Comparison of three methods of phytic acid analysis with various extraction solutions

	% Phytic Acid		
	Precipitate analysis	Supernatant difference	Anion-Exchange
Experiment 1:			
1.2% HCl	1.12 ± 0.02[a]	1.41 ± 0.03	1.43 ± 0.03
Experiment 2:			
3.0% TCA + 10% Na_2SO_4	1.40 ± 0.02	1.61 ± 0.01	1.42 ± 0.03
Experiment 3:			
1.2 % HCl + 10% Na_2SO_4	1.34 ± 0.04	1.48 ± 0.03	–

[a] Mean ± S.D. (N = 3)

Anion-exchange separation and analysis

Phytate analysis after separation of phytate from other phosphorus compounds by anion-exchange column chromatography was chosen as the technique to evaluate methods previously examined. The anion-exchange resin allowed more specific separation of the phytate anion than could be achieved with ferric ion. Table 3 shows the results of a typical analysis of soybean meal. Note that free phosphorus is eluted through the column with the extract elution while essentially all of the phytate phosphorus is eluted with 0.7M NaCl. On the basis of these results the accuracy of the modified method was assumed.

Comparison of three methods

Results of experiments designed to compare the three previously described methods are given in Table 4. For 1.2% HCl extraction the supernatant difference and the anion-exchange results were in close agreement, whereas the precipitate analysis results were much lower. Precipitate analysis results agreed most closely with the anion-exchange results for the 3% TCA + 10% sodium sulfate extraction.

The precipitate analysis results cited in Table 2 indicated that Na_2SO_4 was needed in the extraction for acceptable phytic acid values. Precipitate analysis results in Table 4 showed that extraction with 3% TCA + 10% Na_2SO_4 gave results in better agreement with anion exchange than either of the extractions using HCl. On the other hand, results for the supernatant difference method were higher than anion exchange results with Na_2SO_4 present. The supernatant difference method agreed best with anion-exchange results when extraction is done with 1.2% HCl.

Application to other soy products

The supernatant difference method using HCl for extraction was used to analyze other soybean products. Data in Table 5 show that the supernatant difference method is capable of generating more information regarding precipitation behavior of phosphorus-containing compounds than a precipitation analysis method. For example, tempeh made from dehulled beans has less phytic acid than the original dehulled material. The supernatant difference method shows that although total phosphorus extracted is less for tempeh than for the original dehulled material, the supernatant phosphorus is greater. These seemingly incongruous results could lead one to further examine a product about which little is known, but in this case the results are easily explained by knowledge of tempeh making. Prior to fermentation the beans were soaked overnight and then boiled for 30 min. Thus some of the phosphorus compounds were leached into the water, which was then discarded, giving a somewhat lower total phosphorus content. Because super-

Table 5—Phytic acid of various soybean materials analyzed by the supernatant difference method[a]

	Phytic acid[b]	P_{total}[c]	$P_{supernatant}$[c]
Dehulled[d]	1.54%	4840	510
Hulls[d]	N.D.[h]	340	350
Hypocotyls[d]	0.88%	4000	1530
Tempeh[d]	1.08%	3950	920
Tofu[e]	1.96%	5850	340
Isolate[f]	1.63%	7440	2850
Flour, defatted[g]	1.82%	5900	770

[a] HCl-extraction
[b] On dry weight basis
[c] µg P/g dry sample
[d] From Bonus '75 soybeans
[e] Commercially purchased
[f] Edipro A, Ralston Purina
[g] I-200, A. E. Staley Co.
[h] Not detectable

natant phosphorus is higher in free phosphate and lower inositol phosphate esters, a higher supernatant value for tempeh is consistent with phytase activity of *Rhizopus oligosporus* (Sudarmadji nad Markakis, 1977). Thus the lower phytic acid value for tempeh is accompanied by supplemental information regarding different phosphorus-containg compounds in the material.

For tofu low supernatant phosphorus and high total phosphorus values resulted. The calcium precipitation used to manufacture tofu was apparently effective in concentrating phytate relative to inorganic phosphorus. This effect may be caused by the interaction between calcium ion and phytate. A pertinent observation regarding precipitation of ferric phytate from the tofu extract is that precipitation occurred immediately upon $FeCl_3$ addition and heating, whereas no precipitation was noted from other soy materials in the first 30 minutes of heating. A synergistic effect of calcium ion and ferric ion for production of insoluble phytate could be responsible for this precipitation behavior, analogous to the synergistic effect of calcium and zinc on precipitation of Ca/Zn phytate (Byrd and Matrone, 1965). Mineral availability from tofu would be an interesting avenue to pursue in the light of this behavior.

Although the defatted flour was not from the same soybean source as the dehulled meal, the phytic acid value is expected to be higher as a result of the concentration effect after oil removal.

The 1.63% value for phytic acid in the soy isolate is in reasonable agreement with the value of 1.52% obtained for a soy isolate by deBoland et al. (1975). The precipitate analysis method used by deBoland et al. (1975) gives no information that would cause one to question the result. However, the supernatant difference method reveals an unusually large value for supernatant phosphorus. For dehulled soy meal, approximately 90% of the phosphorus extracted was found in the precipitate, whereas only about 60% of the phosphorus extracted from the isolate was found in the precipitate. Either there is much more inorganic phosphate in the isolate than in the meal, or some of the phytate from the isolate remained in the supernatant. This latter alternative bears further examination. It is possible that as a result of processing to form the isolate, some of the phytate may interact with protein and/or metal ions during final drying to form stable complexes (Erdman et al., 1980). From the standpoint of mineral bioavailability, the phytate in these soluble complexes may be more important than the total phytate as determined.

CONCLUSION

IN ORDER TO DEVELOP a reliable and rapid method of determination of phytic acid for a particular material, one should compare the rapid methods based on precipitation of ferric phytate to the more time-consuming but highly specific method of anion-exchange chromatographic separation. Those rapid methods based on phosphorus analysis are most acceptable. The supernatant difference method has the additional advantage that it provides more information than the precipitate analysis methods; consequently it is the preferred rapid method.

One must be wary of directly applying to other food materials the methods developed here for the determination of phytic acid in soybean meal. The extraction conditions must be reevaluated for each foodstuff. However, use of the supernatant difference method would provide the maximum amount of information. The best approach is to perform the anion-exchange separation and analysis in order to evaluate the effectiveness of any rapid method. Once the rapid method is shown to be reliable for the material and extraction solution, the method of choice may be conveniently applied to numerous similar samples.

Based on good agreement with anion-exchange results and acceptable reproducibility, the best rapid methods for soybeans are (1) the precipitate analysis for phosphorus after extraction with 3% TCA + 10% sodium sulfate and (2) the supernatant difference method after extraction with 1.2% HCl.

REFERENCES

Bartlett, G.R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466.

Byrd, C.A. and Matrone, G. 1965. Investigations of chemical basis of Zn-Ca-phytate interaction in biological systems. Soc. for Exp. Biol. and Med. Proc. 119: 347.

Crean, D.E.C. and Haisman, D.R. 1963. The interaction between phytic acid and divalent cations during the cooking of dried peas. J. Sci. Fd. Agric. 14: 824.

deBoland, A.R., Garner, G.B., and O'Dell, B.L. 1975. Identification and properties of 'phytate' in cereal grains and iolseed products. J. Ag. Fd. Chem. 23: 1188.

deLange, D.J., Joubert, C.P., and dePreez, S.F.M. 1961. The determination of phytic acid and factors which influence its hydrolysis in bread. Proc. Nutr. Soc. S. Afr. 2: 69.

Earley, E.B. 1944. Determining phytic phosphorus. Ind. & Eng. Chem., Anal. Ed. 16(6): 389.

Earley, E.B. and deTurk, E.E. 1944. Time and rate of synthesis of phytin in corn grain during the reproductive period. J. Am. Soc. Agron. 36: 803.

Ellis, R., Morris, E.R., and Philpot, C. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal. Biochem. 77: 536.

Erdman, J.W. Jr. 1979. Oilseed phytates. JAOCS 56(8): 736.

Erdman, J.W. Jr., Weingartner, K.E., Mustakas, G.C., Schmutz, R.D., Parker, H.M., and Forbes, R.M. 1980. Zinc and magnesium bioavailability from acid-precipitated and neutralized soybean protein products. 45(5): 1193.

Fiske, D.H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375.

Forbes, R.M. 1978. Personal communication.

Forbes, R.M., Weingartner, K.E., Parker, H.M., Bell, R.R., and Erdman, J.W. Jr. (1979) Bioavailability of zinc, magnesium, and calcium in soy protein diets. J. Nutr. 109(9): 1652.

Harland, B.F. and Oberleas, D. 1977. A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. Cereal Chem. 54: 827.

Heubner, W. and Stadler, H. 1914 Uber eine titrationsmethode zur bestimmung des phytins. Biochem. Z. 64: 422.

Holt, R. 1955. Studies on dried peas, I. The determination of phytate phosphorus. J. Sci. Fd. and Ag. 6: 136.

Lolas, G.M., Palamidis, N., and Markakis, P. 1976. The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. Cereal Chem. 53: 867.

McCance, R.A. and Widdowson, E.M. (1935) Phytin in human nutrition. Biochem. J. 29: 2694.

Makower, R.V. 1970. Extraction and determination of phytic acid in beans. Cereal Chem. 47: 288.

Marrese, R.J., Duell, R.W., and Sprague, M.A. 1961. A comparison of three current methods for the analysis of phytin phosphorus. Crop Sci. 1: 80.

Nahapetian, A. and Bassiri, A. 1975. Changes in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, and zinc in wheat during maturation. J. Ag. Fd. Chem. 23: 1179.

Noor, Z. 1980. Effect of pH manipulation during aqueous extraction of peanut proteins. Ph.D. thesis, University of Illinois, Urbana, IL.

Oberleas, D. 1971. The determination of phytate and inositol phosphates. Methods of Biochem. Analysis. 20: 87.

O'Dell, B.L. 1979. Effect of soy protein on trace mineral availability. In "Soy Protein and Human Nutrition," Ed. H.L. Wilcke, D.T. Hopkins, and D.H. Waggle, p. 187. Academic Press, New York.

O'Dell, B.L. and Savage, J.E. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med. 103: 304.

Omosaiye, O. 1979. Hollow fiber ultrafiltration of aqueous extracts of whole soybeans. Ph.D. thesis, University of Illinois, Urbana, IL.

Pons, W.A., Stansbury, M.F., and Holfpauir, C.L. 1953. An analytical system for determining phosphorus compounds in plant materials. J. Assoc. Off. Agr. Chem. 36; 492.

Reeves, R.J., Carroll, R.T., and Gennaro, G.P. 1979. Titration of phytic acid. Talanta 26: 1033.

Samotus, B. and Schwimmer, S. 1962. Indirect method for determining phytic acid in plant extracts containing reducing substances. Biochem. Biophys. Acata 57: 389.

Sudarmadji, S. and Markakis, P. 1977. The phytate and phytaste of soybean tempeh. J. Sci. Fd. and Agr. 28: 381.

Tangkongchitr, U., Seib, P.A., and Hoseney, R.C. 1981. Phytic acid. I. Determination of three forms of phosphorus in flour, dough, and bread. Cereal Chem. 58: 226.

Wheeler, E.L. and Ferrel, R.E. 1971. A method for phytic acid determination in wheat and wheat fraction. Cereal Chem. 48: 312.

Young L. 1936. The determination of phytic acid. Biochem. J. 30: 252.

Ms received 10/7/80; revised 8/17/81; accepted 10/4/81.

This paper was presented in part at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, June, 1980.

This work was supported in part by the University of Illinois Agriculture Experiment Station Hatch Grant #50-355.

Microbial Changes in Fermented Peanut and Soybean Pastes Containing Kojis Prepared using *Aspergillus oryzae* and *Rhizopus oligosporus*

Y. -S. CAROL SHIEH and L. R. BEUCHAT

ABSTRACT

Microbiological profiles of peanut and soybean misos containing 6 and 12% NaCl and kojis prepared with *Aspergillus oryzae* and *Rhizopus oligosporus* were monitored over a 90-day period of fermentation. Although total plate counts in misos containing *R. oligosporus* koji fluctuated while counts in misos containing *A. oryzae* koji remained constant during the fermentation period, the final microbial populations in sixteen test formulae were similar to the initial counts. Mold populations in misos containing two types of kojis declined similarly during fermentation. There was little difference in populations of *Saccharomyces rouxii* in misos containing the two types of mold kojis during the first month of fermentation; however, higher numbers of *S. rouxii* were noted in misos containing *R. oligosporus* koji compared to those containing *A. oryzae* koji during later stages of fermentation. The final pH values of misos were similar, even though misos containing *A. oryzae* koji had a larger magnitude of drop in pH than did misos containing *R. oligosporus* koji over the 90-day test period.

INTRODUCTION

TRADITIONAL MISO, a popular semi-solid fermented food prepared in the Orient, contains koji, soybeans, salt (sodium chloride) and water (Beuchat, 1978; Hesseltine, 1965). Rice on which *Aspergillus oryzae* is cultured is used to prepare a starter (koji). Washed, soaked, steamed and mashed soybeans are then mixed with koji, salt and water and allowed to ferment as long as 2–3 months. Lactic acid bacteria and osmophilic yeasts (*Saccharomyces rouxii*) are thought to contribute to the development of good miso. The product is used in soup and stews as well as with grains, beans, bean curd and noodles to enhance flavor.

Molds in the genus *Aspergillus* are widely distributed in nature and certain strains are known to produce mycotoxins. *Aspergillus oryzae* is not an aflatoxin producer, but some strains do synthesize other secondary metabolites when grown on certain substrates which may be harmful to human health. Kinosita et al. (1968) examined several strains of *A. oryzae* which were isolated from samples of miso and detected kojic acid and trace amounts of β-nitropropionic acid. These acids are not as toxic as aflatoxin; however, kojic acid is classified as a convulsant (Wilson and Hayes, 1973). Therefore, experiments were designed to compare *Rhizopus oligosporus*, which produces neither aflatoxin nor kojic acid, to *A. oryzae* as a koji mold for preparing miso. *Rhizopus oligosporus* is traditionally used to ferment whole soybeans to produce tempeh (tempé). The feasibility of substituting peanuts for soybeans in miso was also evaluated as was the effect of two levels of NaCl. Although the word "miso" refers to traditional fermented products containing soybeans, cereals and salt, it will be used here to refer to fermented products containing peanuts and/or soybeans and kojis prepared with either *A. oryzae* or *R. oligosporus*.

Authors Shieh and Beuchat are affiliated with the Dept. of Food Science, Univ. of Georgia Agricultural Experiment Station, Experiment, GA 30212.

EXPERIMENTAL

Procedures for fermentation

Preparation of cultures. Cultures of *A. oryzae* NRRL 1988, *R. oligosporus* NRRL 2710 and *S. rouxii* NRRL Y-2547 were obtained from the USDA, Northern Regional Research Laboratory, Peoria, IL. The two molds were grown on yeast extract-malt extract-peptone-glucose (YMPG) agar plates at 30°C for 4 days. The formula for YMPG agar consists of (g/liter water): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; and agar, 20 (pH 7.0). Conidiospores of *A. oryzae* and sporangiospores of *R. oligosporus* were harvested by flooding the surface of plates with phosphate buffer (0.1M, pH 7.0) containing 0.01% Tween 80 (phosphate-Tween buffer) and stored at 4°C until used to inoculate rice.

The medium used for culturing *S. rouxii* was YMPG broth (pH 7.0) containing 6% NaCl (100 ml in 250 ml Erlenmeyer flasks). Cultures were grown under constant agitation on a rotary shaker (Eberbach Co., Ann Arbor, MI) for 60 hr at 30°C before collecting cells by centrifugation (9000 x *g*, 10 min). Cellular suspensions in 0.1M phosphate buffer (pH 7.0) containing 3% NaCl served as inocula for miso formulae.

Preparation of koji. A mixture of water and enriched long-grain rice (1:2 ratio, vol/wt) was sterilized in an autoclave (121°C, 15 psi, 15 min). Approximately 35% moisture in rice is reported to be optimum for mold growth and enzyme production (Beuchat, 1978). Separate aliquots (3.4 kg) of moistened rice was inoculated with 115 ml of the two mold spore suspensions, spread in layers ca. 3 cm deep and incubated for 48 hr at 25°C, with occasional stirring. The final products served as kojis for miso formulae.

Preparation of soybean and peanut pastes. Soybeans (Bragg 79) were soaked in water at room temperature for 18 hr and steamed for 60 min in a Thermascrew Steamer (Rietz Manufacturing Co., Santa Rosa, CA). Cooled, whole soybeans were then mashed to a paste form by grinding in a Morehouse mill (Morehouse Industries, Los Angeles, CA).

Peanuts (Florunner) were heated in a rotisserie oven at 24°C for 10 min. After the skins were removed, peanuts were soaked in water for 18 h, steamed 70 min, cooled and reduced to a paste using the same procedure described for soybeans.

Preparation of formulae. Rice koji was ground twice with a chopper (Toledo Scale Co., Toledo, OH) and then mixed with water, NaCl, *S. rouxii* inocula and soybean and/or peanut paste (Table 1). Sixteen different miso formulae were prepared using two types of mold koji (*A. oryzae* and *R. oligosporus*), two levels of NaCl (6 and 12%) and two types of oilseeds (peanut and soybean, or their combination). A flow sheet for preparation of miso is shown in Fig. 1. All misos contained 50% moisture.

Conditions for fermentation. Samples were sealed in double-layered plastic bags and incubated at 25°C for various periods of time ranging up to 90 days. Gas release, sample mixing and subjective observations of aroma, texture and color were made during the 90-day fermentation period.

Analyses

Enumeration of total microbial population. Plate count agar (PCA, pH 7.0) supplemented with 3% NaCl was used to enumerate total populations of aerobic microorganisms. Phosphate buffer (0.1M, pH 7.0) was used as a diluent in all microbiological analyses. Colonies were counted after 3–5 days incubation at 30°C.

Enumeration of molds. Potato dextrose agar (PDA, pH 5.6) supplemented with 3% NaCl, 100 ppm chlortetracycline HCl and 100 ppm of chloramphenicol was used for enumerating mold propagules in fermenting misos. Plates were incubated at 30°C and examined after 2 days at daily intervals; enumeration of colonies was made before plates became overgrown with mold mycelia.

Enumeration of *S. rouxii.* Antibiotic- and NaCl-supplemented PDA was used to enumerate *S. rouxii.* Colonies were counted after 5 days of incubation at 30°C.

Enumeration of lactic acid bacteria. Analyses for lactobacilli in miso were made using overlayed LBS agar acidified with glacial acetic acid to pH 5.5. After a 7-day period of incubation at 30°C, colonies were counted.

RESULTS & DISCUSSION

Total microbial population

Initial populations of 1.0×10^6 and 5.0×10^7 colony forming units (CFU) per gram of miso formula inoculated with *R. oligosporus* (A to H, Table 1) and *A. oryzae* (I to P, Table 1), respectively, were determined by plating samples on PCA (Fig. 2). Populations consisted mainly of koji molds, since inocula consisting of 48-hr kojis contained luxuriant growth. Viable populations of 1.2×10^5 *R. oligosporus* spores/g and 1.4×10^5 *A. oryzae* spores/g of rice had been used to initiate koji fermentation.

The total microbial populations after 90 days of fermentation were very similar to initial counts for respective types of misos regardless of the type of mold koji, oilseed composition or NaCl level. However, the total plate counts for misos inoculated ith *R. oligosporus* increased early in the fermentation period, then gradually decreased to about 10^6 CFU/g after 90 days. Misos containing *A. oryzae* koji remained at about 10^7 CFU/g throughout the 90-day incubation period. Changes in total microbial population in misos differed depending on the type of koji mold.

—Continued on next page

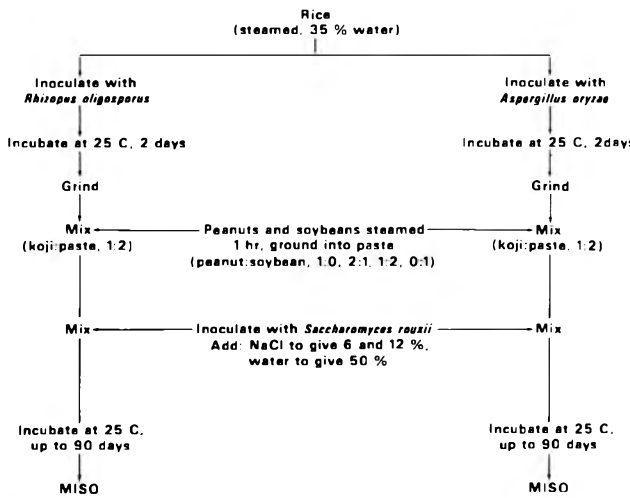


Fig. 1—Flow sheet for preparation of misos.

Table 1—Formulae for soybean/peanut miso

Miso formula code	Ingredient: Rice koji (g)[a]	Soybean paste (g)	Peanut paste (g)	Sodium chloride (g)	Water (ml)[b]
A	1200	2560	0	240	197
B	1200	2320	0	480	483
C	1200	0	2560	240	1499
D	1200	0	2320	480	1663
E	1200	1700	850	240	641
F	1200	1550	770	480	875
G	1200	850	1700	240	1073
H	1200	770	1550	480	1271
I	1200	2560	0	240	197
J	1200	2320	0	480	483
K	1200	0	2560	240	1499
L	1200	0	2320	480	1663
M	1200	1700	850	240	641
N	1200	1550	770	480	875
O	1200	850	1700	240	1073
P	1200	770	1550	480	1271

[a] Kojis for samples A through H were prepared using Rhizopus oligosporus; kojis for samples I through P were prepared using Aspergillus oryzae.
[b] Moisture content of all formulae was 50%.

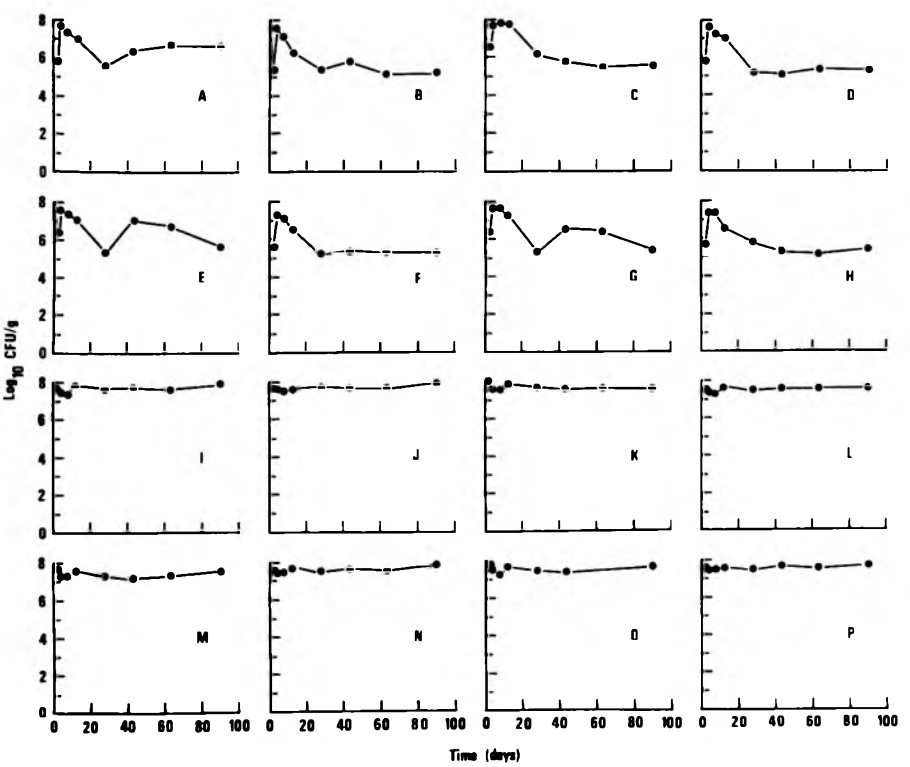


Fig. 2—Total microbial population of sixteen miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

Visual observations of colonies formed on PCA revealed that increased populations in misos inoculated with *R. oligosporus* were due to growth of microorganisms other than mold. Continuous decreases in the percentages of populations which were molds were observed with each increasing period of fermentation. A predominance of *S. rouxii* was noted as fermentation progressed toward 90 days.

Changes in ratios of various types of microorganisms were also observed in misos inoculated with *A. oryzae koji.* Like misos inoculated with *R. oligosporus*, the mold population declined during the early fermentation, probably due to high NaCl concentrations. The presence of both bacterial and yeast colonies was noted on PCA during this period. Other researchers have demonstrated the occurrence of several species of salt-tolerant bacilli and of *Micrococcus* in soy sauce [Matsumoto (1925) and Ishimaru (1933 ab,b) as cited by Yong and Wood, 1974]. In the present study, it is probable that misos contained similar types of microorganisms. Wang et al. (1969) showed that antibacterial compounds produced by *R. oligosporus* could inhibit *Bacillus subtilis* and other Gram-positive bacteria. This may offer an explanation as to why misos containing *R. oligosporus* koji had lower populations of bacteria during the 90-day fermentation period compared to misos containing *A. oryzae* koji.

The total microbial populations in low-salt (6%) formulae containing *R. oligosporus* koji may have been slightly higher than those in high-salt (12%) formulae. However, the level of NaCl had no effect on populations in misos containing *A. oryzae* kojis. Likewise, the type of oilseed ingredient (peanut or soybean, or their combination) did not influence the total microbial count of misos during the 90-day fermentation period.

Rusmin and Ko (1974) studied the microflora of tempeh fermented with *R. oligosporus* and found that bacterial contaminants at levels as high as 10^8 per g of cooked soybean did not affect the fermentation. *Bacillus mycoides, Escherichia coli, Pseudomonas cocovenenans, Pseudomonas pyoceanea* and *Proteus* sp., inoculated separately into soybeans at populations up to 2×10^8 per g, failed to cause significant changes in the quality of the fermented product. However, they warned that appropriate hygienic procedures should be observed, such as frying in oil or boiling, before the product is consumed. A good quality fermented oilseed food can be produced after appropriate pasteurization, even if relatively high levels of microorganisms which may not have contributed to desired sensory characteristics were present in the formula at some point prior to its final form. Nevertheless, good hygienic practices should be observed through fermentation procedures.

Mold population

After 48 hr of incubation at 25°C, the mycelium of *R. oligosporus* and *A. oryzae* covered the rice koji. Within 2 hr after final miso formulations were prepared, samples were analyzed for mold populations. The initial population of *R. oligosporus* was ca. 2×10^5 CFU/g of sample and the *A. oryzae* count was ca. 5×10^6 CFU/g (Fig. 3). During the fermentation, the mold count declined to less than 10 CFU/g of sample after 28 days of incubation. At 43 days of incubation, no molds were detected in 10^{-1} dilutions of samples. In other words, molds survived only about 1 month in the miso formulae due to the lethal effect of high salt concentration and anaerobic conditions.

Populations of *R. oligosporus* (sample A to H) and *A. oryzae* (I to P) declined similarly during fermentation. It is likely that mold inocula in kojis consisted almost entirely of vegetative cells, since the presence of spores would have caused substantial darkening in color. Spores would also have been expected to retain viability for periods longer than 43 days. Low-salt samples had higher mold populations at 4 and 8 days of fermentation compared to high-salt samples. The type of oilseed did not affect mold population over the duration of the period of analysis.

Yeast population

The initial population of *S. rouxii* was 1.0×10^6 per g of formula. Potato dextrose agar plates used to enumerate initial populations of *S. rouxii* in samples I to P were overgrown with *A. oryzae*, making determination of initial populations difficult.

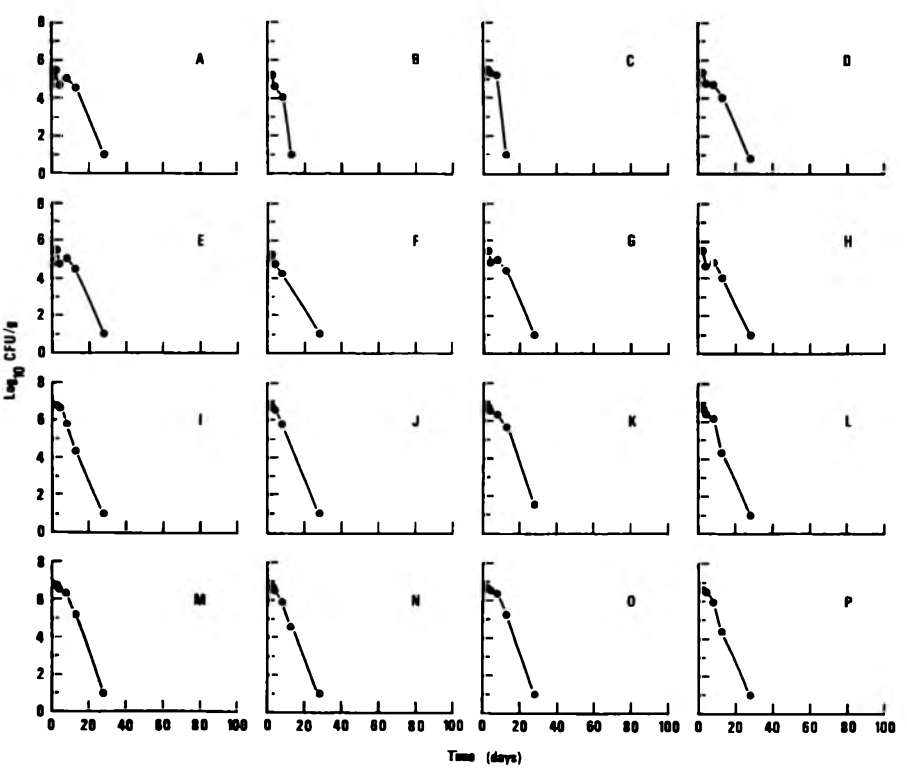


Fig. 3—Total populations of R. oligosporus (A to H) and A. oryzae (I to P) in sixteen miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

The population of *S. rouxii* increased early during the fermentation period, then gradually declined as fermentation progressed (Fig. 4). Comparing the two mold kojis, there was little difference in yeast populations during the first month of fermentation. Thereafter, however, *S. rouxii* declined more rapidly in misos inoculated with *A. oryzae* koji. With the exception of miso formulae A and I, at the end of the 90-day fermentation, *S. rouxii* populations were higher in misos containing *R. oligosporus* koji than in respective misos containing *A. oryzae* koji.

Salt level affected the *S. rouxii* population significantly. With exceptions of formulae G/H and K/L, higher counts were noted in misos containing 6% NaCl compared to those containing 12% NaCl. The type of oilseed used in various batches of misos did not appear to have a marked affect on yeast population.

The contribution of *S. rouxii* to miso fermentation has been studied. Mochizuki et al. (1972) made a comparison of the chromatograms of typically aromatic and nonaromatic miso samples, and found that *i*-butyl, *n*-butyl and *i*-amyl alcohols were important constituents of miso aroma. *Saccharomyces rouxii* contributed to the accumulation of these and other alcohols. An increase in the ethanol content in miso followed by its reaction with hydrolyzed free fatty acids to form the ethyl esters further contributes to miso flavor.

Lactic acid bacteria population

There was no consistent trend in any of the sixteen miso formulae with regard to populations of bacteria isolated on LBS agar. For example, one sample would have a zero count in the 10^{-1} dilution on the first day of fermentation, then increase to over 10^3/g on the fourth day and decline to 10^2 on the eighth day. In some misos, zero counts (10^{-1} dilution) were recorded throughout the fermentation period. Therefore, selected biochemical tests were run to determine if bacteria responsible for forming colonies on LBS agar were indeed lactobacilli. Misos A, C, I and K were examined. Ten colonies were picked from each LBS plate which had been prepared from samples collected after 0, 4, 8 and 13 days of fermentation. The shape, motility, catalase reaction, Gram stain and ability to form spores were determined. Results showed that none of the bacteria examined belonged to the genus *Lactobacillus*.

Although it is not certain at what point lactic acid bacteria and yeasts enter oilseed fermentation schemes, the most likely sequence of dominant microorganisms appears to be fungi, then lactic acid bacteria and finally yeasts (Yong and Wood, 1974). These workers evaluated *A. oryzae* and one strain each of *Lactobacillus delbrueckii* and *S. rouxii* with regard to their sequential contributions to the manufacturing of good soy sauce. These microorganisms (koji mold, lactic acid bacteria and yeast) gave a product judged entirely acceptable by themselves and by colleagues from Hong Kong, Malaysia, Korea and Thailand.

In the present study, the absence of *Lactobacillus* (probably due to pasteurization of soybeans and peanuts) would tend to retard the rate of pH decline during fermentation. However, the activities of lactic acid bacteria may be of secondary importance to those of fungal enzymes and yeasts in the manufacturing of miso. Yong and Wood (1976) tested five soy sauce formulae to determine contributions by various groups of microorganisms. Formulae consisted of: (1) fungal mash with no yeasts and no lactic acid bacteria as control; (2) fungal mash plus *S. rouxii* and *L. delbrueckii*; (3) fungal mash with yeasts alone; (4) fungal mash with lactic acid bacteria alone; and (5) fungal mash with yeast and chemical lactic acid (adjusted to pH 4.5). They reported that the formula containing *S. rouxii* without lactic acid bacteria resulted in soy sauce with good sensory characteristics.

pH Values

Organic acids (0.6–1.5%) such as lactic, succinic, acetic and phosphoric are present in miso (Hesseltine and Wang, 1967). For this reason, the pH values of fermented products tend to be lower than those of raw materials. Although in the present study *Lactobacillus* was not detected in ferments, the pH of all formulae decreased during the 90-day period of analysis (Fig. 5). Acids contributing to lower pH undoubtably came from autolysis of microbial cells. The accumulation of free fatty acids, amino acids and pep-

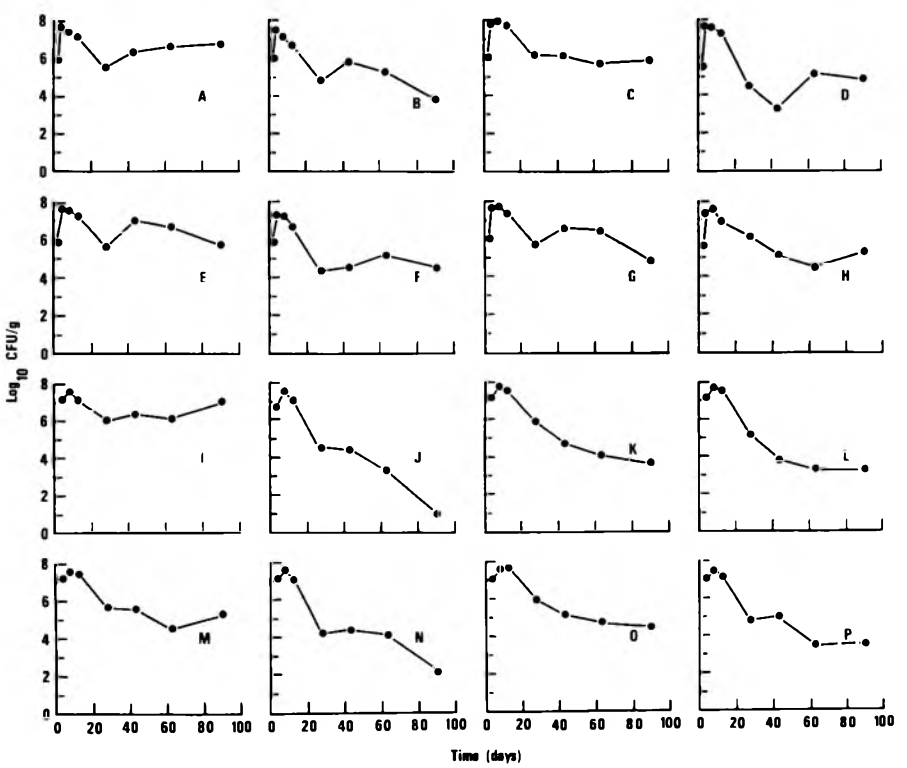


Fig. 4—Total population of S. rouxii in sixteen miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

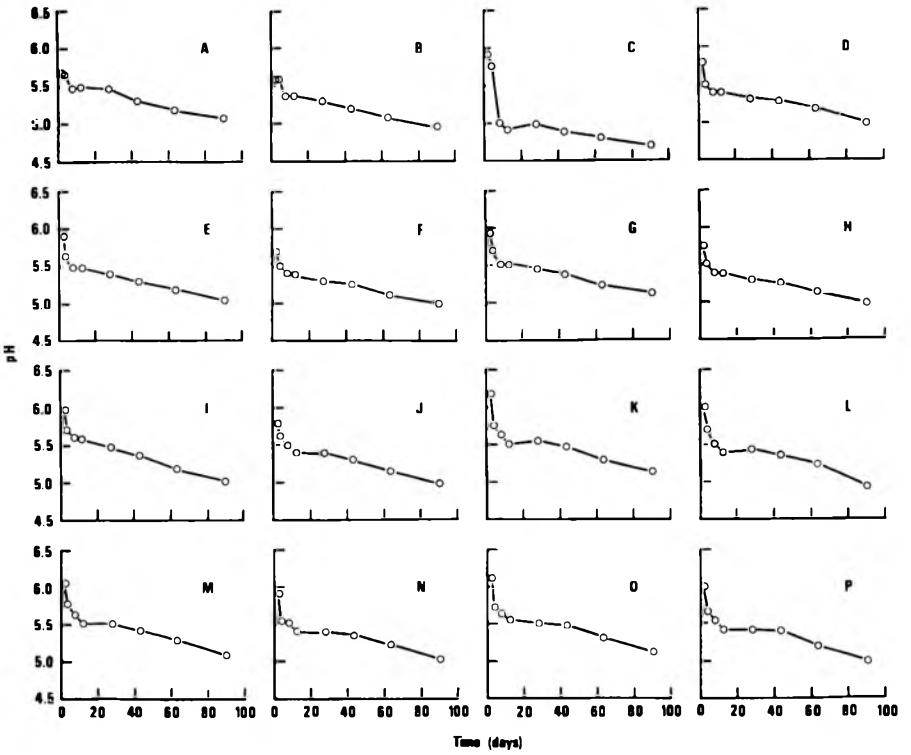


Fig. 5—The pH values of sixteen miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

tides containing carboxylic side chains as a result of hydrolysis of miso constituents could also have contributed to increased hydrogen ion concentrations. Also, other bacteria capable of producing lactic acid may not have been detected on LBS agar. The presence of these bacteria in formulae may have resulted in decreased pH values.

The pH values in low-salt misos declined somewhat quicker than those in high-salt misos. This is in agreement with data reported by Okada et al. (1975). The pH of peanut misos appeared to decline quicker than the pH of soybean misos. Comparing the two types of koji molds, except for formulae C and K, misos containing *A. oryzae* koji had a larger magnitude of decline in pH values from 0–90 days than did misos containing *R. oligosporus.* However, the final pH of all misos ranged from 4.71–5.15 after 90 days of fermentation. The final pH can be related to the buffer capacity of fermented miso (Ebine, 1971). Results from the present study are similar to those reported by Shibasaki and Hesseltine (1962). They observed that the pH of fermented and aged miso ranged from 5.1–5.3.

In summary, considering the microbiological profiles of misos, it appears that *R. oligosporus* would be an acceptable alternative to *A. oryzae* as a koji mold. The type of oilseed had no apparent effect on profiles; however, the highest level of NaCl evaluated (12%) retarded the growth of *S. rouxii.* Data from analyses of chemical and physical changes occurring in fermented soybean and peanut pastes are presented in a separate report (Shieh et al., 1982). Results indicate that a good quality low-salt miso can be prepared with *R. oligosporus* as a koji mold and peanuts as the oilseed ingredient.

REFERENCES

Beuchat, L.R. 1978. Traditional fermented food products. In "Food and Beverage Mycology," Ed. L.R. Beuchat, p. 224. Avi Publishing Co., Westport, CT.

Ebine, H. 1971. Miso. In "Proc. Intl. Symp. Conversion and Manufacture of Foodstuffs by Microorganisms," Ed. M. Fujimaki and H. Mitsuda, p. 127. Saikon Publishing Co., Tokyo.

Hesseltine, C.W. 1965. A millennium of fungi, food and fermentation. Mycologia 57: 149.

Hesseltine, C.W. and Wang, H.L. 1967. Traditional fermented foods. Biotechnol. Bioeng. 9: 275.

Kinosita, R., Ishiko, T., Sugiyama, S., Seto, T., Igarasi, S., and Goetz, I.E. 1968. Mycotoxins in fermented food. Cancer Res. 28: 2296.

Mochizuki, T., Yasukira, H., Hondo, S., Ouchi, I., Rokugawa, K., and Itoga, K. 1972. Studies on the changes of several components during miso making. In "Fermentation Technology Today, Proc. IV Intl. Ferment. Symp., Kyoti," Ed. G. Terui, p. 663. Soc. Ferment. Technol., Osaka, Japan.

Okada, Y., Yokoo, Y., and Tokeuchi, T. 1975. Studies on the reduction of salt concentration in fermented foods. Part 2. Trail making of none and low salted soybean miso. J. Jpn. Food Ind. 22: 379.

Rusmin, S. and Ko, S.D. 1974. Rice-grown Rhizopus oligosporus inoculum for tempeh fermentation. Appl. Microbiol. 28: 347.

Shibasaki, K. and Hesseltine, C.W. 1962. Miso fermentation. Econ. Bot. 16: 180.

Shieh, Y.-S.C., Beuchat, L.R., Worthington, R.E., and Phillips, R.D. 1982. Chemical and physical changes in peanut and soybean misos containing kojis prepared using Aspergillus oryzae and Rhizopus oligosporus. J. Food Sci. 47(2): 523.

Wang, H.L., Ruttle, D.I., and Hesseltine, C.W. 1969. Antibacterial compound from a soybean product fermented by Rhizopus oligosporus. Proc. Soc. Exptl. Biol. Med. 131: 159.

Wilson, B.J. and Hayes, A.W. 1973. Microbiol toxins. In "Toxicants Occurring Naturally in Foods," p. 372. National Academy of Sciences, Washington, DC.

Yong, F.M., and Wood, B.J.B. 1974. Microbiology and biochemistry of soy sauce fermentation. Adv. Appl. Microbiol. 17: 151.

Yong, F.M., and Wood, B.J.B. 1976. Microbial succession in experimental soy sauce fermentation. J. Food Technol. 11: 525.

Ms received 5/7/81; revised 9/25/81; accepted 9/28/81.

Physical and Chemical Changes in Fermented Peanut and Soybean Pastes Containing Kojis Prepared Using *Aspergillus oryzae* and *Rhizopus oligosporus*

Y. -S. CAROL SHIEH, L. R. BEUCHAT, R. E. WORTHINGTON, and R. D. PHILLIPS

ABSTRACT

Studies were made to determine and compare physical and chemical changes occurring during 90 days of fermentation of miso-like products containing peanuts and soybeans as well as a combination of these oilseeds. Two koji molds (*Aspergillus oryzae* and *Rhizopus oligosporus*) and two levels of NaCl (6 and 12%) were evaluated. The L color values decreased more rapidly in misos containing *A. oryzae* koji compared to misos containing *R. oligosporus* koji and changes occurred earlier in low-salt formulae than in high-salt formulae. The type of mold koji had no apparent effect on changes in viscosity. The free fatty acid content of misos increased dramatically during the first 4 days of fermentation. Misos containing *A. oryzae* koji had higher soluble nitrogen and free amino acid content in the final product than did those containing *R. oligosporus* kojis. Peanut miso products had higher soluble nitrogen contents than did respective soybean products; however, the type of oilseed had little effect on accumulation of free amino acids.

INTRODUCTION

THE DELIBERATE MODIFICATION of grains and oilseeds via fermentation with fungi and bacteria has been practiced for many centuries. Descriptions of traditional fermented foods can be found in reviews by Hesseltine (1965) and Beuchat (1978). A fermented soybean or soybean/rice paste known as miso is commonly used in parts of the Orient as a flavoring agent in otherwise bland vegetable diets. Miso as well as soy sauce fermentation involve the use of *Aspergillus oryzae* to prepare a koji (source of enzymes), lactic acid bacteria and yeasts, namely *Saccharomyces rouxii*. *Rhizopus oligosporus* is used to ferment whole soybeans to produce tempeh (tempé) and sometimes as a mold to ferment peanut presscake to produce oncom (Beuchat, 1976).

The chemical composition and nutritional value of fermented soybean and peanut products have been studied by several researchers. Soluble nitrogen and free amino acid contents change significantly during fermentation (Steinkraus et al., 1961; Murata et al., 1967; Mochizuki et al., 1972; Hesseltine, 1965; Hesseltine and Wang, 1967; Beuchat et al., 1975). Free fatty acid levels increase (Wagenknecht et al., 1961; Beuchat and Worthington, 1974) and changes in levels of carbohydrates occur (Shallenberger et al., 1966; Worthington and Beuchat, 1974). Reports detailing physical and chemical changes occurring during fermentation of miso prepared from *R. oligosporus* koji and from peanuts instead of soybeans are lacking. Experiments were therefore designed to evaluate the feasibility of using peanuts in place of soybeans and *A. oryzae* in place of *R. oligosporus* for preparing modified miso products. Since high levels of NaCl (as high as 14%) in traditional misos may be detrimental to persons suffering from hypertension, two concentrations of NaCl (6 and 12%) in miso formulae were tested. Reported here are the results of analyses of physical and chemical changes occurring in sixteen miso formulae during a 90-day fermentation period. While it is recognized that traditional miso consists of soybeans, cereals and salt fermented with selected strains of *A. oryzae*, the term "miso" will be used in the following text to describe fermented peanut and/or soybean products containing either *A. oryzae* or *R. oligosporus* kojis.

Authors Shieh, Beuchat, Worthington, and Phillips are with the Dept. of Food Science, Univ. of Georgia Agricultural Experiment Station, Experiment, GA 30212.

EXPERIMENTAL

Procedures for fermentation

Detailed procedures for preparation of ingredients and conditions for fermentation are outlined in a companion report (Shieh and Beuchat, 1982). Briefly summarized, rice kojis were prepared using two molds, *A. oryzae* and *R. oligosporus*. Soybeans (Bragg 79) which had been soaked in water at room temperature for 18 h were steamed for 60 min, cooled and then reduced to a paste by grinding. Skins were removed from peanuts (Florunner) before soaking in water, steaming and grinding.

Sixteen formulae containing various ratios of soybean and peanut paste were evaluated (Table 1). The ratio of koji:oilseed paste was 1:2 (w/w) in all test formulae. Moisture was adjusted to 50% and a cellular suspension of *S. rouxii* was added to all formulae. Two levels of NaCl (6 and 12%) were examined. Samples were sealed in plastic bags and incubated at 25°C for various periods ranging to 90 days. Gas release, mixing and subjective and objective measurements of aroma, texture and color were made during the course of fermentation.

Physical analyses

Samples of miso were mixed with water (2:1 ratio, miso:water, wt/vol) before analyzing for color, viscosity, and soluble solids content.

Color measurement. Indices of color (L, *a* and *b* values) were determined using a Gardner Color Difference Meter (Hunter Laboratories, Inc., Reston, VA). For all samples, the same position

Table 1—Simplified listing of composition of miso

Koji mold	Ratio of soybean:peanut in paste	Sodium chloride (%)	Miso formula[a] code
R. oligosporus	1:0	6	A
	1:0	12	B
	0:1	6	C
	0:1	12	D
	2:1	6	E
	2:1	12	F
	1:2	6	G
	1:2	12	H
A. oryzae	1:0	6	I
	1:0	12	J
	0:1	6	K
	0:1	12	L
	2:1	6	M
	2:1	12	N
	1:2	6	O
	1:2	12	P

[a] All formulae contained a ratio of koji:oilseed paste of 1:2 (w/w) and 50% water.

of placemnt of the container on the instrument and depth of sample in container were used in order to keep experimental errors at a minimum.

Viscosity measurement. Samples (25g) were deposited on a clear pane of glass which had been placed over a template consisting of concentric circles, 0.2 cm apart. The diameter of samples after 3 min at 22°C was recorded.

Soluble solids measurement. A refractometer (Bausch and Lomb Optical Co., Rocheter, NY) was used to monitor changes in soluble solids content of misos during fermentation. The percentage of soluble solids was read directly from the refractometer after depositing a few drops of sample filtrate on the prism at room temperature.

Chemical analyses

All samples of miso were freeze-dried and pulverized before the following chemical analyses were conducted.

Total lipid content. The total lipid content of 6g of sample was determined in duplicate by extracting for 24 h with Skelly F (petroleum ether, b.p; 30–60°C) using a Goldfish Extractor (Laboratory Construction Co., Kansas City, MO). Solvent was removed by evaporation in a vacuum oven at 50°C.

Profile of fatty acids. Fatty acid methyl esters were prepared in Carius combustion tubes by combining one drop of ether extract of samples with 5 ml of a 3:1 mixture of methanol-benzene containing 3% H_2SO_4 (Worthington et al., 1972). The tubes were immersed in a 90°C water bath and shaken at approximately 20–30 min intervals over a 1.5-hr period. After cooling, phase separation was effected by the addition of 1 ml of redistilled Skelly B (n-hexane, b.p. 60–68°C) and 2 ml of distilled water. The organic phase was removed and filtered through anhydrous sodium sulfate to remove traces of water. The aqueous phase was re-extracted twice with 2 ml of redistilled Skelly B and the ether extracts were likewise filtered and combined with the first extract. Solvent was removed by a stream of nitrogen and the methyl esters were then stored in a refrigerator under nitrogen.

Methyl esters of fatty acids were analyzed on a Micro-Tek 220 gas chromatograph equipped with dual flame ionization detectors (Tractor Inc., Austin, TX) and a CRS–100 integrator (Infotronics Corp., Houston, TX) which reports the data as relative proportions (percent) of total fatty acids. A glass column, 180 cm x 0.4 cm i.d., was packed with 105 Silar 5-CP on 80/100 mesh Chromsorb W (Acid washed and treated with dimethyl dichlorosilane). Detector and injection part temperatures were maintained at 306°C and 275°C, respectively. Chromatographic separation of fatty acid esters in samples (0.1 μl) was carried out at an isothermal temperature of 245°C with a helium flow rate of 100 ml/min.

Free fatty acids. Ether extracts (0.5g) of samples were dissolved in 15 ml of methylene chloride and 15 ml of neutralized 95% ethyl alcohol containing 0.01% phenolphthalein. The mixture was titrated with standardized ethanolic NaOH to determine the concentration of free fatty acids (modified from AOCS, Official Method, 1970). The percentage of free fatty acids calculated as linoleic acid in soybean and oleic acid in peanut were then determined for each sample.

Soluble nitrogen. Samples (2 g/100 ml distilled water) were adjusted to pH values ranging from 2–11 in one-unit increments by adding small amounts of HCl or NaOH. Suspensions were agitated during a 45-min period at 25° C and then centrifuged at 22,000 x *g* for 10 min at room temperature using a modification of the procedure reported by Quinn and Beuchat (1975). The supernant was filtered through Whatman No. 2 paper and the nitrogen in known volumes of filtrate was determined using the macro-Kjeldahl procedure (AOAC, 1970).

Free amino acids. An internal standard (0.5 ml of DL-norleucine) was added to tared samples. The samples were then combined with 25 ml of 3% sulfosalicylic acid and agitated for 1 hr on a rotary shaker (Eberbach Co., Ann Arbor, MI). Suspensions were centrifuged at 12,000 x *g* for 10 min (4°C); the supernant was then adjusted to pH 2.2, passed through a Millipore membrane filter (0.2 μm exclusion) and analyzed for amino acid content.

A Durrum D-500 amino acid analyzer (Dionex CO., Sunnyvale, CA) equipped with a column (50 cm x 0.175 icm i.d.) packed with DC-4A (8μ ± 1) ion exchanger resin was used to identify and quantitate amino acids in miso samples. The injection volume as 20μl. The analytical procedure was that recommended by the instrument manufacturer for protein hydrolyzates. Three buffers (A: pH 3.28, 0.2N Na^+; B: pH 4.25, 0.2N N^+; C: pH 7.90, 1.10N Na^+) and two column temperatures were employed. Aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, and valine were eluted by buffer A at 50.6°C; methionine, isoleucine, leucine, tyrosine and phenylalanine were eluted by buffer B at 64.0°C; and histidine, lysine, NH_4 and arginine were eluted by buffer C at 64.0°C. Total analysis time including column regeneration was 100 min per sample. Elutates were monitored at 590 mμ to determine concentrations of all amino acids except proline, which was monitored at 440 mμ. Results were determined by the instrument (dedicated PDP8m computer) as nmols of amino acid per injected sample. Further data manipulation yielded results of g amino acid per 100 g of dry sample.

Reducing sugars. The pH 5.0 filtrate prepared for soluble nitrogen measurement was also used for reducing sugar analysis. Dextrose (National Bureau of Standards) solution (100 μg/ml) was used as a standard for plotting the standard curve. The Nelson test (Clark, 1964) was used to quantitate the reducing sugar content of miso samples. A Spectronic 20 photometer (Bausch and Lomb Co., Rochester, NY) was used to determine the concentration of reducing sugars in miso extracts.

RESULTS & DISCUSSION

Physical properties

Color. The color of miso is expressed as L, *a* and *b* values (Hunter system). The L values of misos changed significantly during the 90-day fermentation period (Fig. 1). Positive L values signify that color is approaching white, whereas negative L values indicate that the color is approaching black. All sixteen miso formulae became darker in color as the fermentation progressed. The unfermented peanut paste initially had a lighter color than did the soybean paste. For example, the L value of miso formula C (no soybeans) was higher than that of formula A (no peanuts). The unfermented pastes containing *A. oryzae* koji were much darker than corresponding pastes containing *R. oligosporus.* For example, the L value of formula I was lower than that of formula A. This was due in part to slight differences in the color of mycelia in the kojis. The color of koji prepared with *A. oryzae* was slightly greenish-yellow whereas *R. oligosporus* produced a pure white koji.

A comparison of misos prepared with the two types of koji fungi shows that L values of formulae containing *A. oryzae* decreased by a larger magnitude than did values in respective formulae containing *R. oligosporous* koji, i.e., the arithmetic differences in L values between 0 day and 90 days in *A. oryzae* misos (I to P) were larger than those in *R. oligosporus* misos (A to H). L values of low salt miso formulae may have decreased more quickly than did L values of high-salt products. This is consistent with a report by Okada et al. (1975) that changes in miso color correspond in part to salt concentration. The magnitude of change in L values over the 90-day fermentation period did not appear to be influenced by the type of oilseed in the formulae.

A positive *a* value signifies red whereas a negative *a* value signifies green; positive *b* values signify yellow and negative *b* values signify blue. Changes in *a* and *b* values in miso formulae were not significant over the 90-day fermentation period. The variation in *a* values between initial and final days of fermentation was less than 4.6 in any sample. Likewise, the *b* values of formulae changed only slightly throughout the 90-day fermentation period.

Viscosity. As fermentation progressed, the viscosity of the miso formulae decreased (Table 2). In general, misos became more liquid in character, especially during the first 13 days of fermentation. The viscosities of misos fluctuated somewhat between 28 and 90 days of fermentation. With the exception of formulae C and K (peanut, low salt), the viscosities of all misos decreased at 90 days. This can be attributed to hydrolysis of oilseed constituents into smaller molecular weight materials which would in turn influence flow properties. Misos C and K contained higher levels of lipid than did the other fourteen misos. Conversely, misos

C and K contained a lower percentage of carbohydrate and protein compared to other misos. After a period of fermentation, larger amounts of free fatty acids in misos C and K may have acted to emulsify these systems and thus influenced changes in viscosity.

Initially, peanut pastes had lower viscosities than did soybean pastes. This may have been due to the higher lipid content in peanut pastes and also to differences in flow properties of peanut and soybean lipids at room temperature. No significant differences were noted in viscosities over the 90-day fermentation period that could be attributed to the type of koji or level of salt.

Soluble solids. The constituents in miso were broken down into smaller units during the course of fermentation, causing the soluble solids content to increase (Fig. 2). This observation is consistent with that of Van Buren et al. (1972) who reported that the proportion of water-soluble solids reached about half that of the total solids at the end of tempeh fermentation.

In the unfermented formulae, soybean paste contained a higher percentage of soluble solids than did peanut paste, and high-salt pastes showed a slightly higher percentage of soluble solids than did low-salt pastes. Different oil seed ingredients (soybean or peanut) and salt levels did not influence the magnitude of change in soluble solids content over the 90-day period of fermentation. However, misos containing *R. oligosporus* kojis appeared to have a more rapid increase in soluble solids content compared to respective formulae containing *A. oryzae* koji.

Chemical properties

Total lipid content. According to several reports, changes in total lipid content of fermented oilseeds are minor (Hesseltine, 1965; Wagenknecht et al., 1961; Yoshida and Kajimoto, 1972). Table 3 lists the total lipid contents of misos after various periods of fermentation up to 90 days. The percentage of ether-extractable materials increased as fermentation progressed; however, these increases were probably due to decreases in nonlipid materials rather than to an actual increase in lipid during fermentation. The amount of ether-extractable material appeared to increase more rapidly in misos containing *R. oligosporus* kojis compared to respective miso formulae containing *A. oryzae* kojis.

—Continued on next page

Table 2—Viscosity of miso.

Miso formula code	Diameter (cm) of samples[a] after various periods (days) of fermentation							
	0	4	8	13	28	43	63	90
A	6.2	6.6	6.8	7.0	7.2	7.0	7.3	7.3
B	7.4	8.2	8.6	9.0	8.8	9.2	8.8	9.2
C	11.4	12.2	11.8	11.8	11.6	11.8	11.8	11.4
D	12.8	13.8	14.0	14.2	13.6	12.8	13.4	13.2
E	8.2	9.2	9.0	9.4	9.4	8.8	8.8	11.0
F	9.4	10.0	10.8	11.2	11.2	11.4	10.8	10.8
G	8.8	9.8	11.2	10.8	10.8	10.6	10.2	10.6
H	11.6	12.0	12.4	12.6	12.6	11.8	11.8	12.0
I	6.2	6.6	7.2	7.4	7.0	7.4	7.2	7.6
J	7.2	7.8	8.2	8.6	8.2	8.4	8.4	8.4
K	11.2	10.8	10.8	11.2	10.8	11.4	11.0	10.4
L	12.6	12.8	12.6	12.8	12.8	12.6	13.4	13.6
M	7.6	7.8	8.6	8.8	8.8	9.0	8.8	8.4
N	8.6	9.4	9.6	10.2	10.2	9.8	10.0	10.2
O	8.8	9.6	10.0	10.4	10.2	10.4	10.6	9.8
P	11.0	11.0	11.6	12.0	11.8	11.8	11.2	11.4

[a] Samples consisted of 25g of a mixture of fermented producted: water (2:1, w/v). Samples were deposited on a flat surface at room temperature and diameters were recorded after 3 min.

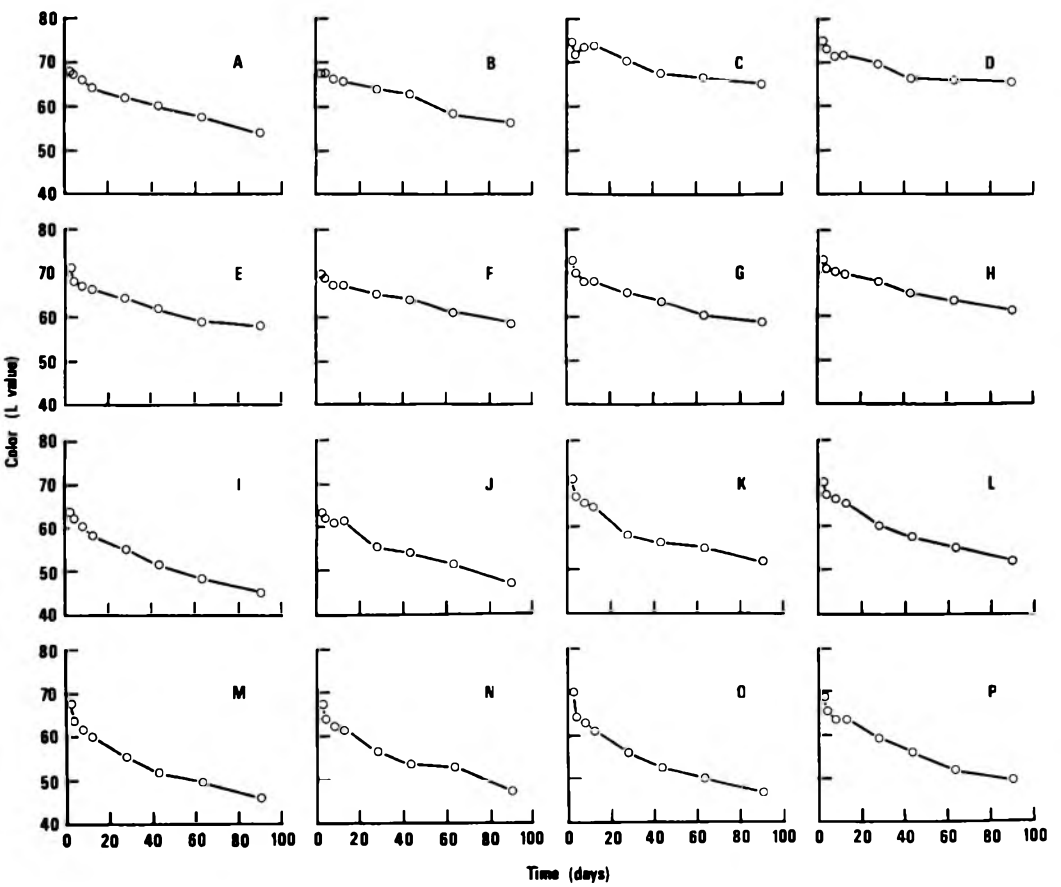


Fig. 1—Color (L values) of sixteen miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

Miso formulae containing peanuts had higher levels of lipid than did respective formulae containing soybeans. The high-salt misos had lower lipid content than the low-salt misos due to the fact that part of the soybean (peanut) component in high-salt misos was substituted by sodium chloride.

Fatty acid profiles. Shaw (1966) reported that the major fatty acids in lipid of *Rhizopus* mycelium are oleic, linoleic, linolenic, stearic and palmitic, whereas the major fatty acids in mycelial lipid of *Aspergillus* are oleic, linoleic, palmitic and stearic. The relative percentage of these fatty acids could contribute to the fatty acid profiles of misos. However, because of the large quantities of lipid in soybeans or peanuts, the fatty acid profiles of these major ingredients would be expected to most significantly influence the fatty acid composition of misos. Listed in Table 4 are the percentages of fatty acids in misos containing only soybeans (A, B, I, J) and peanuts (C, D, K, L). Soybean misos contained the largest amount of linoleic acid (18:2) whereas peanut misos contained oleic acid (18:1) as the major fatty acid.

Table 3—Lipid content of miso.

Miso formula code	Lipid content (%, w/w) after various periods (days) of fermentation				
	0	4	8	28	90
A	11.83	12.94	13.16	12.81	13.35
B	10.13	10.50	11.23	11.90	11.21
C	35.63	37.50	40.30	43.10	42.04
D	31.50	33.29	35.57	37.82	37.79
E	21.71	23.11	24.89	25.76	25.86
F	18.76	19.67	20.91	22.15	22.02
G	28.75	30.85	32.65	35.01	34.49
H	25.67	27.17	29.00	30.73	31.04
I	11.81	12.54	13.93	15.33	11.94
J	10.35	10.32	11.35	11.81	10.25
K	35.49	35.78	38.42	34.75	33.66
L	31.55	32.62	36.63	37.51	32.66
M	21.39	22.32	23.55	26.02	22.39
N	18.33	19.18	20.56	21.84	19.62
O	28.84	28.60	31.96	33.76	29.69
P	25.68	25.81	27.83	30.76	28.47

The composite total amount (% of total fatty acids) of major fatty acids (18:1 and 18:2) in misos containing *R. oligosporus* koji decreased slightly during the first 4 days of the 90-day fermentation period. The relative percentages of other fatty acids increased. Changes in percentages of fatty acids may have been due to preferential utilization of linoleic and oleic acids by microorganisms and/or production of other fatty acids in *Rhizopus* mycelium. Changes in percentages of fatty acids in misos containing *A. oryzae* were less than those observed in misos containing *R. oligosporus* koji. Changes in fatty acid content of miso lipids were not affected by salt concentration.

Free fatty acid content. The concentration of titratable free fatty acid increased during the fermentation of miso formulae A, B, C, D, I, J, K and L (Fig. 3). The free fatty acid contents in unfermented formulae were relatively high. However, these levels were observed, in part, probably because of the action of lipase from the koji during the 1 to 2 h period between mixing the ingredients and freezing samples for eventual lipid analyses. A dramatic increase in free fatty acid content was noted in all misos during the first 4 days of fermentation, indicating strong lipase activity in the kojis. After 4 or 8 days of fermentation, decreases in free fatty acid content were clearly evident in all soybean misos (A, B, I, J) and perhaps also in peanut misos containing *A. oryzae* koji (K, L). The free fatty acid content in peanut misos containing *R. oligosporus* koji

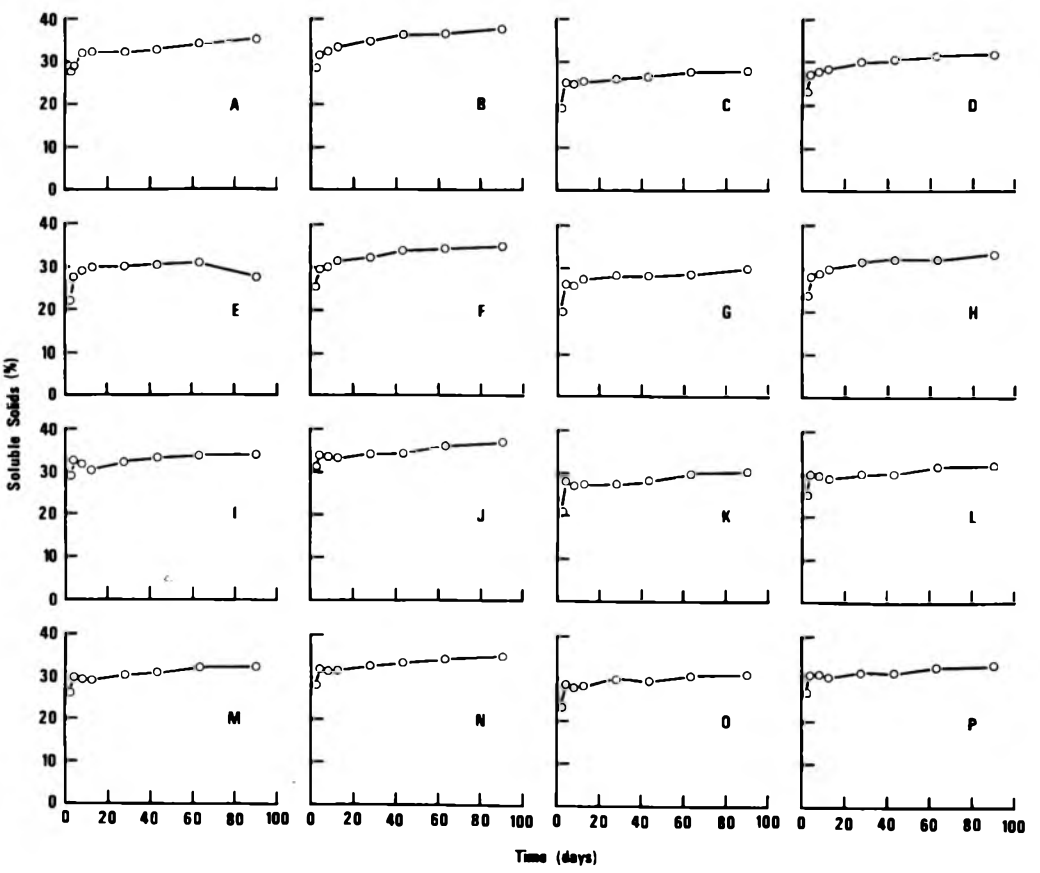


Fig. 2—Soluble solids content in sixteen miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

(C, D) was high at the end of the 90-day fermentation. Since neither koji mold preferentially utilizes any particular fatty acid (Beuchat and Worthington, 1974), decreases in free fatty acid content are probably due to the formation of fatty acid esters. Mochizuki et al. (1972) found that decreases in the free fatty acid content of miso coincided with increases in fatty acid ethyl ester content. They reported that glycerides were hydrolyzed to release free fatty acids which in turn continued to form the ethyl esters. This process resulted in a fluctuation in concentration of free fatty acids. The fatty acid esters derived from fatty acids and ethanol or higher alcohols are important in giving miso its characteristic odor (Mochizuki et al., 1972; Shibasaki and Hesseltine, 1962).

Different salt levels did not substantially influence the accumulation of free fatty acids. The type of oilseed ingredient had no effect on free fatty acid production in *R. oligosporus* misos; however, production was higher in soybean misos compared to peanut misos containing *A. oryzae* kojis.

Soluble nitrogen content. The soluble nitrogen content is the index of hydrolysis and solubilization of protein. All the fermented misos analyzed had much higher soluble nitrogen contents at pH values near the isoelectric points of soybean and peanut protein compared to respective control formulae (Fig. 4). Increases were due mainly to microbial protease activity on oilseed proteins. Apparently, protease activity in misos containing *A. oryzae* koji (I, J, K, L) was higher than in misos containing *R. oligosporus* koji (A, B, C, D). Low-salt misos had higher soluble nitrogen contents than high-salt misos did, which is consistent with a report by Okada et al. (1975) indicating that enzyme activities were higher in ripened miso of lower salt concentration.

Mochizuki et al. (1972) stated that soluble nitrogen content increased in the early stage of miso maturation and reached a maximum value of approximately 60% on the 50th day of incubation. All of the misos analyzed in the present study had soluble nitrogen contents greater than 60%, even at pH values near the isoelectric points of oilseed proteins. Soluble nitrogen was higher in peanut misos compared to soybean misos.

—Continued on next page

Table 4—Fatty acid content of miso

Miso formula code	Fermentation period (days)	Fatty acid (% of total fatty acids)[a]									
		16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0	Others[b]
A	0	12.45	3.62	16.82	59.29	7.15	T[c]	0.10	0.35	0	0.22
	4	12.37	4.07	19.31	54.06	8.17	T	0.23	0.44	0	1.35
	8	13.33	3.94	17.94	56.83	7.82	0	0	0.19	0	0
	28	12.06	3.77	17.94	56.90	8.87	T	0.06	0.25	T	0.15
	90	12.74	3.78	17.58	56.92	8.65	0	0.04	0.20	T	0.09
B	0	11.47	3.70	16.93	59.84	7.11	0.32	0.09	0.28	0	0.26
	4	12.37	4.22	19.65	53.99	7.89	T	0.18	0.46	0.03	1.21
	8	11.95	3.85	18.02	56.41	8.87	0	0.36	0.32	0.16	0.06
	28	12.31	3.75	18.03	56.71	8.73	0	T	0.26	T	0.21
	90	12.44	3.71	17.57	56.69	9.01	T	0.09	0.26	0.04	0.19
C	0	10.78	2.05	54.41	28.31	0	0.85	0.87	1.78	0.86	0.09
	4	10.99	2.19	49.94	30.94	0	0.92	0.99	2.50	1.20	0.33
	8	11.29	2.18	49.97	30.84	0	0.95	0.89	2.44	1.25	0.19
	28	11.16	2.16	50.18	30.96	0	0.85	0.92	2.46	1.15	0.16
	90	11.33	2.16	49.85	31.19	0	0.88	0.96	2.30	1.16	0.17
D	0	9.24	1.68	52.74	31.18	0	0.96	0.91	1.88	1.20	0.21
	4	11.14	2.17	49.78	30.44	0	1.08	1.09	2.40	1.29	0.61
	8	11.48	2.18	50.56	30.73	0	0.85	0.85	2.29	1.01	0.05
	28	11.27	2.16	50.11	31.34	0	0.77	0.94	2.21	1.07	0.13
	90	11.65	2.12	50.12	30.94	0	0.92	1.02	2.16	0.93	0.14
I	0	11.68	3.64	20.36	55.47	8.15	T[c]	0.13	0.39	T	0.28
	4	11.96	3.58	20.05	55.29	8.30	T	0.12	0.44	0.08	0.18
	8	12.02	3.52	19.49	55.91	8.25	0	0.07	0.32	0.51	0
	28	12.15	3.62	19.97	55.25	8.05	0	0.11	0.42	0.08	0.35
	90	12.50	3.57	19.97	54.92	8.12	0	0.34	0.40	0.22	0
J	0	11.70	3.72	18.19	57.10	8.52	T	0.13	0.29	T	0.35
	4	12.51	3.80	18.36	56.49	8.06	T	0.07	0.26	0	0.45
	8	12.13	3.66	17.68	56.99	8.88	0	0.06	0.32	T	0.28
	28	12.06	3.63	17.75	57.24	8.75	0	0.04	0.30	0.21	0.20
	90	12.41	3.57	17.30	57.13	8.83	0	0.28	0.26	0.14	0.08
K	0	10.90	2.13	50.13	31.23	0	0.82	0.93	2.50	1.25	0.11
	4	11.39	2.14	49.92	31.50	0	0.77	0.90	2.24	1.06	0.08
	8	11.14	2.14	50.15	31.35	0	0.75	0.89	2.17	1.19	0.23
	28	11.64	2.13	50.84	30.91	0	0.73	0.70	2.12	0.80	0.15
	90	11.08	2.12	49.92	31.66	0	0.74	0.89	2.27	1.14	0.18
L	0	11.07	2.17	51.08	30.41	0	1.01	1.11	2.54	1.31	0
	4	11.50	2.13	50.92	30.50	0	0.86	0.87	2.22	0.96	0.04
	8	11.27	2.08	50.71	30.90	0	0.83	0.85	2.24	1.07	0.05
	28	12.40	2.11	50.84	30.91	0	0.73	0.70	2.12	0.80	0
	90	11.55	2.09	50.20	31.62	0	0.74	0.77	2.14	0.87	0.02

[a] Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), eicosanoic acid (20:0), eicosenic acid (20:1), docosanoic acid (22:0), and tetracosanoic acid (24:0);
[b] Includes trace and unidentified fatty acids.
[c] Trace

Free amino acid content. The free amino acid contents of eight miso formulae after 90 days of fermentation are listed in Table 5. Misos containing *R. oligosporus* kojis (A, B, C, D) had lower concentrations of total amino acids than did respective formulae (I, J, K, L) containing *A. oryzae* kojis. A positive correlation was observed regarding the relative levels of free amino acids and the amounts of soluble nitrogen in misos containing the two types of kojis (compare Table 5 and Fig. 4). Okada et al. (1975) stated that the contents of total and free amino acids and the color of miso changed (darkened) in accordance with a reduction of salt concentration. The same trend was noted in the present study. The type of oilseed (soybean or peanut) did not appear to greatly influence the amount of free amino acids accumulated after 90 days of fermentation.

The largest amount of total free amino acids was observed in miso containing 6% sodium chloride and *A. oryzae* koji (code I); this miso also contained the largest amount of free essential amino acids. Except for tryptophan, which was not analyzed, all other essential amino acids were detected in the eight formula C. The free amino acid in highest concentration in formulae A, C, D, I, J and K was glutamic acid, whereas leucine was highest in formula B and arginine was highest in formula L. Mochizuki et al. (1972) stated that free glutamic and aspartic acids were abundant among miso amino acids. This is consistent with data presented here for misos containing *A. oryzae* koji (I, J, K, L) but not for misos containing *R. oligosporus* koji (A, B, D, D). The latter formulae had reduced concentrations of free aspartic acid. Mochizuki et al. (1972) also reported that glutamic and aspartic acids increased gradually until the 90th and 120th day of fermentation but that other amino acids maintained constant levels or decreased after about 20–35 days of fermentation. Glutamic acid is a very important contributor to miso flavor and therefore would be assumed to enhance the flavor of all misos listed in Table 5.

Reducing sugar content. The reducing sugar contents of misos after 90 days of fermentation are listed in Table 6. Misos containing *A. oryzae* kojis had higher amounts of reducing sugars compared to respective formulae containing *R. oligosporus* kojis. Worthington and Beuchat (1974) also reported that *A. oryzae* utilized carbohydrates in peanuts to a greater extent than did *R. oligosporus*.

Miso formulae containing *A. oryzae* kojis also had larger amounts of free amino acids than did formulae containing *R. oligosporus* kojis. These higher levels of free amino acids and reducing sugars would be more likely to lead to aminocarbonyl reactions and thus a more rapid darkening in color.

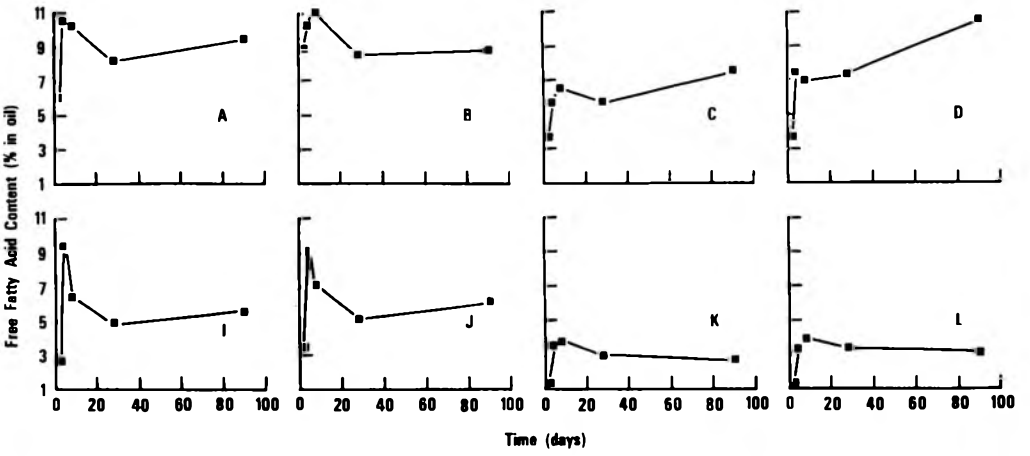


Fig. 3—Free fatty content in eight miso formulae during 90 days of fermentation. See Table 1 for explanation of codes.

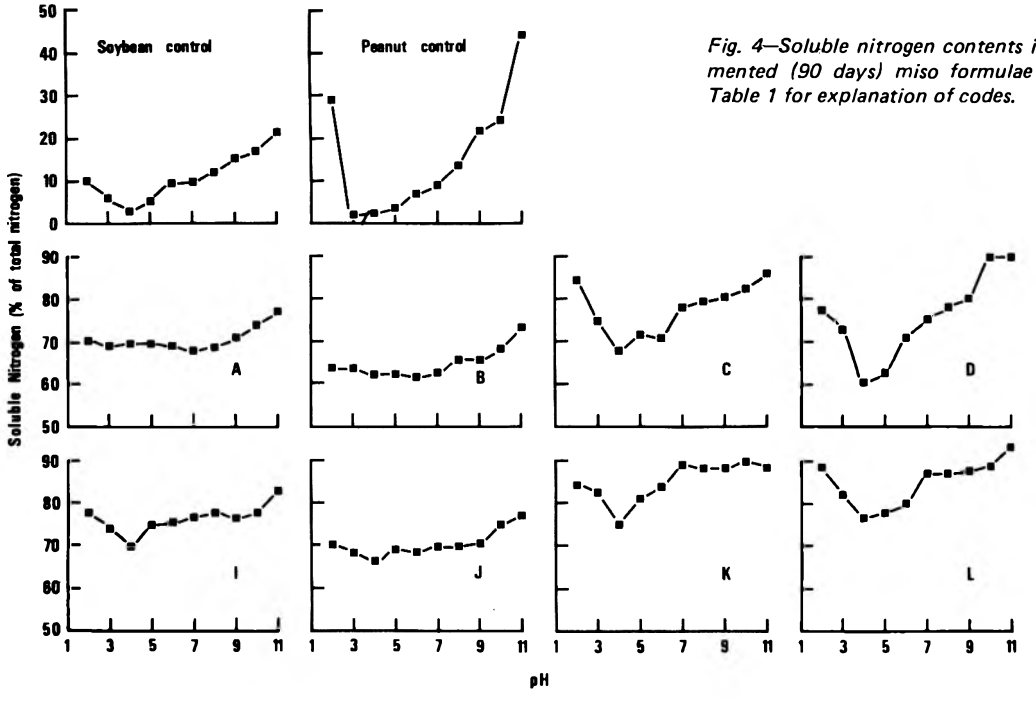


Fig. 4—Soluble nitrogen contents in control (unfermented) and fermented (90 days) miso formulae over a pH range 2.0–11.0. See Table 1 for explanation of codes.

Table 5—Free amino acid content of selected miso formulae after 90 days of fermentation

Amino acid	Miso formula code (g/100 g)[a]							
	A	B	C	D	I	J	K	L
Aspartic acid	0.43	0.11	0.36	0.20	1.60	0.85	1.38	0.99
Threonine[b]	0.46	0.20	0.27	0.17	0.64	0.40	0.36	0.28
Serine	0.97	0.23	0.60	0.45	0.90	0.55	0.70	0.58
Glutamic acid	1.51	0.37	1.01	0.75	2.22	1.34	1.81	1.53
Proline	0.33	0.21	0.15	0.26	0.55	0.32	0.48	0.29
Glycine	0.31	0.06	0.23	0.15	0.50	0.29	0.61	0.44
Alanine	0.62	0.20	0.36	0.29	0.77	0.50	0.58	0.46
Cystine	0	0	0	0	0	0	0	0
Valine[b]	0.67	0.30	0.34	0.30	0.82	0.50	0.55	0.43
Methionine[b]	0.11	0.05	0.11	0.06	0.18	0.13	0.16	0.12
Isoleucine[b]	0.61	0.31	0.35	0.31	0.78	0.50	0.64	0.51
Leucine[b]	1.04	0.53	0.71	0.59	1.28	0.87	0.95	0.75
Tyrosine[b]	0.56	0.25	0.10	0.36	0.75	0.52	0.78	0.59
Phenylalanine[b]	0.66	0.34	0.49	0.43	0.84	0.52	0.62	0.52
Histidine	0.15	0.02	0.10	0.06	0.36	0.25	0.28	0.20
Lysine[b]	0.61	0.14	0.33	0.13	1.04	0.65	0.51	0.41
Arginine	0.90	0.28	0	0.70	1.40	0.96	1.76	1.55
Tryptophan[b]	–[c]	–	–	–	–	–	–	–
NH_4	0.11	0.06	0.22	0.07	0.21	0.14	0.18	0.14
Total	10.06	3.66	5.71	5.28	14.83	9.29	12.88	9.79

[a] Dry weight basis
[b] Essential amino acid
[c] Not determined

In this study, high salt levels resulted in higher levels of reducing sugars in final products. This observation was different from that of Okada et al. (1975) who reported that the amylase content was lower in high salt misos than in low-salt ones. Soybean misos had higher reducing sugar contents compared to respective peanut misos, probably due in part to differences in amounts of carbohydrates found in soybeans and peanuts.

Sensory evaluation of various miso formulae would contribute substantially to judgements concerning the feasibility of using *R. oligosporus* as a koji mold and peanuts instead of soybeans for preparing miso. Partially defatted peanuts may be more suitable than full fat kernels for preparing miso with viscosity characteristics more similar to those of soybean miso. However, results presented here indicate that good-quality low-salt miso can be prepared with *R. oligosporus* as a koji mold and peanuts as the oilseed ingredient.

Table 6—Reducing sugar content of miso.

Miso formula code	Reducing sugar (mg/g)[a]
Soybean control	2.65
Peanut control	0.45
A	47.20
B	72.75
C	7.00
D	46.15
I	69.35
J	95.30
K	48.30
L	52.00

[a] Calculated on a dry weight basis. Samples were anlayzed after 90 days of fermentation.

REFERENCES

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

AOCS. 1970. "Official and Tentative Methods of the American Oil Chemists' Society, Chicago, IL.

Beuchat, L.R. 1976. Fingal fermentation of peanut press cake. Econ. Bot. 30: 227.

Beuchat, L.R. 1978. Traditional fermented food products. In "Food and Beverage Mycology," p. 224. Avi Publ. Co., Westport, CT.

Beuchat, L.R., Young, C.T., and Cherry, J.P. 1975. Electrophoretic patterns and free amino acid composition of peanut meal fermented with fungi. Can. Inst. Food Sci. Technol. J. 8: 40.

Beuchat, L.R. and Worthington, R.E. 1974. Changes in the lipid content of fermented peanuts. J. Agric. Food Chem. 22: 509.

Clark, J.M. Jr. 1964. Part one: Carbohydrates: Reactions of reducing sugars. In "Experimental Biochemistry," p. 12. W.H. Freeman and Company, San Francisco, CA.

Hesseltine, C.W. 1965. A millennium of fungi, food and fermentation. Mycologia 57: 149.

Hesseltine, C.W. and Wang, H.L. 1967. Traditional fermented foods. Biotechnol. Bioeng. 9: 275.

Mochizuki, T., Yasukira, H., Hondo, S., Ouchi, I., Rokugawa, K., and Itoga, K. 1972. Studies on the changes of several components during miso making. In "Ferment. Symp., Kyoto, Ed. G. Terui, p. 663. Soc. Ferment. Technol., Osaka, Japan.

Murata, K., Ikehata, H., and Miyamoto, T. 1967. Studies on the nutritional value of tempeh. J. Food Sci. 32: 580.

Okada, Y., Yokoo, Y., and Takeuchi, T. 1975. Studies on the reduction of salt concentration in fermented foods. Part II. Trials of no and low salted soybean miso. J. Jpn. Food Ind. 22: 379.

Quinn, M.R. and Beuchat, L.R. 1975. Functional property changes resulting from fungal fermentation of peanut flour. J. Food Sci. 40: 475.

Shallenberger, R.S., Hand, D.B., and Steinkraus, K.H. 1966. Changes in sucrose, raffinose and stachyose during tempeh fermentation. Eighth Dry Bean Research Conference, USDA ARS-74-41. p. 68.

Shaw, R. 1966. The polyunsaturated fatty acids of microorganisms. Adv. Lipid Res. 4: 107.

Shibasaki, K. and Hesseltine, C.W. 1962. Miso fermentation. Econ. Bot. 16: 180.

Shieh, Y.-S.C. and Beuchat, L.R. 1982. Microbial changes in peanut and soybean misos containing kojis prepared using Aspergillus oryzae and Rhizopus oligosporus. J. Food Sci. 47(2): 518.

Steinkraus, K.H., Hand, D.B., Van Buren, J.P., and Hackler, L.R. 1961. Proc. conf. soybean products for protein in human food. ARS-71-22, p. 83.

van Buren, J. P., Hackler, L. R., and Steinkraus, K.H. 1972. Solubilization of soybean tempeh constituents during fermentation. Cereal Chem. 49: 208.

Wagenknecht, A.C., Mattick, L.R., Lewis, L.M., Hand, D.B., and Steinkraus, K.H. 1961. Changes in soybean lipids during tempeh fermentation. Food Sci. 26: 373.

Worthington, R.E., and Beuchat, L.R. 1974. α-Galactosidase activity of fungi on intestinal gas-forming peanut oligosaccharides. J. Agric. Food Chem. 22: 1963.

Worthington, R.E., Boggess, T.S. Jr., and Heaton, E.K. 1972. Fatty acids of channel catfish. J. Fisheries Res. Bd. Can. 29: 113.

Yoshida, H. and Kajimoto, G. 1972. Changes in lipid components during miso making studies on the lipids of fermented foodstuffs. (part I). J. Jpn. Soc. Food Nutr. 25: 415.

Ms received 5/7/81; revised 9/25/81; accepted 9/28/81.

Effect of Heating Time of Soybean on Vitamin B-6 and Folacin Retention, Trypsin Inhibitor Activity, and Microstructure Changes

U. SOETRISNO, Z. A. HOLMES, and L. T. MILLER

ABSTRACT

Heating treatments of boiling 20 min or autoclaving 5 min, 10 min, or 20 min, of soaked (25°C for 10 hr) soybeans, significantly influenced vitamin B-6, free folacin, trypsin inhibitor activity, water absorption, moisture content and blue, green and amber color values in the cooked soybeans. Analysis of covariance showed a relationship ($P \leq 0.05$) between water absorption after cooking with total folacin in cooked soybeans and water absorption after cooking with blue color values. Other relationships ($P \leq 0.05$) were observed in cooked soybeans between texture and total folacin, moisture and trypsin inhibitor activity, and trypsin inhibitor activity and free folacin.

INTRODUCTION

IN THE LAST 30 YEARS, the increasing popularity of soybeans in the United States is reflected by the tremendous expansion in planted acreage (McArthur, 1980). Soybeans are used in a variety of ways and in many different products (Nelson et al., 1978; Willet, 1976). These may range from consumption of the cooked bean in soups or casseroles to products such as bean curd, fermented bean, soybean concentrates or soybean isolates. Soybean flour and defatted flour have been widely used in improving nutritional status of children and adults in many developing countries (Sabin, 1961), as well as in the United States.

Because of its extensive use as a high quality protein supplement in human diets, an investigation of the influence of selected preparation and processing procedures on other select nutrients, folacin and vitamin B-6, would be pertinent. Soybeans are rich in these two vitamins (Bailey et al., 1935; Harris and Karmas, 1975; Perloff and Butrum, 1977). Folacin and vitamin B-6 are water soluble and heat labile vitamins (Herbert and Bertino, 1967; Storvick et al., 1964). The conditions of soybean preparation for consumption would permit the leaching of these vitamins in the soaking and cooking water. Studies by a number of workers have reported vitamin B-6 and folacin retention in pinto beans (Miller et al., 1973) and in lima, blackeye and pink beans (Rockland et al., 1977).

Although there is decreased vitamin retention with soaking and cooking soybeans, they may enhance the nutritive value of the beans due to destruction of both an antinutritional factor and undesirable factors which are formed upon digestion. Trypsin inhibitors present in soybeans reduce the nutritional quality of the protein (Rackis, 1981). Other components such as urease and hemagglutinin may be toxic to animals (National Research Council, 1973). Trypsin inhibitors, urease, and hemaglutinin are heat labile. The oligosaccharides, such as raffinose and stachyose, which cause flatulence, can be leached out or converted to monosaccharides upon cooking (Hymowitz, 1976; Ku et al., 1976; Steggerda et al., 1970).

A nutritionally important aspect to any food is its edibility. Soaking beans such as soybeans (Wang et al., 1979; Perry et al., 1976) prior to cooking has been reported to improve both color and texture of cooked beans. One-hundred percent hydration of soybean, equal to soaking 5.5 hr at 20°C, gave better results than unsoaked beans, while complete hydration (140% hydration) did not give further improvement in cooking quality of the beans. Wang et al. (1979) reported the texture of cooked soybean which had been soaked prior to cooking to be more tender beans at the same cooking time than the soybean without soaking.

The purpose of the research reported in this article was to study the effect of four selected heat treatments on vitamin B-6 and folacin retention in soybeans. Additionally, the effect of the heat treatments on soybean trypsin inhibitor activity, texture, color, and microstructure were studied. The possible relationship and/or interaction of vitamin B-6 or folacin retention and color or texture or microstructure changes were evaluated.

Authors Soetrisno, Holmes, and Miller are with the Dept. of Foods & Nutrition, Oregon State Univ. Corvallis, OR 97331.

EXPERIMENTAL

PACIFIC COAST SOYBEANS (Walla Walla, WA 99362) used in this experiment were selected for soundness and wholeness (Wang et al., 1979). One-hundred grams of selected soybeans were weighed and placed in each of 32 plastic bags and sealed. These were randomly assigned to the four treatments with eight replications for each treatment.

Soybeans were boiled for 20 min (B-20) or autoclaved (Precision Surgical Supply Sterilizer; American Sterilizer Company, Erie) for 5 (A-5), 10 (A-10), or 15 (A-15) min. For each treatment, the 100-g portion of soybeans was soaked in 500 mL redistilled water (25°C) in a Precision Scientific (Freas 815; Precision Scientific, Subsidary of GCA Corp.) incubator for 10 hr. The soaked beans were drained for 10 min, then gently blotted with Kim Wipes (Kimberly-Clark, Stock Number 34155), and the beans were weighed. The blotted, soaked soybeans were cooked according to treatment in a 1L glass beaker in 500 mL redistilled water.

The boiling method was done using a preheated (30 min) hot plate (Pyro Magnetic King Size, stirring speed, off, heating set, maximum). The 20 min boiling time was counted after boiling was initiated. Cooking in the autoclave was done at 102 kPa/117°C for 5, 10, or 15 min.

Cooked soybeans were cooled (30–36°C) for 20 min, drained, and blotted dry as for soaked beans. After weighing for water absorption determination, and removal of samples for texture and color evaluation, the cooked soybeans were frozen using liquid nitrogen (−196°C) prior to grinding the vitamin assay, trypsin inhibitor assay (TIA), and moisture determination. These frozen bean samples were ground to a fine powder using an Osterizer-duel (range 10 at grind speed) blender.

Vitamin B-6 determination

The total vitamin B-6 was determined by the AOAC method (Horowitz, 1970) without chromatography strip after hydrolyzing 2g of samples with 0.44N HCl. *Saccharomyces carlsbergensis ATCC-9080* was used as test organism, and the "Difco" Casamino Acids (Difco #0288-02, Difco Laboratories, Detroit, MI) substituted for acid-hydrolyzed casein for making the basal medium. Vitamin B-6 values of the samples were calculated from a pyridoxine standard curve using a Hewlett-Packard Calculator, Model 41-c, (Hewlett-Packard Calculator, Model 41-C, Loveland, CO 80537), and the power curve fit program.

Folacin determination

Folacin activity was assayed microbiologically with Lactobacillus casei ATCC-7469. Free folacin was measured in samples

without conjugase treatment; total folacin was assayed in samples treated with "Difco" chicken pancrease (Difco #0459-12, Difco Laboratories, Detroit, MI) (Hurdle et al., 1968). The chicken pancrease solution was added to the sample supernatant as suggested by Butterfield and Calloway (1972), which then incubated for 6 hr at 37°C. Ascorbic acid was added to the buffer used for extracting the vitamin from food and for the assay growth medium at the concentration of 150 mg/100 mL buffer (Herbert and Bertino, 1967; Hurdle et al., 1968).

The folacin value of the samples was calculated from a pteroylglutamic acid (Biochem. 101725) standard curve and a Hewlett-Packard Calculator, Model 41-c, with a power curve fit program.

Trypsin inhibitor activity (TIA)

TIA was determined using AACC method number 71-10 (Kakade et al., 1974). Trypsin and the substrate benzoyl-DL-arginine-p-nitroanalide (Sigma-B4875, Sigma Chemical Company, St. Louis, MO) was used. The results are presented as trypsin inhibitor units (TIU) per gram of sample.

Texture measurement

The Instron Universal Testing Machine (Model 1132, Instron Corporation, Canton, MA) and Kramer cell attachment were used (crosshead speed, 12 in/min; chart speed, 20 in/min) to measure the force required to shear triplicate samples of 25g each of cooked soybeans. The shear force was determined as pounds per 25g.

Color measurement

Color of cooked soybeans was determined as percent reflectance by using a Photovolt Reflection Meter (Photovolt, New York, NY 10010) with an enamel plaque (blue, 51.5; green, 50.0; amber, 48.5) standard. Triplicates for each treatment replication of 50g of cooked soybeans in an identical 100 mL Pyrex glass beaker were used with the search unit in the upward position.

Water absorption

Measurement of water absorption was determined as the percentage increase in weight of the soybean sample from the initial weight. Absorption was determined for percentage increase after soaking and after cooking.

Moisture determination

Moisture content of 5g ground cooked soybeans was determined using a vacuum oven (National Appliance Co., Model 58301, Portland, OR) (20 ± 22 psi) at 60°C for 8 hr. The loss of weight was reported as percent moisture (Eq. 1). Determinations were done in triplicate and an average reported.

$$\%\ \text{Moisture of soybean} = \frac{\text{Initial wt} - \text{Final wt}}{\text{Initial wt}} \times 100$$

Scanning Electron Microscopy (SEM)

Crosswise and lengthwise 1.5 mm thick cuts of soaked or cooked soybean samples and fragments of raw soybeans were used for SEM evaluation. Scanning electron micrographs were obtained using an AMRAY 1000A scanning electron microscope with the procedure reported by Holmes and Soeldner (1981). Magnifications of 150x and 1000x were recorded.

Experimental design and statistical analysis

An incomplete block design for the eight replications of the four treatments was used (Federer, 1955). The total blocks were 16 with two treatments each laboratory period. Data were statistically analyzed by standard deviation and least square analysis of variance and covariance and t-test (Niess, 1980).

RESULTS & DISCUSSION

THE HEATING TREATMENTS in this experiment tended to affect vitamin B-6 and folacin retention, trypsin inhibitor activity, color values, texture, percent water absorption, and moisture content. These data are reported in Table 1 and Table 2.

Vitamin B-6 retention

The values of vitamin B-6 in the beans, in the cooking water or in the soaking water are shown in Table 1. These values of vitamin B-6 in the cooked soybeans are lower than those reported by other researchers (Miller and Khabirmaidensyah, 1981). All heat treatments in the current experiment reduced the vitamin B-6 content calculated on a dry weight basis approximately 40% from that in the raw bean. This is to be expected as other workers have indicated that vitamin B-6 is destroyed by heat (Perera et al., 1979; Storvick et al., 1964). Miller and Khabirmaidensyad (1981) autoclaved soaked beans in the soaking water for 20 min and reported 2.7 ng/g vitamin B-6 in wet weight. The differences from the current experimental result could be due to the differences in cooking water concentration or to differences in moisture content. *—Continued on next page*

Table 1—Vitamin B-6 (μg/g), total folacin (μg/g), free folacin (μg/g), and trypsin inhibitor activity (TIA) cooked soybean, cooking water, and soaking water

Parameter	Treatment[a,b] B-20	A-5	A-10	A-20
Vitamin B-6[c]				
Soybean	1.31±0.11 (4.02±0.39)	1.48±0.18 (4.24±0.54)	1.37±0.17 (4.13±0.52)	1.27±0.40 (4.11±1.43)
Cooking water	0.93±0.26	0.50±0.10	0.59±0.15	0.69±0.20
Soaking water	0.10±0.00	0.10±0.00	0.10±0.01	0.10±0.01
Total folacin[c]				
Soybean	0.50±0.01 (1.54±0.28)	0.60±0.17 (1.73±0.50)	0.57±0.08 (1.72±0.26)	0.48±0.06 (1.53±0.20)
Cooking water	0.35±0.11	0.27±0.12	0.23±0.04	0.35±0.10
Soaking water	0.18±0.05	0.19±0.05	0.20±0.05	0.20±0.03
Free folacin[c]				
Soybean	0.40±0.08 (1.22±0.24)	0.53±0.17 (1.51±0.48)	0.45±0.08 (1.36±0.24)	0.37±0.14 (1.20±0.44)
Cooking water[d]	0.19±0.04	0.11±0.02	0.12±0.03	0.17±0.06
Soaking water	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.02
Trypsin inhibitor Activity (TIA/g)[c,d]	828.0±99.2*	1168.5±85.6#	766.5±87.8*	602.5±57.0†

[a] B-20 represents 20 min boiling. A-5, A-10, A-20 represents an autoclave time of 5, 10, or 20 min, respectively.
[b] Value on wet weight basis with soybean values in parentheses being on a dry weight basis.
[c] Raw soybean value for vitamin B-6, total folacin, free folacin, and trypsin inhibitor activity was 6.27±0.28, 1.68±0.0, 1.27±0.22 μg/g and 61,349.7 TIU, respectively.
[d] Symbols not in common denote statistical significant ($P \leq 0.05$).

There are no significant differences (Table 1) among the means of the bean vitamin B-6 of each heating treatment. Heat treatment did significantly influence the vitamin B-6 content in their respective cooking water. Vitamin B-6 content in A-20 or B-20 cooking water is significantly higher than all other treatments, the vitamin B-6 content in the B-20 cooking water is higher than the cooking water from the autoclaved heat treated beans (A-5, A-10, A-20). The vitamin B-6 content in the soaking water, as expected, did not differ significantly.

Actual destruction of vitamin B-6 by heat, has been evaluated by totaling the content in soak water, cook water and the bean and then comparing the totals to the value in the raw bean. Miller et al. (1973) reported that during the processing and canning of pinto and lima beans, loss of vitamin B-6 was mostly to the cooking water. In the current experiment, based on dry weight comparisons, the total recovery of vitamin B-6 from beans, cooking water and soaking water for all treatments were 71.6%, 68.8%, 68.6%, and 69.5% for B-20, A-5, A-10, and A-20, respectively.

Folacin retention

In the current experiment, the total and free folacin values (Table 1) in the beans and soaking water did not differ significantly. However, as with vitamin B-6 values, as the samples were autoclaved for greater lengths of time, the total and free folacin content decreased in the bean samples. DeRitter (1976) has indicated that total folacin decreases during cooking. In preparing soybeans, there were some losses of folacin into soaking and cooking water, since folacin is water soluble. Actually, heat treatment may be advantageous in folacin determination. Holmes et al. (1979) obtained data from blanching of green beans that suggests that the heating in water may increase the availability of total folacin to *L. casei*, the assay organism. These results are supported by work reported by Leichter et al. (1978) who noted similar trends in their experiment on spinach. The recovery in the current experiment is greater than 100% total folacin.

Although total folacin values did not differ significantly (Table 1) in the cooking water, free folacin did show some differences. B-20 had a significantly ($P \leqq 0.05$) higher free folacin value than A-5 but was not significantly different with A-10 or A-20. The reason for the difference in significance with treatment of total folacin versus free folacin could possibly be attributed to the ease of leaching. Free folacin is more easily leached out than the conjugated form (Herbert and Bertino, 1967). However, values obtained in the current experiment were similar to those reported by Perloff and Butrum (1977). They reported 171 μg of folacin per 100g edible portion of raw soybean.

Trypsin inhibitor activity (TIA)

Collins and Beaty (1980) reported that boiling whole soybean for 9 min inactivated 96.1% of trypsin inhibitor activity. There was evidence that trypsin inhibitor activity decreased drastically in the first 3 min. In the current experiment, over 98% (Table 1) of the trypsin inhibitor activity (TIA), expressed as trypsin inhibitor units (TIU), was inactivated. As Albrecht et al. (1966) observed, the boiling method can be as effective as pressure cooking in destroying the trypsin inhibitor. B-20 and A-10 were significantly ($P \leqq 0.05$) different from the values for A-5 or A-20. A-5 was significantly ($P \leqq 0.05$) higher in activity than B-20, A-10 or A-20. While B-20 and A-10 are not significantly different they were higher ($P \leqq 0.05$) than A-20. As have been reported in many experiments, the trypsin inhibitor activity is decreased by heating. For example, Albrecht et al. (1966) using soybeans (20% moisture), reported that 99% of its trypsin inhibitor activity was destroyed when steamed at 100°C for 15 min. Inactivation was accelerated to 5–7 min when the soaked whole beans (62–65% moisutre) were immersed and boiled in water.

Texture measurement

There were no significant ($P \leqq 0.05$) differences in the texture (Table 2), as measured by shear pressure, of the soybeans from the four heat treatments. In comparing the pressure cooked beans, the greatest decrease of approximately 28% occurred during the 5–10 min cooking period; however, a 7% further decrease in pressure occurred from A-10 to A-20. These results concur with those reported by Sefa-Dedeh et al. (1978) who, using an Instron Testing Machine (Model TM-M; texture test attachment of the Ottawa Texture Measuring System), observed that the longer the cooking time of a soaked bean the more tender the bean became. The longer cooking time of soaked beans permitted more water absorption into the beans. Additionally, this tenderization of the soybean during cooking may be due to the breakdown of middle lamella (Sefa-Dedeh et al., 1978; Rockland and Jones, 1974) or the softening of the protein matrix (Sefa-Dedeh et al., 1979) in the cotyledon. It also might be due to the bursting of the hulls after full absorption of water.

Color measurement

In the current experiment, blue (B), green (G) and amber (A) percent reflectance values were obtained using the Photovolt Reflectance Meter. The blue values (Table 2) appear to be the color value of greatest discrimination. The mean blue value of B-20 is significantly ($P \leqq 0.05$) higher than that of A-5. However, the mean blue value for B-20 was not significantly different from those of A-10 or A-20. The green and amber values of B-20 were also significantly ($P \leqq 0.05$) higher than any of the pressure heat treated samples. The higher blue, green and amber values indicated a whiter, lighter brown soybean with the boiling heat treatment. Possibly, this type of heating caused less occur-

Table 2—Texture, color value, moisture content, and percent of water absorption after soaking and after cooking

Treatment[a]	Texture (lb/25 g)	Color[d]			Water absorption[b]		
		Blue[b,e]	Green[b,e]	Amber[b,e]	Moisture (%)	After soaking (%)	After cooking (%)
B-20	121.3±4.2	15.8±0.4*	31.8±0.7*	35.3±1.0*	67.3±0.95*	126.8±0.95	143.1±2.39*#
A-5	160.3±18.4[c]	15.0±1.0#†	31.1±1.4	34.6±1.4	65.1±0.85#	126.8±1.02	141.4±2.11*
A-10	115.4±40.8	15.4±0.7*†	30.6±1.0	34.1±1.0	66.8±0.78*	127.4±1.62	146.5±2.87#
A-20	107.4±42.5	14.9±0.7*†	29.4±1.6	32.9±1.6	68.9±1.07†	127.4±1.18	154.0±2.88†

[a] B-20 represents boiling 20 min. A-5, A-10, or A-20 represent autoclaving for 5, 10, or 20 min, respectively.
[b] Symbols not in common denote statistical significance ($P \leqq 0.05$).
[c] Average value of six replications instead of eight replications.
[d] Average value of percent reflectance.
[e] Blue, green, and amber raw soaked soybean values were 13.6±0.8, 26.2±1.0, and 30.0±0.5, respectively.

rence of the browning reaction due to lower temperature (100°C) employed as compared to that in the autoclave (117°C).

Water absorption and moisture content

The relationship of moisture content and/or water absorption has been related to this legume's quality (Wang et al., 1979; Sefa-Dedeh et al., 1978, 1979). In the current experiment (Table 2), the beans' moisture content was significantly ($P \leq 0.05$) affected by the heat treatment. The values are within the range of those reported in other experiments (Albrecht et al., 1966; USDA, 1975). As noted in Table 2, the moisture content of the pressure cooked samples differed significantly from each other.

Water absorption (Table 2) was evaluated at the end of the 10 hr (25°C) soaking period and upon completion of cooking. Beans at the completion of the soak period increased an average of 127%, with no difference ($P \leq 0.05$) due to block. The type of heat treatment significantly influenced percent water absorption. The value for A-20 is significantly higher. A-5 was the lowest value but B-20 was not significantly different from A-5 or A-10. The higher value of A-20 in the pressure heat treatment comparisons is likely due to the longer cooking time, which gave more time for water absorption (Junek et al., 1980).

Microstructure appearance

A number of scanning electron micrographs (SEM) were evaluated for each treatment. Representative photographs are in Fig. 1. The SEM of the raw cotyledon revealed the cell structure because of their slight rigidity. As noted by the insert (Fig. 1D), the cell contains round bodies. These may be either protein bodies enmeshed in a protein network or lipid. The SEM's appear similar to those of

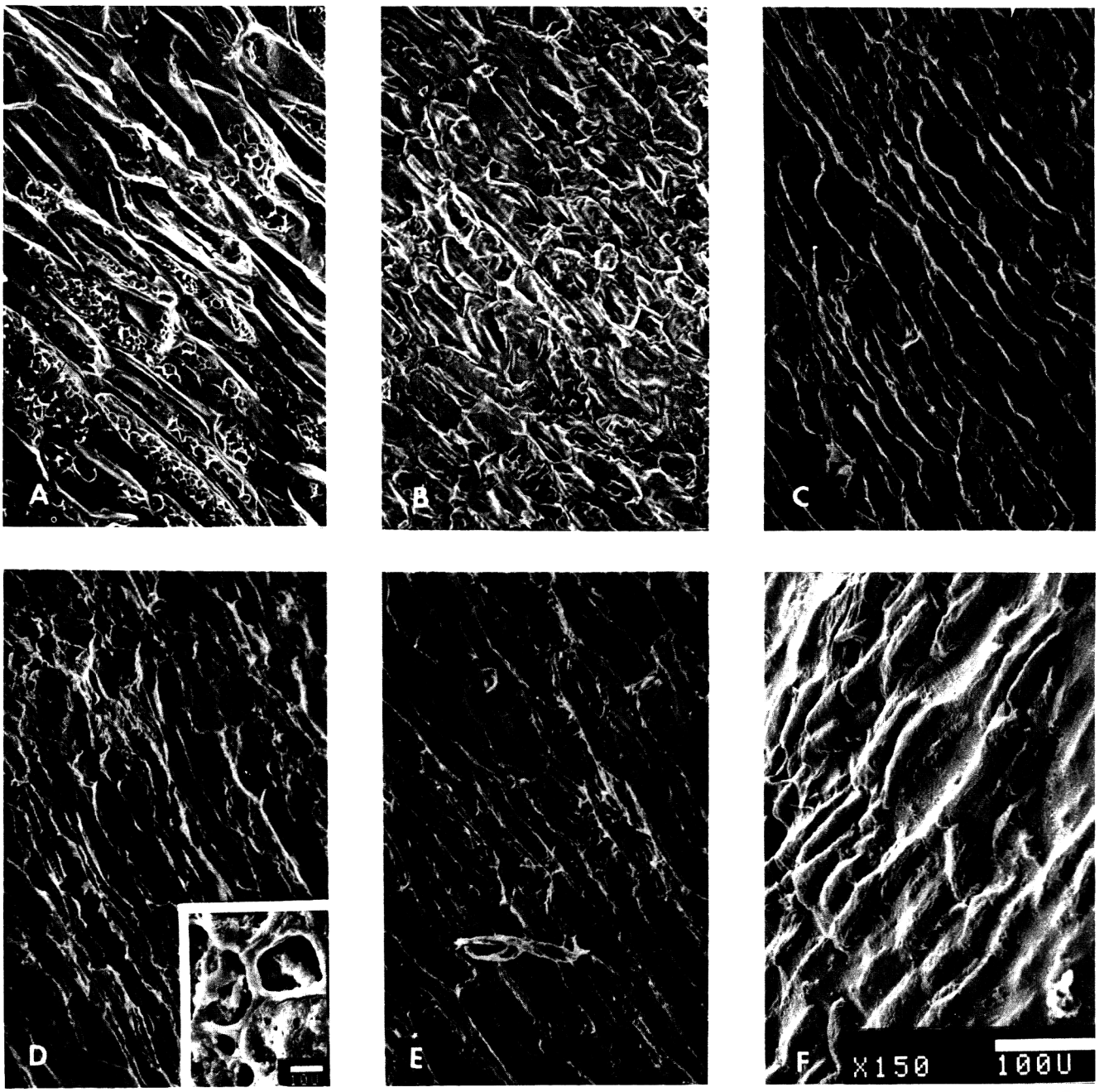


Fig. 1—Scanning electron micrographs (150X) of soybeans: (A) raw; (B) soaked; (C) boiled 20 min: (D) autoclaved 5 min (insert, 1000X); (E) autoclaved 10 min; (F) autoclaved 20 min.

soybeans described by Wolf and Baker (1972; 1980). A comparison of treatment in the current experiment would indicate that soaking and increasing the cooking stress decreased the definition of the soybean cell structure. The insert (Fig. 1D) in the SEM indicates the protein network ridges surrounding the protein bodies are apparent. Wolf and Baker (1972) indicated that these ridges likely enclosed oil-containing spherosomes. The meshlike network on the protein bodies is also present in the current experiment.

INTERRELATIONSHIPS OF OBJECTIVE TESTS

MANY STUDIES have related the influence of nutrient retention and the treatment variable or stress. For example, the effect of heat upon vitamin retention is well documented (DeRitter, 1976; Hein and Hutchings, 1974). However, a minor portion of the investigations have evaluated the extent of the relationships or influence of secondary factors to vitamin retention. Using a covariance type of statistical analysis, an attempt was made to evaluate the interactive relationships of texture or color changes, trypsin inhibitor inactivation or moisture or water absorption changes to vitamin B-6 or folacin retention.

Texture had a significant negative ($P \leqq 0.05$) relationship to the total folacin content. Although not significant, texture values and free folacin or vitamin B-6 were positively related. A possible critical factor in this relationship was the correlation ($P \leqq 0.05$) between texture and water absorption. Texture values, indicating decreased firmness, decreased as water absorption and moisture content increased. Total folacin content was significantly ($P \leqq 0.05$) increased with increasing water absorption. Both free folacin and vitamin B-6 tended to decrease as the percent water absorption and moisture content increased. These relationships may occur due to the disruption of the soybean cell integrity. As the cell's integrity decreases it is likely more easily sheared indicating a more tender product. Additionally, with the possible loss of integrity, the cell exudes more of the free folacin and vitamin B-6 or/and makes it more susceptible to heat destruction and/or to more easy analysis.

Folacin or vitamin B-6 retention and color appeared to show a relationship. With the exception of the green values to total folacin content, all color values (blue, green, amber) had a positive relationship to total and free folacin. As the color values increased, the free and total folacin values also increased. This generally positive relation between color values and folacin could possibly be attributed to the relationship of color to water absorption and water content. The negative relationship between color values and vitamin B-6 retention also appeared, however, there is no statistical significance. Compared to that of green or amber values, the blue value has less effect on the retention of vitamin B-6. The blue values had a significant ($P \leqq 0.05$) relationship with percent water absorption. Although not significant, the other two color values, green and amber, also had a positive relationship. Blue values generally are considered to be analogous to hue in our perception. Thus, the significance of blue values to water absorption is expected.

Free folacin has a significant ($P \leqq 0.05$) negative relationship with trypsin inhibitor activity. Evidently, the more moisture in the beans, the more media available for increasing the effectiveness to inactivate the trypsin inhibitor. There also is a negative relationship between TIA with free and total folacin content; which in this case free folacin has a significant ($P \leqq 0.05$) negative relationship to TIA. Vitamin B-6 tended to be positively but not significantly ($P \leqq 0.05$) decreased as the TIA increased.

REFERENCES

Albrecht, W.J., Mustakas, G.C., and McGee, J.E. 1966. Rate studies atmospheric steaming and immersion cooking of soybeans. Cereal Chemistry 43: 400.

Bailey, L.H., Capen, R.G., and LeClerc, J.A. 1935. The composition and characteristics of soybeans, soybean flour, and soybean bread. Cereal Chemistry 12(5): 441.

Butterfield, S. and Calloway, D.H. 1972. Folacin in wheat and selected foods. JADA 60: 310.

Collins, J.L. and Beaty, B.F. 1980. Heat inactivation of trypsin inhibitor in fresh green soybeans and physiological responses of rats fed the beans. J. Food Sci. 45: 542.

De Ritter, E. 1976. Stability characteristics of vitamins in processed foods. Food Technol. 30: 48.

Federer, W.T. 1955. "Experimental Design," First ed., p. 415. MacMillan Company, New York, NY.

Harris, R.S. and Karmas, E. 1975. "Nutritional Evaluation of Food Processing." Avi Publishing Company, Inc., Westport, CT.

Hein, R.E. and Hutchings I.J. 1974. Influence of processing on vitamin-mineral content and bioavailability in processed foods. In "Nutrients in Processed Foods," p. 59. American Medical Association, Publishing Sciences Group, Inc., Boston, MA.

Herbert, V. and Bertino, J.R. 1967. Folic acids. in "The Vitamin Chemistry, Physiology, Pathology, Methods," Vol. 7, Second ed., p. 243. Ed. P. Gyorgy and W.N. Pearson. Academic Press, New York, NY.

Holmes, Z.A., Miller, L., Edwards, M., and Benson, E. 1979. Vitamin retention during home drying of vegetables and fruits. Home Economics Res. J. 7: 258.

Holmes, Z.A. and Soeldner, A. 1981. Macrostructure of selected raw starches and selected heated starch dispersions. JADA 78: 153.

Horowitz, W. 1970. "Official Methods of Analysis." Association of Official Analytical Chemists, P.O. Box 540, Washington, DC 20044.

Hurdle, A.D.P., Barton, D., and Searles, I.H. 1968. A method for measuring folate in food and its application to a hospital diet. Am. J. Clin. Nutr. 21(10): 1202.

Hymowitz, T. 1976. Extraction of oligosaccharides during cooking of whole soybeans. J. Food Sci. 41: 361.

Junek, J.J., Sistrunk, W.A., and Neely, M.B. 1980. Influence of processing methodology on quality attributes of canned dry bean. J. Food Sci. 45: 821.

Kakade, M.L. Rackis, J.J., McGhee, J.E., and Puski, G. 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51: 376.

Khabirmaidensyah, H. 1981. Personal communication, Unpublished data. Oregon State Univ., Corvallis, OR 97331.

Ku, S., Wei, L.S., Steinberg, M.P., Nelson, A.I., and Hymowitz, T. 1976. Extraction of oligosaccharides during cooking of whole soybeans. J. Food Sci. 41: 361.

Leichter, J., Switzer, V.P., and Landymore, A.F. 1978. Effect of cooking on folate content of vegetables. Nutr. Report Intl. 18(4): 475.

McArthur, W.C. 1980 Oct. Soybean Production Practices and Costs in the United States. Research Report 360. Agricultural Experiment Station, Univ. of Georgia, Athens, GA 30602.

Miller, C.F., Guadagni, D.G., and Kon, S. 1973. Vitamin retention in bean products: Cooked, canned and instant bean powders. J. Food Sci. 42: 25.

Miller, L.T. and Khabirmaidensyah, H. 1981. Personal Communication, Unpublished data. Oregon State Univ. Corvallis, OR 97331.

National Research Council, Committee on Food Protection. 1973. Toxicants Occurring Naturally in Foods, p. 109. National Academy of Sciences, Washington, DC.

Nelson, A.I., Steinberg, M.P., and Wei, L.S. 1978. Whole soybean foods for home and village use. In Soy Series No. 14., International Agricultural Publications, Urbana, IL 61801.

Niess, D.G. 1980. Personal communication (Dec. 16). Computer Center, Oregon State Univ., Corvallies, OR 97331.

Perera, A.D., Leklem, J.E., and Miller, L.T. 1979. Stability of vitamin B-6 during bread making and storage of bread and flour. Cereal Chem. 56: 577.

Perloff, B.P. and Butrum, R.R. 1977. Folacin in selected foods. JADA. 70: 161.

Perry, A.K., Peters, C.R., and Van Duyne, F.O. 1976. Effect of variety and cooking method on cooking times, thiamin content and palatability of soybeans. J. Food Sci. 41: 1330.

Rackis, J.J. 1981 Protease inhibitors: Physiological Properties and Nutritional Significance. In "Antinutrients and Natural Toxicants in Foods, " Ed. R.L. Ory, p. 203. Food & Nutrition Press, Inc., Westport, CT.

Rockland, L.B. and Jones, F.T. 1974. Scanning electron microscope studies on dry beans. Effect of cooking on the cellular structure of cotyledons in rehydrated large lima beans. J. Food Sci. 39: 342.

Rockland, L.B., Jones, F.T., and Hahn, D.M. 1977. Light and scanning microscope studies on dry beans: Extracellular gelatinization of lima bean starch in water and a mixed salt solution. J. Food Sci. 42: 1204.

Sabin, D.R. 1961. Implementation of the WHO/FAO/UNICEF Protein Rich Foods Program. In "Conference on Soybean Products for Protein in Human Foods," Peoria, IL.

Sefa-Dedeh, S., Stanley, D.W., and Voisey, P.W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (Vigna unquilata). J. Food Sci. 43: 1832.

Sefa-Dedeh, S., Stanley, D.W., and Voisey, P.W. 1979. Effect of storage time and conditions on the hard-to-cook defect in cowpeas (Vigna unguicultata). J. Food Sci. 44: 790.

Steggerda, F.R., Shimizu, T., Anderson, J., and Pearl, S.L. 1970. Soybean factors relating to gas production by intestinal bacteria. J. Food Sci. 35: 634.

Storvick, C.A., Benson, E.M., Edwards, M.A., and Woodring, M.J.

—Continued on page 537

Phytic Acid Hydrolysis and Soluble Zinc and Iron in Whole Wheat Bread As Affected by Calcium Containing Additives

MICHAEL B. ZEMEL and LEORA A. SHELEF

ABSTRACT

The effects of nonfat dry milk, $CaCO_3$, $CaCl_2$ and $MgCl_2$ on phytate hydrolysis and on Zn and Fe availability was evaluated in whole wheat bread and in a model fermentation system. $CaCO_3$ and $CaCl_2$ both depressed phytate hydrolysis to an equivalent degree, but the milk had a greater effect than could be accounted for by its Ca content. $MgCl_2$ had a less pronounced effect than the Ca salts in both the bread and the model system. Increasing fermentation time in the model system increased phytate hydrolysis, but the effect was delayed when milk or $CaCl_2$ was added. Supplementing the bread dough with Ca equivalent to that typically contributed by Ca-containing additives caused 50% reductions in the available quantities of soluble ("free") zinc and iron; the decreases observed with varying added amounts of Ca or milk exhibited a high degree of correlation with the observed increases in residual phytate P.

INTRODUCTION

PHYTATES have long been known to have potentially adverse effects on the bioavailability of multivalent cations, especially Zn^{++}, Ca^{++}, Fe^{++} and Fe^{+++}, due to the formation of insoluble complexes in the gastrointestinal tract (Harrison and Mellanby, 1939; Sharpe et al., 1950; O'Dell and Savage, 1960; Oberleas et al., 1966; Reinhold et al., 1973). Formation of these phytate complexes is believed to be the cause of zinc and iron deficiency in some populations which subsist on unleavened whole grain bread, and rely on it as a primary source of these two minerals (Prasad et al., 1961, 1963; Reinhold, 1971, 1972; Haghshenass et al., 1972; Reinhold et al., 1973). In yeast leavened breads, the formation of these cation-phytate complexes is minimized due to phytate hydrolysis catalyzed by a yeast phytase during the fermentation process (Reinhold, 1975). However, calcium is frequently added to bread, usually in the form of nonfat dry milk, calcium propionate and/or calcium phosphate; these additives typically increase the calcium content of whole wheat bread by 50–100 mg/100g bread, or up to 50%. Calcium and zinc exhibit a synergism with respect to phytate precipitation, and in vitro studies demonstrate that calcium causes increased precipitation of an insoluble zinc-phytate complex at intestinal pH's (Oberleas et al., 1966; Oberleas, 1973). Furthermore, the addition of calcium to diets high in phytic acid causes a decrease in phytic acid hydrolysis and reduces zinc absorption in animals (Oberleas et al., 1966; Oberleas, 1973; Nahapetian and Young, 1980). On the other hand, a more recent study indicates that increasing the level of calcium intake of humans by the use of dairy products increases the absorption of zinc from whole wheat bread (Sandstrom et al., 1980).

The objectives of the present study were to determine whether the additon of calcium or milk to a whole wheat yeast bread dough will result in the formation of a stable calcium-phytate or calcium-zinc-phytate complex resistant to hydrolysis by yeast.

Authors Zemel and Shelef are with the Food Science & Human Nutrition Div., Dept. of Family and Consumer Resources, Wayne State Univ., Detroit, MI 48202.

EXPERIMENTAL

Studies in bread

Loaves of bread were prepared using the following basic ingredients: 100g whole wheat flour, 70g glass distilled water, 15g margarine, 5g sugar, 2g NaCl and 2g yeast. The loaves contained either (a) no additives; (b) 6.6 or 13.2 dried skim milk which provided 86 or 172 mg calcium, respectively; (c) 22, 43, 86 or 172 mg calcium from $CaCl_2$; (d) 22, 43, 86 or 172 mg calcium from $CaCO_3$; or (e) 86 or 172 mg magnesium from $MgCl_2$. After fermentation (1 hr), shaping, and baking (170°C) to uniform weight, the loaves were oven dried at 100°C to constant weight and homogenized in a blender. Aliquots were analyzed for phytate phosphorus, total zinc, total iron, soluble zinc and soluble iron.

Studies in a model system

The model fermentation system consisted of 2g whole wheat flour and 5 ml of a suspension containing 2g yeast, 1g NaCl and 3g sucrose in 100 ml distilled water. The protocol of the model system paralleled that used in the bread with respect to additions of dried skim milk, calcium and magnesium; in addition, the effect of fermentation time was evaluated in systems containing no additives, dried skim milk or calcium salts. Phytate phosphorus was was analyzed directly on the entire mixture following interruption of fermentation.

Phytate phosphorus determination

Phytate phosphorus was determined using a modification of the method described by Oberleas (1971) and modified by Nahapetian and Bassiri (1975). Phytate was extracted with 1.2% HCl/10% Na_2SO_4 and then precipitated in a boiling water bath with 0.4% $FeCl_3 \cdot 6H_2O$ in 0.07N HCl. The precipitated ferric-phytate salt was isolated via centrifugation, washed with 4% Na_2SO_4 in 0.07N HCl and re-centrifuged. The precipitate was dissolved in 4–5 ml nitric acid and boiled for approximately 2 hr in a ceramic crucible. The sample was then ashed overnight (ca 14 hr) at 500°C in a muffle furnace. After cooling, the ash was dissolved in concentrated hydrochloric acid, diluted to an appropriate volume and analyzed for phosphorus using the colorimetric technique of Fiske and Subbarow (1925). The recovery of phytate phosphorus added to samples treated in this manner was 94 ± 6% (mean ± sd).

Total zinc and iron determination

Samples for the analysis of total zinc and iron were ashed as described above, and analyzed by atomic absorption spectrophotometry using a Varian AA-6 atomic absorption spectrophotometer (Varian Associates, Inc., Park Ridge, IL.); all samples contained lanthanum chloride at a final concentration of 1% (w/v) to avoid interference from phosphorus.

Soluble zinc and iron determination

"Free" zinc and iron in the dried bread samples were estimated as soluble zinc and iron using a method similar to that of Lee and Clydesdale (1979) for soluble iron. Samples (10–20g) and distilled water (30–40g) were placed into 250 ml Erlenmeyer flasks which were agitated for 3 hr on a shaker. The samples were then allowed to settle for 30 min and centrifuged at 1000 × *g* for 30 min. The resulting supernatants were filtered through Whatman #1 paper and analyzed for zinc and iron using atomic absorption spectrophotometry.

Statistical treatment of data

All data were analyzed using analysis of variance and the least

significant difference (LSD) test to separate means at the 1% alpha level.

RESULTS & DISCUSSION

Phytate hydrolysis

The effects of the various supplements on phytate hydrolysis as indicated by residual phytate P are shown in Tables 1–3. The addition of nonfat dry milk to either the bread dough (Table 1) or model fermentation system (Table 2) resulted in a decrease in phytate hydrolysis as indicated by a significant ($p < 0.01$) elevation in residual phytate phosphorus. This decrease in phytate hydrolysis could not be attributed entirely to the effect of calcium in the milk, since adding 86 mg Ca in the form of either $CaCO_3$ or $CaCl_2$ caused a 24% increase, while adding sufficient nonfat dry milk to contribute an equivalent amount of Ca caused a significantly ($p < 0.01$) greater increase in residual phytate phosphorus. Furthermore, the effect of calcium on phytate hydrolysis appeared to be independent of pH, since supplementation by either $CaCO_3$ or $CaCl_2$ produced identical effects (Table 1). When the level of calcium supplementation was increased two-fold to 172 mg/loaf, there was a further increase in residual phytate phosphorus, with the greatest effect again being from the nonfat dry milk.

Although magnesium supplements also depressed phytate hydrolysis in both the model system (Table 2) and in the bread dough (Table 3), the effect was significantly ($p < 0.01$) less pronounced than that of calcium.

Table 1—Effect of calcium supplementation on phytate P in whole wheat bread[a,b]

Supplemental Ca/loaf (mg)	Source	Phytate P/loaf (mg)	% Increase
0 (control)	–	218a	–
22	$CaCl_2$	240b	10a
44	$CaCl_2$	247c	13b
86	$CaCl_2$	270d	24c
86	$CaCO_3$	270d	24c
86	dried skim milk	277e	27d
172	$CaCl_2$	297f	36e
172	$CaCO_3$	294f	35e
172	dried skim milk	306g	40f

[a] Nonmatching letters in each column denote significant differences ($p < 0.01$).
[b] Triplicate analyses performed on each of three loaves at each level of calcium or milk supplementation.

Table 2—Effect of supplements on residual phytate P in whole wheat flour-yeast suspension after 1 hr of fermentation at 30° C[a,b]

Dry milk powder (g/100g flour)	Increase in residual phytate P (%)
0	9a
8	22b
23	31c
46	46d
$CaCl_2$ (g/100g flour)	
0.3	23b
0.6	39e
0.9	59f
$MgCl_2$ (g/100g flour)	
0.3	18g
0.6	31c
0.9	36h

[a] Nonmatching letters denote significant differences ($p < 0.01$).
[b] Triplicate analyses performed on each of three suspensions for each level of supplementation.

Fermentation time

As expected, decreasing the fermentation time significantly depressed phytate hydrolysis, while extending fermentation time to 3 hr resulted in a substantial increase in hydrolysis (Table 4). Similar trends were found when fermentation time was extended in samples containing either $CaCl_2$ or dried-milk powder, although hydrolysis was delayed. For example, after 90 min of fermentation, only 43% of the original phytate phosphorus remained in the control system, as compared to 57 and 78% in the systems with the $CaCl_2$ and nonfat dry milk powder, respectively. Reducing phytate phosphorus to approximately 59% of that originally present required 60 min of fermentation in the control system, 90 min in the system with added $CaCl_2$ and 120 min in the system with added dried-milk powder.

Soluble zinc

Supplementing the bread dough with calcium resulted in a significant ($p < 0.01$) decrease in soluble zinc (Table 5). When no calcium was added to the bread dough, the loaves contained 0.47 mg free Zn, representing 14% of the total Zn present. However, when 86 mg calcium ($CaCl_2$ or $CaCO_3$) were added to the dough, soluble zinc represented only 6.5% of the total. A calcium supplement of 172 mg effected only a slight further decrease in soluble zinc. Adding dried-milk powder also caused a decrease in soluble zinc. However, although the effects of the calcium salts and dried-milk powder on free zinc were identical when expressed as a percentage of total zinc, the dried milk did not depress total free zinc as much as the calcium salts did, since the milk contributed additional soluble zinc to the bread dough.

Soluble iron

As with soluble zinc, supplementing dough ingredients with calcium caused a significant ($p < 0.01$) decrease in

Table 3—Effect of magnesium supplementation on phytate P in whole wheat bread[a,b]

Supplemental mg/loaf (mg)	Phytate P/loaf (mg)	% increase
0 (control)	218a	–
44	240b	10a
86	262c	20b
172	279d	28c

[a] Nonmatching letters in each column denote significant differences ($p < 0.01$).
[b] Triplicate analyses performed on three loaves at each level of magnesium supplementation.

Table 4—Effects of fermentation time on residual phytate phosphorus[a,b]

Fermentation time at 30° C (min)	Residual phytate phosphorus (%)		
	Control[c]	3 mg CaCl/g flour[b]	0.083g dried-milk powder/g flour
0	100a	100a	100a
30	85c	96b	96b
60	59e	81d	86c
90	43f	57e	78d
120	36g		
240	6h		

[a] Nonmatching letters denote significant differences ($p < 0.01$).
[b] Triplicate analyses performed on each of three suspensions at each fermentation time/additive combination.
[c] 2.7 mg phytate P per g flour

Table 5—Effect of calcium supplementation on soluble ("free") Zn in whole wheat bread[a,b]

Supp. Ca (mg/loaf)	Source	Zn (mg/loaf)	Soluble Zn (mg/loaf)	% soluble Zn
0 (control)	–	3.44a	0.47a	14a
22	$CaCO_3$	3.49a	0.40b	11b
44	$CaCl_2$	3.42a	0.32c	9c
86	$CaCl_2$	3.45a	0.23d	7d
86	$CaCO_3$	3.52a	0.22d	6d
86	Dried skim milk	4.35b	0.32c	7d
172	$CaCl_2$	3.50a	0.19e	5e
172	$CaCO_3$	3.48a	0.19e	5e
172	Dried skim milk	5.10c	0.25d	5e

[a] Nonmatching letters denote significant differences ($p < 0.01$).
[b] Triplicate analyses performed on each of three loaves at each level of calcium supplementation.

Table 6—Effect of calcium supplementation on soluble ("free") iron in whole wheat bread[a,b]

Supp. Ca (mg/loaf)	Source	Fe (mg/loaf)	Soluble Fe (mg/loaf)	% soluble Fe
0 (control)	–	2.30a	0.71a	31a
22	$CaCl_2$	2.29a	0.54b	24b
44	$CaCl_2$	2.30a	0.48c	21c
86	$CaCl_2$	2.31a	0.36d	16d
86	$CaCO_3$	2.30a	0.35d	15d
86	Dried skim milk	2.31a	0.40d	17d
172	$CaCl_2$	2.28a	0.32e	14e
172	$CaCO_3$	2.29a	0.31e	14e
172	Dried skim milk	2.31a	0.33e	14e

[a] Nonmatching letters in each column denote significant differences ($p < 0.01$).
[b] Triplicate analyses performed on each of three loaves at each level of calcium supplementation.

soluble iron (Table 6). However, unlike soluble zinc, there was no significant difference between the effects of calcium (as $CaCO_3$ or $CaCl_2$) and dried-milk powder on soluble iron. The addition of 86 mg Ca from any of the three sources caused a 50% decrease in soluble iron, from 0.71 mg/loaf to about 0.37 mg/loaf.

The results of the present study demonstrate that the common practice of incorporating nonfat dry milk into whole wheat bread ingredients results in reduced hydrolysis of phytic acid. This reduction can be largely attributed to the calcium content of the milk, presumably due to the formation of Ca-phytate complexes resistant to attack by yeast phytase. As expected, extending fermentation time caused increased hydrolysis of phytates, but nonfat dry milk depressed phytate hydrolysis even at longer fermentation times.

If one considers the solubility of zinc and iron as predictors of their bioavailability, supplementing whole wheat bread with calcium salts or nonfat dry milk may cause a substantial reduction in the bioavailablity of iron and a modest reduction in that of zinc. The most likely explanation for these reductions is the formation of insoluble complexes of zinc and/or iron with the calcium stabilized phytate (Sharpe et al., 1950; O'Dell and Savage, 1960; Prasad et al., 1963; Reinhold et al., 1973). This concept is further supported by the significant ($p < 0.01$) negative correlations found between the percent increase in residual phytate phosphorus and the fractions of zinc and iron remaining in the soluble form ($r = -0.91$ and -0.92, respectively). It therefore appears that the effects of milk added to bread should be considered when estimating the total available iron and zinc in the diet. Modest improvements in iron and zinc availability from whole wheat bread may be accomplished by replacing calcium with other cations in compounds, such as propionates and phosphates, which are added to bread dough.

REFERENCES

Fiske, C.H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 63: 375.
Haghshenass, M., Mahloudji, M., Reinhold, J.G., and Mohammedi, N. 1972. Iron-deficiency anemia in an Iranian population associated with high intakes of iron. Am. J. Clin. Nutr. 25: 1143.
Harrison, D.C. and Mellanby, E. 1939. Phytic acid and the rickets-producing action of cereals. Biochem. J. 33: 1660.
Lee, K. and Clydesdale, F.M. 1979. Quantitative determination of elemental, ferrous, ferric, soluble, and complexed iron in foods. J. Food Sci. 44: 549.
Nahapetian, A. and Bassiri, A. 1975. Changes in concentration and interrelationships of phytate, phosphorus, calcium and zinc in wheat during maturation. J. Agric. Food Chem. 23: 1179.
Nahapetian, A. and Young, V.R. 1980. Metabolism of ^{14}C-phytate in rats: effect of low and high dietary calcium intakes. J. Nutr. 110: 1458.
Oberleas, D., Muhrer, M.E., and O'Dell, B. 1966. Dietary metal complexing agents and zinc availability in the rat. J. Nutr. 90: 56.
Oberleas, D. 1971. The determination of phytate and inositol phosphates. Methods Biochem. Anal. 20: 87.
Oberleas, D. 1973. Phytates. In "Toxicants Occurring Naturally in Foods," p. 363. National Academy of Sciences, Washington, DC.
O'Dell, B.L. and Savage, J.E. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med. 193: 304.
Prasad, A.S., Halsted, J.A., and Nadimi, M. 1961. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am. J. Med. 31: 532.
Prasad, A.S., Miale, A. Jr., Farid, Z., Sandstead, H.H., and Darby, W.J. 1963. Biochemical studies on dwarfism, hypogonadism and anemia. Arch. Intern. Med. 111: 407.
Reinhold, J.G. 1971. High phytate content of rural Iranian bread: a possible cause of human zinc deficiency. Am. J. Clin. Nutr. 24: 1204.
Reinhold, J.G. 1972. Phytate concentrations of leavened and unleavened Iranian breads. Ecol. Food Nutr. 1: 187.
Reinhold, J.G. 1975. Phytate destruction by yeast fermentation in whole wheat meals. J. Am. Dietetic Assoc. 66: 38.
Reinhold, J.G., Lahimgarzadeh, A., Nasr, K., and Hedayati, H. 1973. Effects of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus, and nitrogen in man. Lancet i: 283.
Sandstrom, B., Arvidsson, B., Cederblad, A., and Bjorn-Rasmussen, E. 1980. Zinc absorption from composite meals. 1. The significance of wheat extraction rate, zinc, calcium and protein content in meals based on bread. Am. J. Clin. Nutr. 33: 739.
Sharpe, L.M., Peacock, W.C., Cooke, R., and Harris, R.S. 1950. The effect of phytate and other food factors on iron absorption. J. Nutr. 41: 433.

Ms received 8/3/81; revised 10/6/81; accepted 10/22/81.

SOYBEAN HEATING . . . From page 534

1964. Chemical and microbiological determination of vitamin B-6. In "Methods of Biochemical Analysis," Vol. 12, p. 183. Palo Alto, CA.
USDA. 1975. "Nutritive Value of American Foods in Common Units," Handbook No. 456, Washington, DC.
Wang, H.L., Swain, E.W., Hesseltine, C.W., and Heath, H.D. 1979. Hydration of whole soybeans affects solids losses and cooking quality. J. Food Sci. 44: 1510.
Willet, J.W. 1976. Research to meet U.S. and world food needs. In "The World Food Situation. Problems and Prospects to 1985," p. 857. Oceana Publications, Incorporated. Dobbs Ferry, NY.
Wolf, W.J. and Baker, F.L. 1972. Scanning electron microscopy of soybeans. Cereal Sci. Today 17: 125.
Wolf, W.J. and Baker, F.L. 1980. Scanning electron microscopy of soybeans and soybean protein products. Scanning Electron Microscopy 3: 621.

Ms received 7/8/81; revised 9/25/81; accepted 9/28/81.

Oregon Agricultural Experiment Station Technical Paper No. 5916.
Presented by U. Soetrison in partial fulfillment for the Master of Science Degree of U.S.

Effect of Four Anticaking Agents on the Bulk Characteristics of Ground Sugar

A. M. HOLLENBACH, M. PELEG and R. RUFNER

ABSTRACT

Fine silicon oxide, sodium aluminum silcate, tricalcium phosphate and calcium stearate powders were admixed with dry ground sugar at four concentration levels between 0.1 to 2%. Appreciable increase in loose bulk density and decrease in compressibility were noticeable at 0.1% concentration in all four agents. The effect reached an apparent peak or a flat maximum at an agent concentration of about 0.5–1%. With the exception of silicon oxide treated powders, increase in density was accompanied by a corresponding decrease in compressibility. Bulk parameters (i.e., density and compressibility) were more sensitive indices to changes occurring in powders as compared to parameters determined in compacted specimens (i.e., yield in shear, internal friction and relaxation pattern). Results are explained in terms of possible bed arrays and their scatter by differences in particle size and shape distributions. Support for these explanations is presented in scanning electron micrographs of sugar treated and untreated particles.

INTRODUCTION

ACCORDING TO THE Code of Federal Regulations (1980) "anti-caking agents and free flowing agents" are "substances added to finely powdered or crystalline food powders to prevent caking, lumping or agglomeration." Commonly the anticaking agents are very fine powders (particle size in the order of few microns) of silicon oxide, silicates, insoluble phosphates and the bi or tri valent salts of stearic acid. They are commercially available in different types or grades that are distinct with respect to particle size, bulk density and absorptive capacity as well as other less important properties. Most of them are fairly inert substances and are classified as GRAS. Their legally permitted concentration is restricted to a proven, useful level as anticaking agents (Code of Federal Regulations, 1980) and in practice this level is usually in the order of 1% or less.

There is no established method for evaluating the effectiveness of anticaking agents. The main reason is that the major factors that regulate powders cohesion and caking tendency, namely moisture and temperature, are independent variables. Therefore, the term effectiveness has meaning only under a very specific range of composition and storage conditions. Under such conditions the anticaking agent effect can be quantified in terms of flowing time through a funnel (a commercial test), by flowability related physical parameters (Peleg and Mannheim, 1973; York, 1975), or by sieving and weighing of the lumps formed after exposure to moisture (Irani et al., 1959; Irani and Callis, 1960). For qualitative evaluation, the mere observation of whether lumps have been formed under controlled environmental conditions is a simple and convenient method (Peleg and Mannheim, 1969; Peleg and Mannheim 1977). This procedure is also commonly reported in the manufacturers technical publications. Its major shortcoming (and that of the sieving method as well) is that the hardness of the agglomerates is not taken into account.

Authors Hollenbach and Peleg are with the Dept. of Food Engineering, and author Rufner is with the Electron Microscopy Laboratory, Massachusetts Agricultural Experiment Station, Univ. of Massachusetts, Amherst, MA 01003.

Powdered sugars are known to be fairly cohesive materials and with strong caking tendency. Because of being a frequent ingredient in the food and pharmaceutical industries the flowability and physical characteristics of these powders is of concern in a variety of processes. The effect of two types of anticaking agents (or flow conditioners) on the properties of sucrose was previously reported (Peleg and Mannheim, 1973). The results of this work indicated that at 1–3% concentration the effect of aluminum silicate and calcium stearate was practically independent of the conditioner concentration. The suggested explanation was that at 1% conditioner concentration the sucrose particles had already been effectively covered by the conditioner and therefore additional coverage could not produce any significant effect. York (1975) who worked with conditioned powdered crystalline lactose found that a saturation situation indeed existed and that beyond a certain critical concentration level (in his report about 1–2%) the flowability could not be further improved. It ought to be memtioned, however, that such a behavior cannot be assumed as typical to all conditioned powders and there are reports of an actual decline in flowability once a critical concentration has been exceeded (e.g. Kristensen and Jensen, 1969; Danish and Parrott, 1971).

In this work the concentration effect of four anticaking agents (conditioners) was studied in dry powdered sucrose. Unlike in the previous work on sucrose the selected concentration levels were such as to cover the range in which saturation or optimal conditions could be found. The study focussed on the induced modifications in the physical characteristics of the conditioned powders with special emphasis on their bulk density.

EXPERIMENTAL

COMMERCIAL SURCOSE was purchased at a local store and was pulverized by a laboratory mill prior to testing. The powder was sieved and the fraction between 50 and 300 micron was collected. This fraction was admixed with amorphous fumed silicon oxide, (CAB-O-SIL®, EH-5, Cabot Corp., Tuscola, IL) sodium aluminum silicate (ZEOLEX® 7, Huber Corp., MD), tricalcium phosphate (Stauffer Chemical Co., Westport, CT, and calcium stearate (Mallinckrodt® Co., St. Louis, MO) at agent concentrations of 0.1, 0.5, 1 and 2% on weight basis. In each series of experiments, part of the powdered sugar was tested untreated and the results were used as a reference. In each powder specimen (treated and untreated) the moisture content was determined by drying in a vacuum oven at 70°C for at least 48 hr.

Mechanical analysis

Loose bulk density was measured by weighing a cell with a known volume filled with a freely poured powder.

Compressibility was determined by compression of the powder in the cell by a piston mounted on the crosshead of an Instron Universal Testing Machine model TM. (For more details see Moreyra and Peleg, 1980). The force deformation curves were transformed to bulk density (ρ_B) vs normal stress (σ_N) relationships. As previously shown (e.g. Peleg and Mannheim, 1973; Moreyra and Peleg, 1980) the latter could be described by the equation:

$$\rho_B = a + b \log \sigma_N \qquad (1)$$

where a and b are constants. The constant b depicting the change in bulk density as a result of loading is referred to as compressibility.

The stress relaxation curves of the compacted specimens were also recorded. The curves were normalized and linearized by (Peleg, 1977; Peleg and Moreyra, 1979; Moreyra and Peleg, 1980):

$$\frac{F(o)\,t}{F(o)-F(t)} = k_1 + k_2 t \qquad (2)$$

when F(o) is the initial force, F(t) the decaying force after time t and k_1 and k_2 constants. The constant k_2 representing the asymptotic portion of the stress that remains unrelaxed was used as a measure of short term solidity on a scale where $k_2 = 1$ represents liquid properties (the stress totally relaxes) and $k_2 \to \infty$ ideal elasticity (no relaxation at all).

Shear analysis was performed by the Jenike and Johanson Flow Factor Tester model ST-HT. Results are reported in terms of yield stress in shear under given consolidation load and as effective angle of internal friction (Jenike, 1964).

All the mechanical tests were performed in two to four replicates and their results are given as mean values.

Scanning electron microscopy

The dried powders were affixed to aluminum stubs with copper tape, coated with gold-palladium at 15 mA for 3 min (∿495Å) and examined with an ISI Super III-A scanning electron microscope using a tilt angle of 40° and an accelerating voltage of 30V.

RESULTS & DISCUSSION

PHOTOMICROGRAPHS of fumed silica, aluminum silicate, tricalcium phosphate (TCP) and calcium stearate are shown in Fig. 1–4. These demonstrate that the particles of the first three agents have a porous structure while the calcium stearate particles have a distinct flaky or layerly shape. It is also evident from the photographs that because of the very fine size their particles tend to aggregate and form soft agglomerates with a very nonuniform size.

A photomicrograph of untreated sugar particles is shown in Fig. 5. It demonstrates that the particles have an active surface that attracts a significant number of smaller particles, in this case mostly fine sugar particles. This type of particle adherence is characteristic to many cohesive powders especially if composed of water soluble crystalline materials. In the powder technology literatue, such powders are termed and treated as "ordered mixtures" since the fines are found at the surface of the larger particles and therefore cannot be considered as randomly distributed in the strict sense of the expression (Yeung and Hersey, 1979; Lai et al., 1981).

The interaction between fines and the surface of the larger particles is not limited to particles of the same species. It is necessary, however, that chemical or physical compatibility will exist between the adhering particles so that the system will not segregate. Fig. 6 demonstrates that such compatibility indeed exists in the mixtures of sucrose and the tested anticaking agents. Confirmation of the presence of the agent particles at the surface of the sugar particles is crucial to the analysis of the bulk behavior. The reason is that the powder bed structure is strongly affected by interparticle forces, especially in the case of cohesive powders (e.g., Scoville and Peleg, 1981; Moreyra and Peleg, 1981). It is, therefore, important to know whether the agent activity is through surface modification, that alter the interparticle friction and the particles capacity to form bridges or through reducing the bed porosity by being merely a filler of the interparticle space. In our

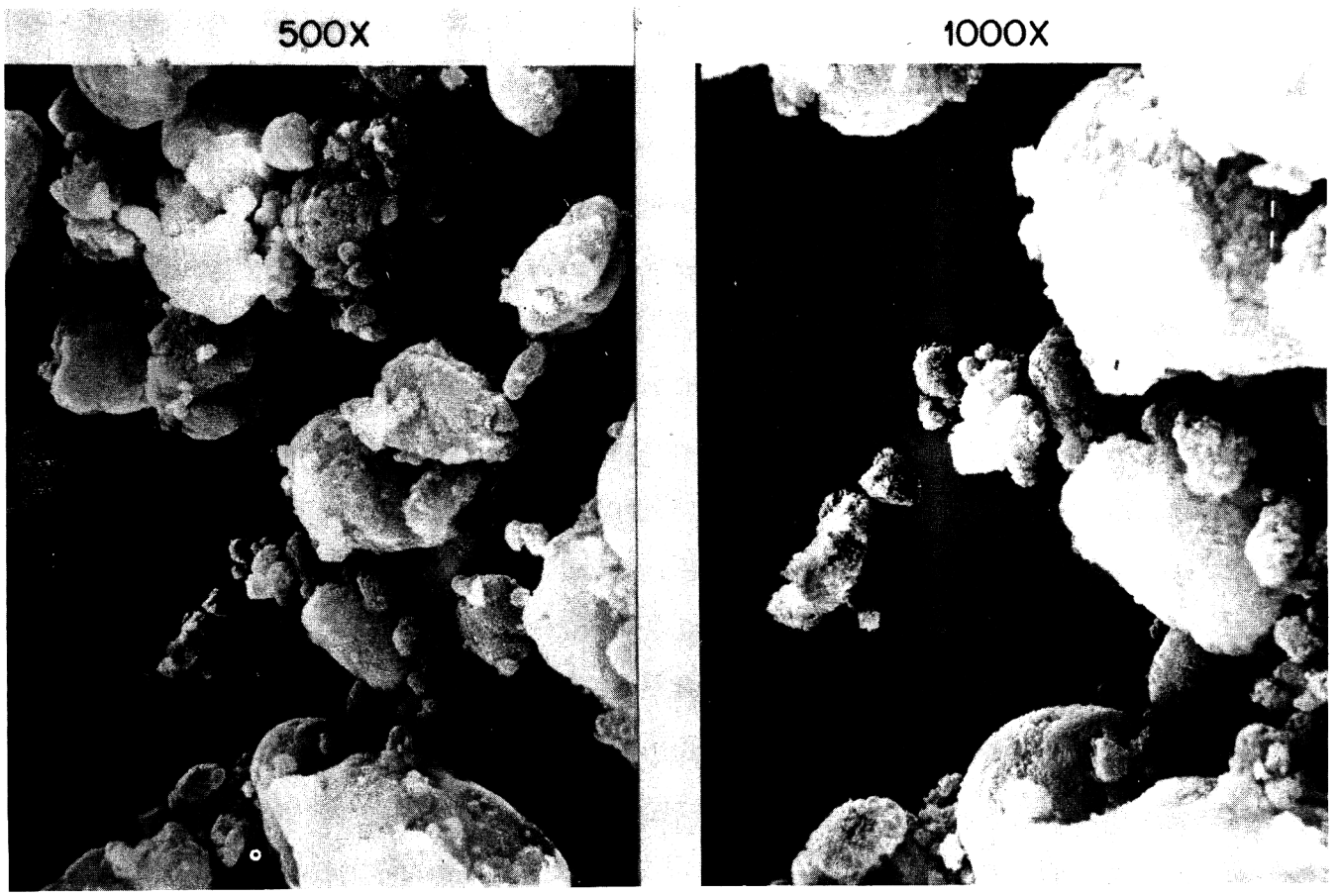


Fig. 1–Scanning electron micrographs of silicon oxide.

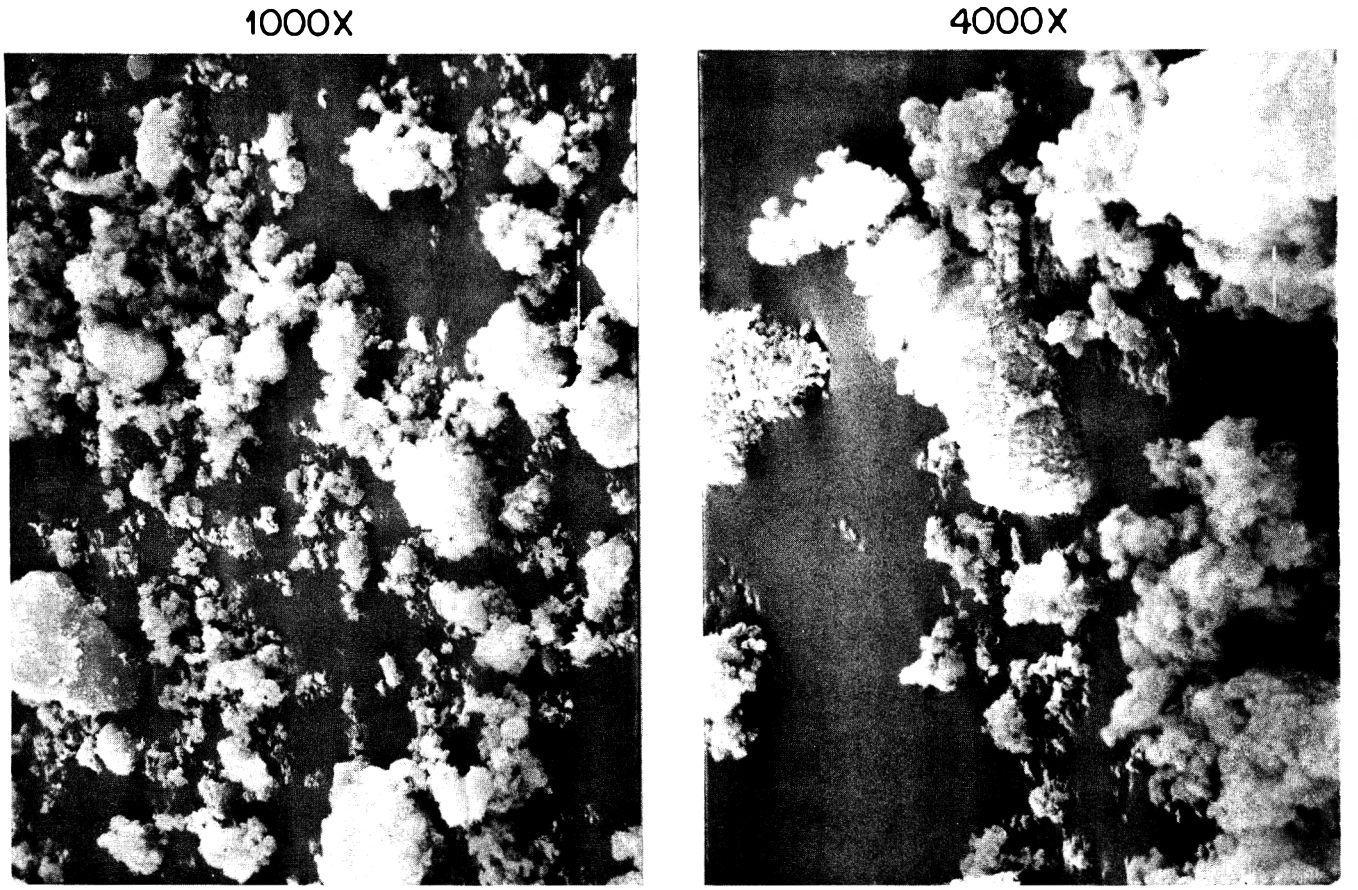


Fig. 2—Scanning electron micrographs of sodium aluminum silcate.

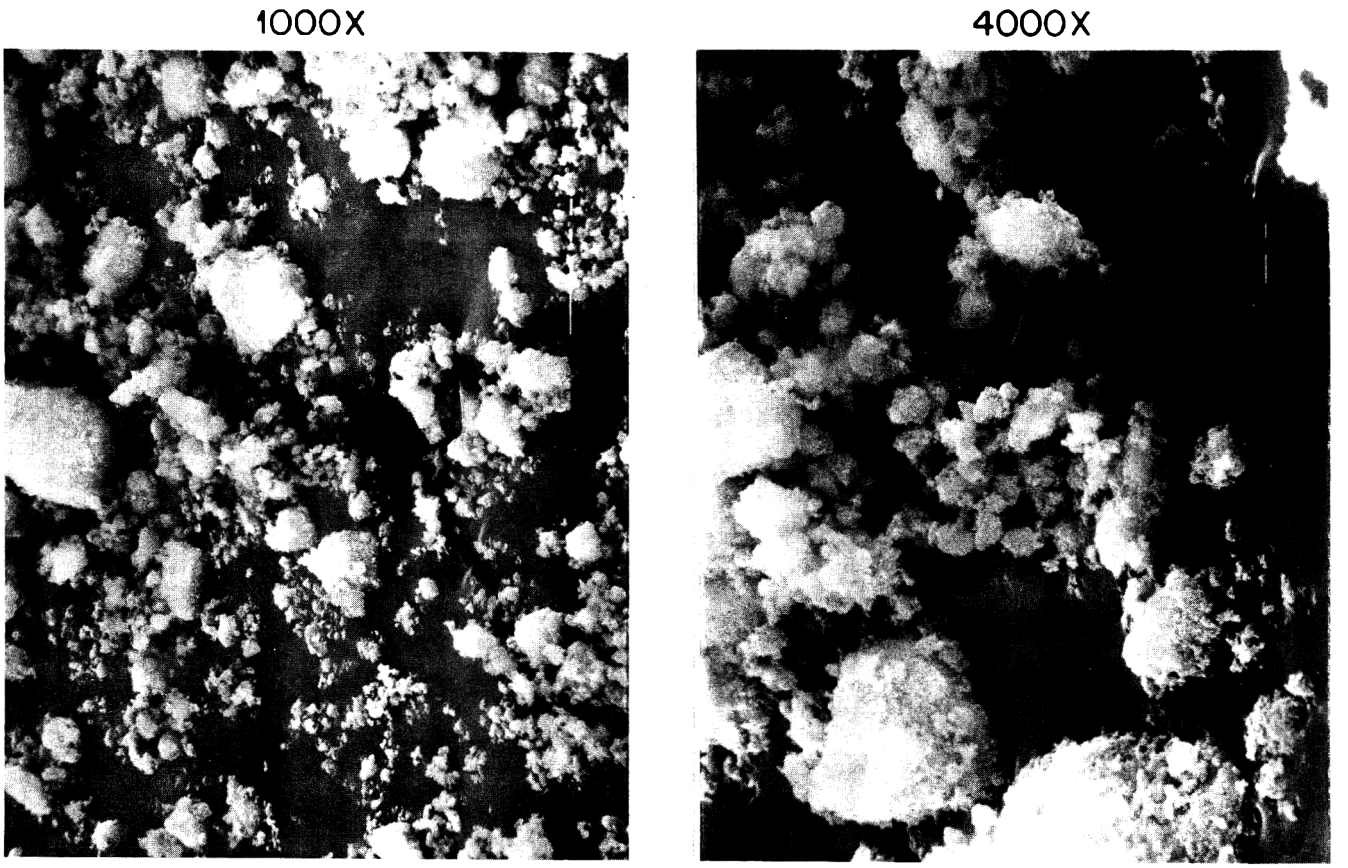


Fig. 3—Scanning electron micrographs of tricalcium phosphate (TCP).

case the fairly uniform distribution of the agents at the sugar particles surface suggests that the former mechanism is dominant. Further support for this hypothesis is provided by the kind of changes in bulk behavior which are reported and discussed below.

Bulk density and compressibility of conditioned powders

The effect of the four anticaking agents on the loose bulk density of ground sugar is shown in Fig. 7. The observed increases in loose bulk density were generally in the order of 5–25% depending on the agent and its concentration. (The exceptional case of silicon oxide will be discussed separately). If the admixture of the agents resulted in the filling of interparticle voids only, the bulk density of the conditioned powder ought to have been given by:

$$\rho_{BT} = \rho_{BU} (1 + X_A) \quad (3)$$

where ρ_{BT} is the bulk density of the treated (conditioned) powder, ρ_{BU} is the bulk density of the untreated powder, and X_A the weight fraction of the agent.

By this equation the expected increases in loose bulk density would have been in the order of 0.1–2% independently of the agent type and density. It is clear, therefore, that the conditioners weight contribution ought to be excluded as a major reason for the increase in bulk density. A more plausible reason is that the presence of the conditioner at the particle's surface physically separates the particle, thus reducing its attractive interparticle forces. Their presence also interferes with the formation of liquid bridges between particles in cases where surface moisture is sufficient to produce such bridges (Peleg and Mannheim, 1973).

The reduction or elimination of interparticle forces also reduces the possibility of maintaining an open bed structure supported by these forces. The result, therefore, in contrast to the effect of added moisture for example (Scoville and Peleg, 1981; Moreyra and Peleg, 1981), is a higher bulk density in which the porosity is largely decided by the geometrical characteristics of the particles and the random voids that are created during the settlement of the particle in the measuring container. This denser structure, which is characteristic to noncohesive powders is also expected to show lower compressibility under relatively small loads. (Conditioners are not expected to modify the host powder particles rigidity.) It is possible, however, that some of them will facilitate interparticle sliding under high pressures (e.g., in tabletting) thus producing denser compacts. This range, however, is out of the scope of this work and will not be discussed here).

The observed decline in compressibility (Fig. 8) is in line with this explanation, and in all but two of the tested powders the increase in bulk density was also accompanied by a corresponding decrease in compressibility (See also Fig. 9).

The case of silicon oxide at 1 and 2% concentration was unique with respect to bulk density. Unlike in the other conditioned powders the density not only did not significantly increase but actually decreased slightly at an agent concentration of 2%. This seems to be an indication of the formation of a new kind of relatively stable bed structure as is clearly evident from low levels of these powders compressibility when compared to that of the untreated powder (Fig. 8).

Effects of agent concentration

Bulk density and compressibility of ground sugar treated with various levels of anticaking agents are shown in Fig. 7 and 8. These figures clearly show that all four agents had a noticeable effect on these physical bulk characteristics at

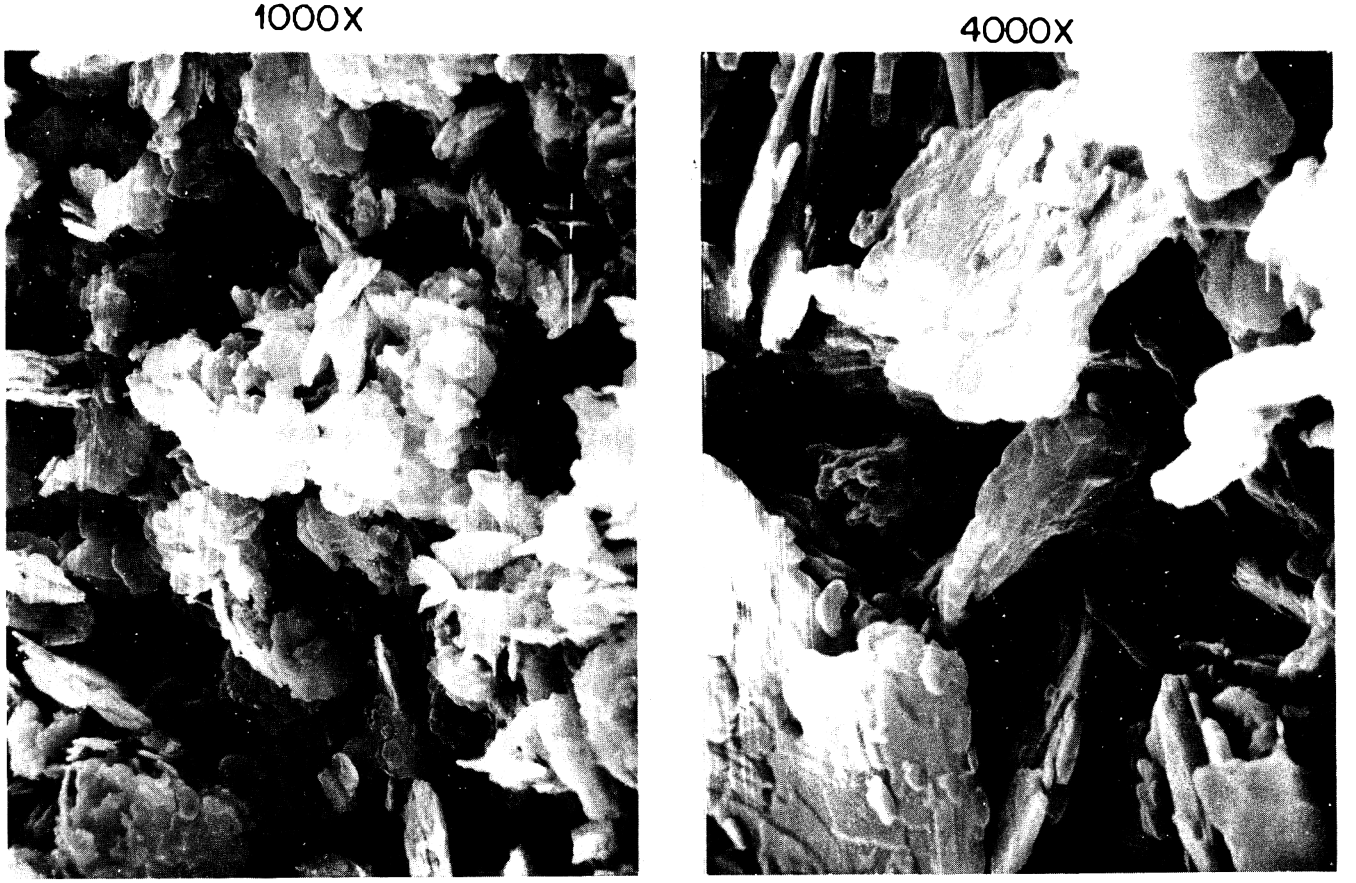


Fig. 4—Scanning electron micrographs of calcium stearate.

concentration as low as 0.1%. It is also evident that maximum effect was reached at agent concentration of about 0.5–1.0%. Beyond this level the effect remained practically the same or even declined. Although this general trend is clearly evident the relationships between the agent concentration and the bulk properties do not appear to be quite smooth. Deviations of this order could well be expected as a result of inevitable random differences in the particles size and shape distributions among the experimental powders (see Fig. 5 and 6) and perhaps in minor differences in moisture too. The total moisture content range was between 0.04–0.12% with almost all the powder falling between 0.05–0.08%. Analysis of the data with respect to a possible moisture effect showed that the latter had been only a minor factor.

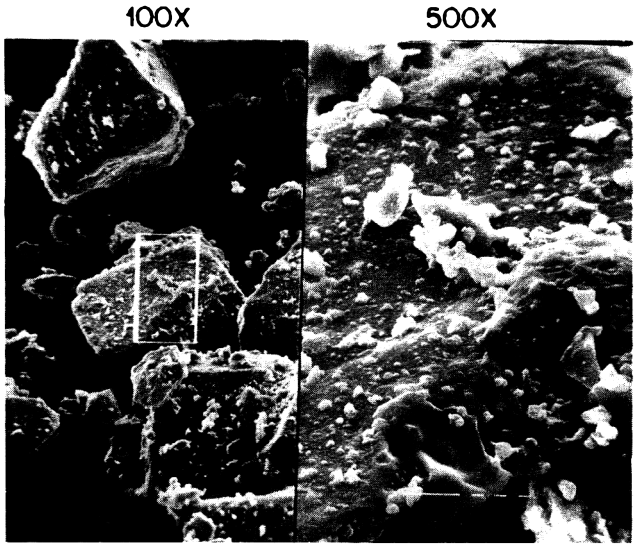


Fig. 5–Scanning electron micrographs of the surface of untreated sugar particles. (Note the adherence of fines).

The existence of a critical agent concentration at around 0.5–1% indicates that effective coverage of the particles surface is obtained at this level. This also helps to explain why in a previous work (Peleg and Mannheim, 1973) no significant differences could be found between the properties of sucrose powders treated at levels of 1–3%. It ought to be mentioned, however, that the critical concentration level is not necessarily fixed for a given anticaking agent and it may well be a function of the host powder chemical nature as well as its particle size and shape distributions.

The critical concentration may also be influenced by segregation of the agent particles in extremely dry host powders or by its uneven distribution in the case of cohesive host powders. The latter may also result in nonuniform bed structures which will be expressed in a large scatter in the measured bulk properties. It appears though that the reproducibility of the density and compressibility measurements was fairly high (deviations of and 0.5–6% from the mean) thus excluding insufficient mixing as a major factor in determining the critical concentration level in the systems that were tested in this work.

Evaluation of the other mechanical parameters

The mechanical characteristics of ground sugar treated with the four anticaking agents at 0.5% concentration are summarized in Table 1. The table presents two groups of mechanical parameters. The first (i.e., loose bulk density and compressibility) solely refers to the properties of the powders original bed structure and the second (i.e., yield stress in shear, angle of friction and the relaxation parameter) is to a large extent a representation of the compact properties. Although it is obvious that the two are interdependent (Jenike, 1964), they do not necessarily have the same sensitivity to changes that occur in the powder bed structure. This can be partly attributed to inherent experimental artifacts (e.g., there can be differences in density between the tested compacts), but it is also due to instrumental limitations (especially in a linear shear cell) and certain theoretical weaknesses of the methodology. (A

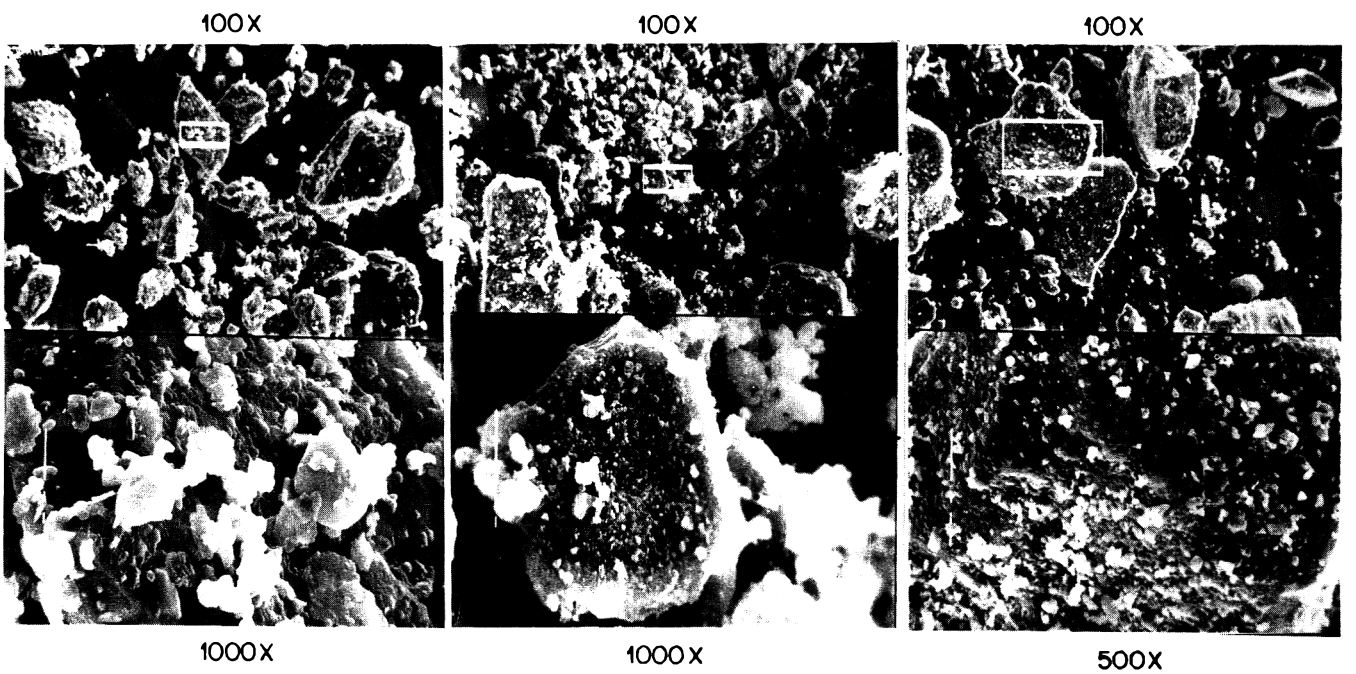


Fig. 6–Scanning electron micrographs of sugar particles coated with 2% calcium stearate (left), tricalcium phosphate (middle) and silicon oxide (right). (Note the adherence of the anticaking agents particles to the sugar surface.)

more detailed review of these aspects of powder testing and their relation to food powders has been published elsewhere (Peleg, 1977)).

The table demonstrates that as far as the powder's cohesiveness is concerned the changes in bulk density and compressibility, were much more sensitive and reliable indices than the compact related parameters. This, of course, does not mean that the compact properties were not affected by the anticaking agents presence. It means that the magnitude of such changes was not big enough to be revealed by a small number of experiments, especially where considerable variability (due to differences in size distribution for example) is an inherent characteristic of the system. It ought to be also added that the ground sugar used in this study was dry and therefore only moderately cohesive. In more moist and cohesive powder, the agents presence was significantly expressed in the compact properties as well as those of the loose powders (Peleg and Mannheim, 1973).

Regarding the relaxation pattern, the only significant effect was found in the silicon oxide treated powder. The expected effect was an increase in the value of k_2, (i.e., or reflecting a more solid compact) and indeed this was what has been found. In the other powders, the trend was unclear. As in the case of the parameters determined by shear the differences may not have been large enough to overcome small differences in moisture and perhaps in density too. It seems, however, that calcium stearate, because of its fatty anion may not only reduce friction by lubrication (Peleg and Mannheim, 1973) but also effect the relaxation pattern by allowing interparticle contacts to flow. However, since this effect is also produced by moisture, its significance can only be established by additional work.

CONCLUSIONS

IN THIS WORK, no effort has been made to compare the effectiveness of the four agents in preventing caking nor to evaluate their capacity to improve flowability. A previous report indicated that anticaking agents in powdered sugar also act like flow conditioners (Peleg and Mannheim, 1973).

–Continued on next page

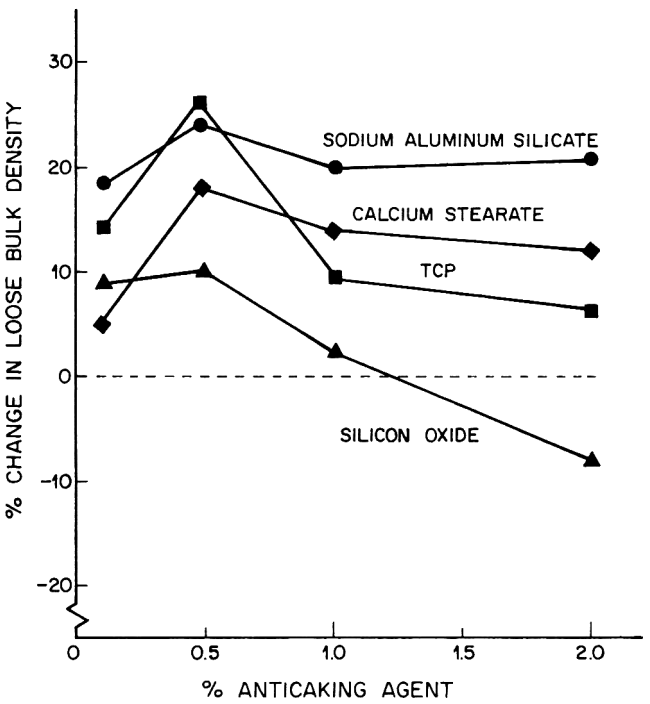


Fig. 7–Effect of the anticaking agent concentration on the bulk density of ground sugar.

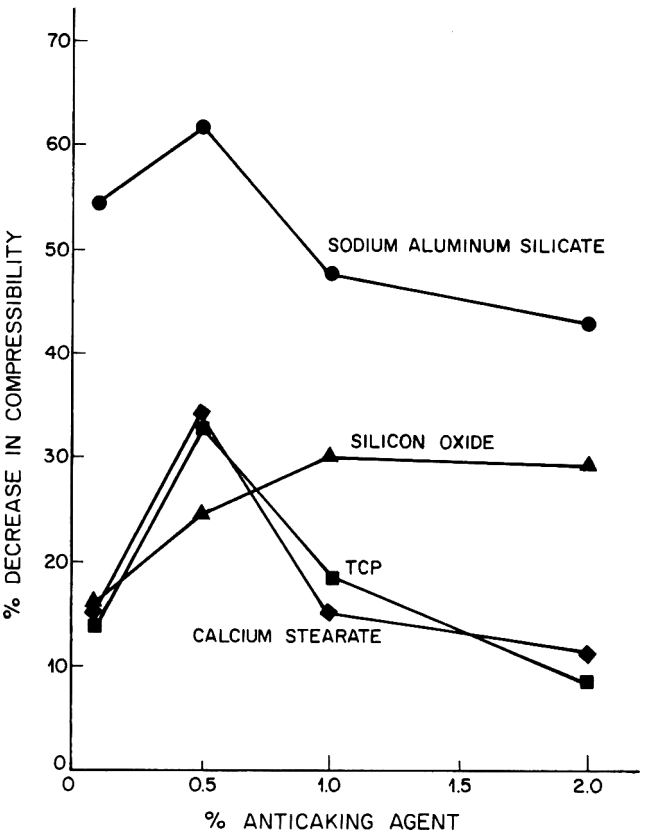


Fig. 8–Effect of the anticaking agent concentration on the compressibility of ground sugar.

Table 1–Some physical parameters of ground sugar conditioned with four anticaking agents at 0.5% concentration

Conditioner	Loose bulk density ($g \cdot cm^{-3}$)	Compressibility[a] (b in Eq. 1)	Yield stress in shear ($kg \cdot cm^2$) under consolidation stress of: 1.2 $kg \cdot cm^{-2}$	0.27 $kg \cdot cm^{-2}$	Angle of internal friction (deg)	Relaxation parameter[b] (k_2 in Eq. 2)
None	0.697	0.066	0.96	0.46	42	1.77
Silicon oxide	0.749**	0.052**	1.10	0.50	43	2.25*
Sodium aluminum silicate	0.872**	0.026**	1.05	0.48	42	1.63
Tricalcium phosphate	0.761*	0.044**	1.10	0.48	43	1.76
Calcium stearate	0.865**	0.039**	0.87	0.43	36	1.61

[a] The regression coefficients in fitting Eq. 1 were between r = 0.992 and r = 0.999 (significant at P = 0.001)
[b] The regression coefficients in fitting Eq. 2 were between r = 0.997 and r = 0.999 (significant at P = 0.001)
*Different from the corresponding value of the untreated powder at significance level of P = 0.05.
**Different from the corresponding value of the untreated powder at significance level of P = 0.01.

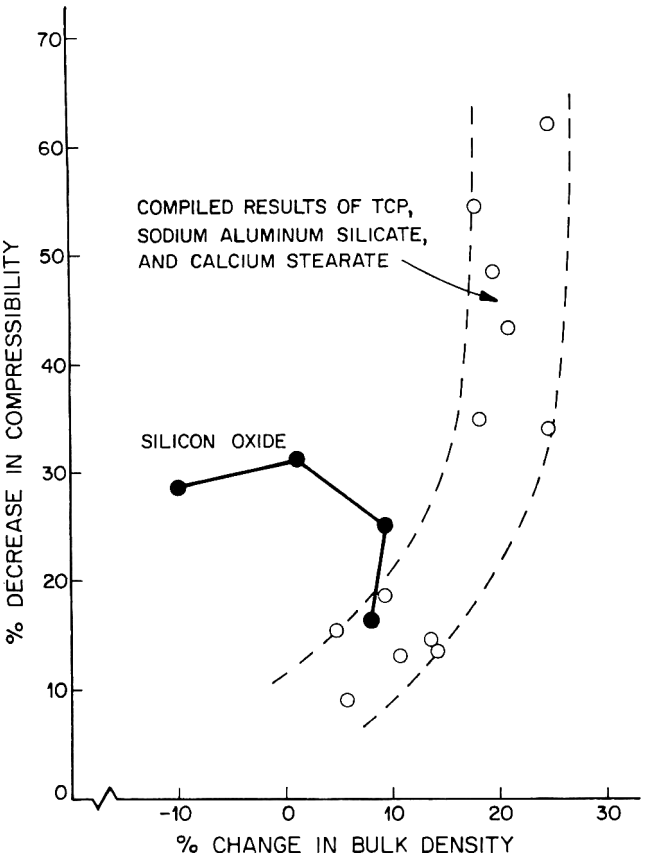


Fig. 9—The relationship between the changes in bulk, density and compressibility of ground sugar treated with anticaking agents constructed from the compiled data of Fig. 7 and 8. (Note the peculiar deviation of 1 an 2% mixtures of silicon oxide where the decrease in compressibility was not accompanied by a corresponding increase in bulk density.)

It is also known, however, that when hygroscopic powders are exposed to an atmosphere with high relative humidity, they cake despite the agents presence (Peleg and Mannheim, 1977). Therefore, the range of humidities in which such agents are effective is inherently limited. In that range or at lower humidity levels where the powder does not cake spontaneously, the addition of the anticaking agent is expected to modify the powder bulk peoperties to a much larger extent than can be assumed by their weight concentration. As shown in this work, the magnitude of such effects can vary according to the conditioner type and its concentration. Therefore, it seems adivsable that such consequences ought to be considered seriously before a powder is treated with an anticaking agent or when one agent is replaced by another.

It would also be worth mentioning that in all the tested powders affinity existed between the sugar surface and the agent particles and this was probably the source of these large effects on density. Such affinity, however, cannot be taken for granted and there are powders (e.g., soyflour treated with calcium stearate) where certain types of anticaking agents will have little or no effect because of segregation of the agent particles.

REFERENCES

Anonymous. 1980. Code of Federal Regulations (FDA) Parts 170.3, 172E and 182.
Danish, F.Q. and Parrot, E.L. 1971. Effect of concentration and size of lubricant on flow rate of granules. J. Pharm. Sci. 60: 752.
Irani, R.R. and Callis, C.F. 1960. The use of conditioning agents to improve the handling properties of cereal products. Cereal Sci. Today. 5: 1980.
Irani, R.R., Callis, C.F., and Liv, T. 1959. Flow conditioning and anticaking agents. Ind. Eng. Chem. 51: 1285.
Jenike, A.W. 1964. Storage and flow of solids. Bull. No. 123. Utah Engng. Exp. Sta., Univ. of Utah, Salt Lake City.
Kristensen, H.G. and Jensen, V.G. 1969. Studies on flow properties of powders. 3. The influence of glidants on the technical properties of tablet granulations. Dansk. Tidsskv. Farm. 43: 205.
Lai, F., Hersey, J.A., and Stainforth, J.N. 1981. Segregation and mixing of fine particles in an ordered mixture. Powder Technol. 28: 17.
Moreyra, R. and Peleg, M. 1980. Compressive deformation patterns of selected food powders. J. Food Sci. 45: 866.
Peleg, M. 1977. Flowability of food powders and methods for its evaluation. (A Review). J. Food Proc. Eng. 1: 303.
Peleg, M. and Mannheim, C.H. 1973. Effect of conditioners on the flow properties of powdered sucrose. Powder Technol. 7: 45.
Peleg, M. and Mannheim, C.H. 1977. The mechanism of caking of powdered onion. J. Food Proc. & Pres. 1: 3.
Peleg, M. and Moreyra, R. 1979. Effect of moisture on the stress relaxation pattern of compacted powders. Powder Technol. 23: 277.
Peleg, Y. and Mannheim, C.H. 1969. Caking of onion powder. J. Food Technol. 4: 157.
Scoville, E. and Peleg, M. 1981. Evaluation of the Effect of liquid bridges in the bulk properties of model powders. J. Food Sci. 46: 174.
Yeung, C.C. and Hersey, J.A. 1979. Ordered powder mixing of course and fine particulate systems. Powder Technol. 22: 127.
York, P. 1975. The use of glidant to improve the flowability of fine lactose powder. Powder Technol. 11: 197.
Ms received 7/27/81; revised 10/19/81; accepted 10/22/81.

Contribution of the Massachusetts Agricultural Experiment Station at Amherst. The project has been supported by the NSF Particulate and Multiphase Processes Program (Grant No. ENG 78-13275).

The authors express their thanks to Mr. R.J. Grant for his graphical aid and to the Cabot Corporation, Huber Corporation, Stauffer Chemical Company, and Mallinckrodt Company for the donations of anticaking agents.

The mention of commercial products in this work does not imply their endorsement by the authors and the sponsoring agency.

Riboflavin Photochemical Degradation in Pasta Measured by High Performance Liquid Chromatography

E. A. WOODCOCK, J. J. WARTHESEN, and T. P. LABUZA

ABSTRACT

A technique utilizing high performance liquid chromatography (HPLC) was developed to determine the kinetics of riboflavin losses in enriched macaroni as a function of temperature, water activity and light. Riboflavin and lumichrome were extracted from macaroni and assayed by reverse-phase HPLC with fluorescence detection. Riboflavin degradation and lumichrome production in macaroni occurred in two phases, a rapid initial phase followed by a slower phase. Photodegradation of riboflavin appears to follow first-order reaction kinetics. Under the experimental conditions used, more than 50% of the riboflavin content of macaroni was lost within one day. Light intensity was the rate determining factor for riboflavin loss and lumichrome production. The riboflavin level decreased with increased temperature in the second phase of breakdown.

INTRODUCTION

PASTA PRODUCTS stored under environmental conditions found in a normal distribution cycle degrade over time to a level of unacceptability, which corresponds with the end of shelf-life of the product. Generally, staling and moisture gain or loss have been used as guides to estimate the shelf-life of pasta, which reportedly ranges anywhere from 6 months to infinite (Anonymous, 1977). Vitamin losses and a decrease in protein quality are two additional modes of deterioration which can occur in pasta products (Chrzanowska, 1974).

Current dietary goals for improved nutritional status, written by the U.S. Senate Select Committee on Nutrition and Human Needs (1977), suggest an increase in consumption of complex carbohydrates and a decrease in consumption of refined sugars, saturated fats and cholesterol. Since many of the richest sources of riboflavin, such as milk, cheese, liver and eggs contain considerable quantities of saturated fats and cholesterol, enriched pasta products may be regarded as potentially significant contributors of riboflavin in the diet.

Riboflavin in solution degrades rapidly when exposed to ultraviolet or visible radiation. Under acidic or neutral conditions, riboflavin loses the ribityl side chain to form lumichrome whereas in alkali solutions, riboflavin is photochemically converted to lumiflavin. These two flavins have not been found to have riboflavin activity (Sebrell and Harris, 1972). Continued irradiation will yield further breakdown products (Metzler, 1960).

Aerobic photodegradation of riboflavin in solution has been investigated by numerous authors (Shimizu, 1950; Sattar and deMan, 1973, 1977; Singh et al., 1975; Allen and Parks, 1979). Evaluation of riboflavin losses demonstrated that degradation proceeds as a first-order rate reaction and increases with increasing temperature. Work done by Singh et al. (1975) with whole milk stored at constant temperature and varying light intensities showed that the rate of riboflavin degradation also increases with increased radiation levels. Singh et al. (1975) found that the rate of loss varied with the type of packaging and reported activation energies of 8.0 and 41.2 Kcal/mole for clear glass and paperboard, respectively.

Kinetic information is available for the loss of riboflavin in only a few dry food systems. Dennison et al. (1977) showed that there was no significant influence of increasing relative humidity on the stability of riboflavin in a simulated dry cereal model system packaged in paperboard cartons. This system was held under aerobic conditins at 30°C without light exposure. Chrzanowska (1974) showed that when enriched macaroni was stored at room temperature in cellophane bags and exposed to diffuse sunlight, it rapidly decreased in riboflavin content. Labuza and Kreisman (1978) estimated a half-life of only 19 days for riboflavin in the Chrzanowska (1974) study.

The approved fluorometric method for riboflavin in foods (AOAC, 1975) requires considerable purification and handling. An improved semi-automated determination for riboflavin was developed independently by Egberg and Potter (1975) and Pelletier and Madere (1975) which combined rapid sample preparation with a continuous flow scheme using in-line permanganate oxidation to eliminate interferences.

Chromatographic techniques have also been used to separate riboflavin from interfering compounds. High performance liquid chromatography (HPLC) with absorbance detection has been successfully utilized for the analysis of multi-vitamin preparations (Williams et al., 1973; Conrad, 1975; Wills et al., 1977). Kamman et al. (1980) reported a riboflavin and thiamin analysis procedure for cereal products using HPLC with absorbance detection at 254 nm. This technique resulted in fewer manipulations and a shorter analysis time than the AOAC (1975) procedure. A high performance liquid chromatographic method for riboflavin using fluorescence detection to increase sensitivity and specificity has been used by Van de Weerdhof et al. (1973) and Osborne and Voogt (1978).

Only a limited amount of method development has been done on the quantitation of lumichrome in food systems. Analysis of lumichrome in foods has been accomplished predominantly by thin-layer chromatography following extraction and spotting on silica gel plates (Parks and Allen, 1977; Treadwell et al., 1968; Suzuki et al., 1979).

The present study developed a procedure for the separation and quantification of riboflavin and lumichrome from enriched macaroni by HPLC equipped with a fluorescence detector. In addition, the effects of light, water activity (a_w) and temperature on the rate of riboflavin degradation in macaroni were studied. In an attempt to better understand the kinetics and mechanism of riboflavin losses, lumichrome production was also measured with respect to these variables.

MATERIALS & METHODS

Sample preparation

Elbow macaroni (The Creamette Co.) was manufactured from semolina enriched with riboflavin, thiamin, niacin and iron. Riboflavin was added at a level to conform to the standards of identity

Authors Woodcock, Warthesen & Labuza are with the Dept. of Food Science & Nutrition, Univ. of Minnesota, 1334 Eckles Avenue, St. Paul, MN 55108.

which is 1.7–2.2 mg/lb of product (U.S. Standards of Identity, 1955).

For extraction, a 3-g ground macaroni sample (passed through a 40 mesh sieve) was placed in a 125 ml Erlenmeyer flask to which 20 ml 0.1N HCl was added. The flask was covered with foil and autoclaved for 30 min at 15 psi. The sample was quantitatively transferred to a 50 ml polyethylene centrifuge tube and centrifuged for 15 min at 550 x *g*. Following centrifugation, the supernatant was decanted into a 50-ml volumetric flask. The remaining sediment was washed two times with 10 ml 0.1N HCl, recentrifuged, and decanted. Combined supernatants were brought up to 50 ml total volume with 0.1N HCl and then filtered through a 0.20 μm micropore membrane filter (Gelman Instrument Co.). All manipulations and analyses were carried out in subdued light and with amber glassware.

HPLC measurement of riboflavin

Ten microliters of the extract were injected into a high performance liquid chromatograph equipped with a Model 6000 A pump (Waters Associates) and a Model 7120 Rheodyne 10 μl loop injector. Fluorescence intensity was measured by a Farrand Ratio Fluorometer 2 fitted with a Farrand 10 μl quartz flow cell assembly. Excitation wavelength was regulated by the primary filter 7-59 and the fluorescent emission was determined by the secondary filters 3-70 and 4-70. These filters correspond to the excitation and emission wavelengths for riboflavin of 450 and 510 nm, respectively (Kirk, 1974; Sebrell and Harris, 1972). A Hewlett Packard 3380A recorder-integrator was used to record and electronically integrate the output from the fluorescence detector. Reverse phase HPLC with a Bondapak C_{18} column (30 cm x 3.9 mm, i.d., Waters Associates) was used to separate and quantify riboflavin from macaroni. The mobile phase was 1% A.C.S. grade glacial acetic acid (Mallinckrodt), 43% A.C.S. grade methanol (Fisher Scientific) and 56% distilled water. At a flow rate of 1.5 ml/min, the elution of riboflavin was completed within 4 min.

Riboflavin (Sigma Chemical Co.) standard stock solution was prepared by dissolving 15 mg in 0.1N HCl to make 100 ml total volume. This solution was stored under toluene at 5°C in an amber volumetric flask to prevent riboflavin deterioration. An external standard was prepared in duplicate weekly from the stock solution by removing one milliliter and diluting to 1000 ml with 0.1N HCl, for a final concentration of 0.15 μg/ml. The riboflavin content of samples was quantified by comparing the peak heights from the macaroni extracts to the peak heights of the standards. A theoretical baseline was constructed at the base of the peak from the point where riboflavin elution began to the end of the peak. Peak height was measured on a line which bisected the peak. The levels of riboflavin studied were shown to have a linear relationship with fluorescent detector response up to a level of 5 μg injected. A recovery study was conducted in which known amounts of riboflavin were added to enriched macaroni (1.67 μg/g) prior to the assay procedure. To compensate for differences in moisture content, results were converted to the solids basis after moisture analysis [AOAC (1975) air oven method].

HPLC measurement of lumichrome

Chromatographic instrumentation and conditions utilized to measure lumichrome was identical to those selected for riboflavin with the exception of mobile phase composition and detector filters. Excitation of lumichrome occurs at 300–350 nm and fluorescent radiation is emitted at a maximum of 479 nm (Kamin, 1971). Filter selection was based upon the characteristic excitation and emission spectrum of lumichrome (primary filter 7-51 and secondary filters 3-72 and 4-70). Optimum lumichrome elution, as judged by peak symmetry and separation from other compounds, was achieved with a mobile phase of 1% acetic acid, 49% methanol and 50% demineralized water. Lumichrome was eluted from enriched macaroni within 7 min. Identification was based on retention time and the use of a macaroni sample that had not been exposed to light and contained no lumichrome. Quantification was accomplished by comparing the peak height of lumichrome in the sample to that found in the external standard. An external standard was prepared weekly by dissolving 30.0 mg of lumichrome (Aldrich Chemical Co.) in 50 ml of methanol. Serial dilutions were made with methanol to obtain a final concentration of 0.6 μg/ml. Detector response was linear within the lumichrome concentration range found in macaroni extracts.

Lumichrome recovery was determined by adding lumichrome (1.67 μg/g) to ground macaroni that had not been previously exposed to light and contained no lumichrome other than what was added. A reproducibility study was conducted to measure the standard deviation of eight determinations of lumichrome in a macaroni sample stored for 21 days at 27.87 lumens/m^2, 55°C, and 0.44 a_w.

Kinetic analysis

The stability of riboflavin and lumichrome was measured under steady state conditions of 25, 35 and 55°C. At each temperature samples were stored over a salt solution at an a_w of 0.44. These samples were then exposed to light intensities of 9.29, 18.58 and 27.87 lumens/m^2 (100, 200, 300 ft-c, respectively). Macaroni was also stored at an a_w of 0.32 at two temperatures but only at 27.87 lumens/m^2.

Storage experiments were conducted in 10 gallon aquarium tanks with sealed plexiglass covers held in constant temperature environmental chambers (± 0.3°C). Incident light transmission was prevented by painting the outside of the aquarium tank black. Inside the aquarium tank, a plexiglass plate (perforated with holes 1 cm in diameter) was placed approximately 5 cm from the bottom of the tank. The holes in the plexiglass plate allowed for air circulation between the saturated salt solution at the bottom of the tank and the macaroni samples on the plate. A single layer of elbow macaroni was positioned in the bottom of petri dishes (4 cm diameter) which were then set on top of the plexiglass plate. Prior to placement in the designated environmental chamber, macaroni samples were equilibrated to the appropriate a_w in desiccators covered with foil. Light intensity was regulated by adjustment of a rheostat attached to three standard fluorescent lamps (15 watt, mercury vapor) mounted on top of the chamber. Illumination levels were set using a General Electric type 214 light meter.

Control samples were equilibrated and held at the appropriate a_w at 25°C with no light exposure. Duplicate control samples were extracted and analyzed along with duplicate macaroni samples exposed to light. Zero time data were established as the average of the riboflavin content of the control sample, stored under a given a_w. Lumichrome was not detected in samples stored in the dark so the minimum detectable level was used as the zero time concentration.

Analysis of variance was used to determine the influence of various factors on riboflavin degradation. Standard linear regression was used to construct the best-fit-line for determining rate constants from a semi-log plot of the amount of riboflavin versus time.

RESULTS & DISCUSSION

HPLC measurement of riboflavin and lumichrome

The extraction and HPLC analysis technique used in this study eliminated the need for pH adjustments, oxidation followed by decolorization, and blank determinations required by the AOAC (1975) riboflavin procedure. Since riboflavin and lumichrome do not have identical fluorescence characteristics, optimum chromatographic and detector conditions for each flavin were established separately. The detection wavelengths used for lumichrome analysis resulted in a number of peaks on the chromatogram, particularly in the area of riboflavin elution. In contrast, the wavelengths used for riboflavin analysis detected relatively few peaks. Through the adjustment of the methanol concentration in the mobile phase, it was possible to select specific isocratic conditions which would afford good separation of riboflavin and lumichrome from other fluorescing substances. Fig. 1 and 2 show the respective separation of riboflavin and lumichrome from enriched macaroni. The lower limits of detectability for the HPLC method using riboflavin and lumichrome standard solutions were 0.01 ng and 0.05 ng, respectively, per 10 μl injection.

Riboflavin recovery in an enriched macaroni sample was 99% with a coefficient of variability of 2–4%. The recovery of lumichrome added to fresh macaroni samples was 85% with a coefficient of variability of 3–6%. The riboflavin level in enriched macaroni as measured by HPLC was 0.562 ± 0.012 mg riboflavin /100g solids. This is within the expected range for enriched macaroni and the

standard deviation is similar to that reported for the semi-automated method (Egberg and Potter, 1975). Lumichrome reproducibility was determined in macaroni held at 55°C, 0.44 a_w and exposed to 27.87 lumens/m^2 for 21 days. The lumichrome level in enriched macaroni held under these conditions was 0.144 ± 0.010 mg lumichrome/100g solids.

Kinetic analysis

The results of this study showed that riboflavin degradation in pasta occurs in two distinct phases. Approximately 50% of the initial riboflavin content was lost within 12 hr. Beyond that time, the rate of riboflavin degradation decreased substantially leading to the conclusion that riboflavin destruction would be best represented by two phases of breakdown. Fig. 3 illustrates the percent of riboflavin remaining after a specified time of irradiation at 27.87 lumens/m^2 at either 25 or 55°C and at 0.32 a_w. Riboflavin degradation appeared to follow first order kinetics. Data collected for riboflavin retention after 0, 4, 8 and 12 hr of light exposure showed the rapid first phase of degradation. The regression line of best fit for this initial period is drawn on the graph but the data points are not shown because of the steep slope. Between days 1 and 35, additional retention data were collected and the points along the line of best fit are illustrated for this second phase of degradation. Riboflavin degradation in macaroni stored at 0.44 a_w and three levels of light intensity is shown in Fig. 4, 5 and 6. The initial phase of degradation at a_w 0.44 for the three temperatures yielded very similar rate constants. Rate constants and half lives for riboflavin measured under the conditions studied are given in Table 1. Because of the rapid initial rate of degradation, the half life of riboflavin for all conditions studied was less than one day.

Since the macaroni was exposed to a constant level of irradiation on only one side, the light probably caused rapid deterioration of the riboflavin on the surface and outer portions. Following the initial stage of degradation, riboflavin losses proceeded at a rate about one hundred times slower, possibly due to the decreased intensity of light in the interior of the macaroni. The light intensities used in this investigation include the levels that might be found in a retail grocery store, and the water activities of 0.32 and 0.44 are what might be expected in a macaroni product (Earle and Rogers, 1941; Taufig, 1977). However, in attempting to apply these reaction rates to packaged macaroni, it should be noted that this study was performed with a single layer of product. The level of riboflavin loss in a package would be a function of packaging material, the amount of actual product exposed to light on the surface, and the penetration of light into the interior of the product.

The results shown in Table 1 and an analysis of variance indicate that the a_w levels used for storage did not have a significant effect upon the rate of riboflavin degradation in either phase of loss. The three levels of light intensity were also shown to be not significantly different from one another in either phase of degradation at a 95% probability level. While the presence or absence of light was a critical factor in riboflavin degradation, over the range of intensities studied the light intensity level was not significant

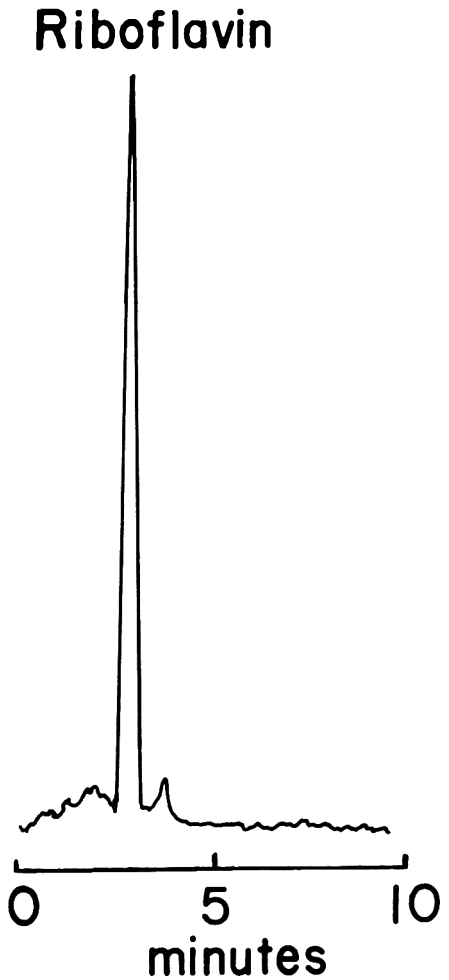


Fig. 1—Riboflavin elution from a sample of enriched macaroni using a mobile phase of 56% water, 43% methanol and 1% acetic acid.

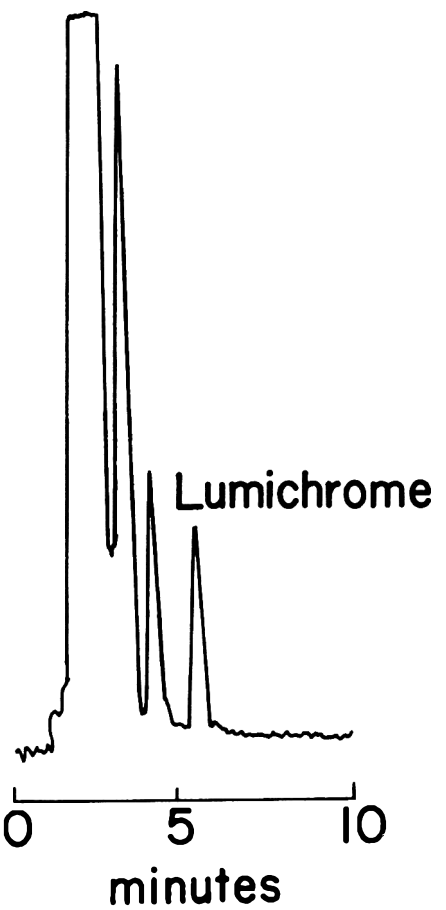


Fig. 2—Lumichrome elution from a sample of enriched macaroni exposed to light. The mobile phase was 50% water, 49% methanol and 1% acetic acid.

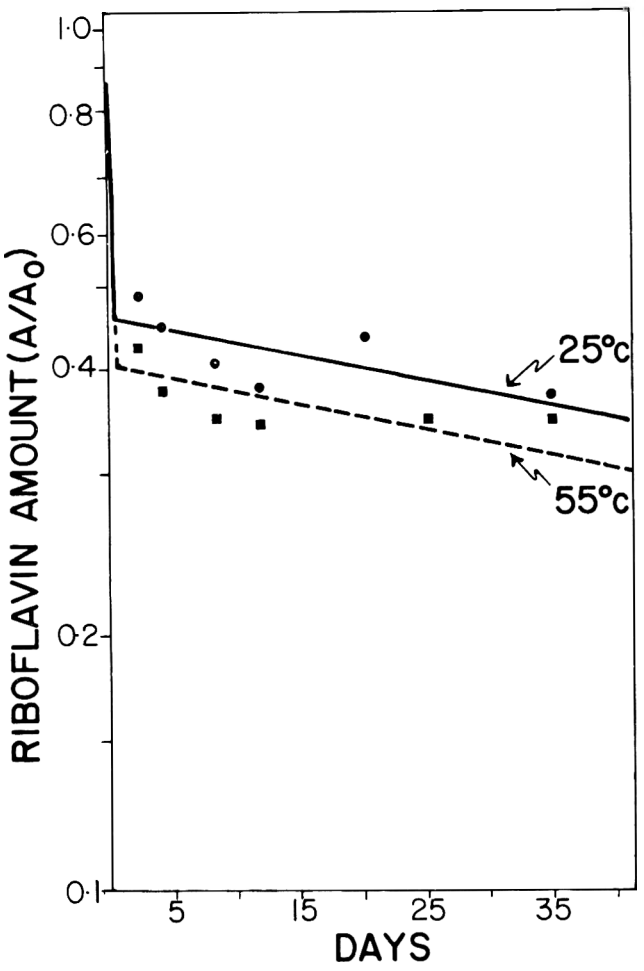


Fig. 3—Retention of riboflavin in enriched macaroni stored at 0.32 a_w, 27.87 lumens/m^2 and 25 and 55° C.

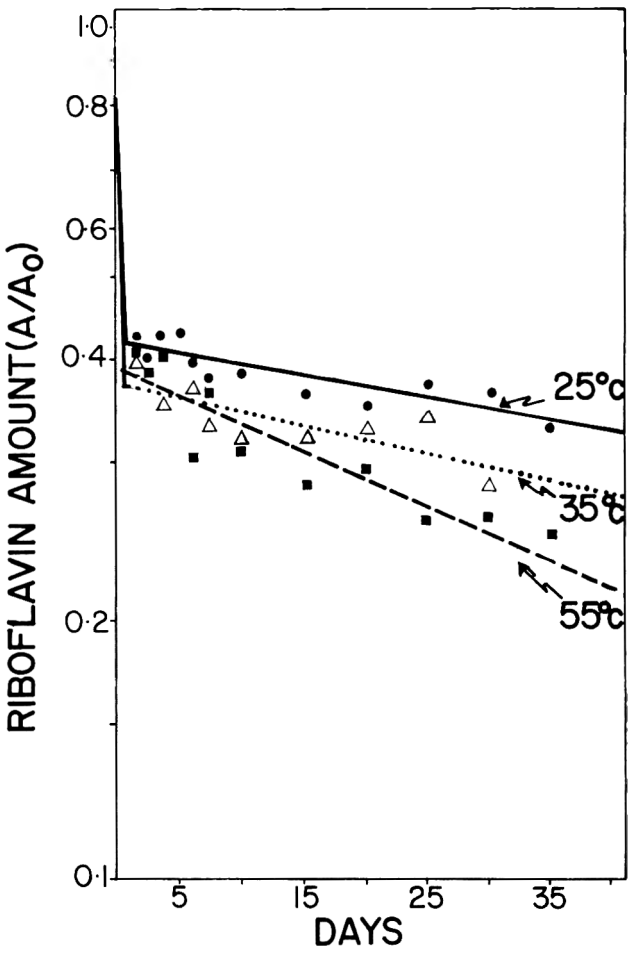


Fig. 4—Retention of riboflavin in enriched macaroni stored at 0.44 a_w, 9.29 lumens/m^2 and 25, 35 and 55° C.

Table 1—Effect of temperature, light intensity, and water activity on rate constant and half-life ($\theta_{1/2}$) for riboflavin loss in enriched macaroni

a_w	°C	Lumens/m^2	1st degradation phase Rate constant k (days^{-1})	2nd degradation phase Rate constant k (day^{-1}) x 10^2	$\theta_{1/2}$ (days) for first phase
0.32	25	27.87[a]	1.26	0.78	0.55
	55	27.87	1.38	0.55	0.50
0.44	25	27.87	1.56	0.57	0.44
	35	27.87	1.52	0.55	0.46
	55	27.87	1.32	0.87	0.53
0.44	25	18.58[a]	1.30	0.84	0.53
	35	18.58	1.27	0.85	0.55
	55	18.58	1.72	1.10	0.40
0.44	25	9.29[a]	1.37	0.70	0.50
	35	9.29	1.53	0.80	0.45
	55	9.29	1.32	1.33	0.52

[a] 27.87, 18.58 and 9.29 lumens/m^2 correspond to 300, 200 and 100 ft-c, respectively.

(P=0.05) with respect to the rate of degradation. There was, however, a significant difference at P=0.05 in the second phase of degradation where increased temperature increased the rate of reaction.

The activation energies for each phase of degradation were calculated and ranged from 0.6 to 2.0 Kcal/mole for the initial phase and 1.9 to 4.3 Kcal/mole for the second phase of the reaction, indicating that increased temperature had little effect on the reaction rate. The activation energies also showed no pattern with respect to the a_w or light intensities studied.

HPLC analysis for lumichrome in the stored macaroni indicates that lumichrome production occurs in two phases. A rapid initial phase of production which appears to be a first order reaction is followed by a second phase of slower increase or, in some cases, a decrease in the concentration of lumichrome. The rate constants k for the first 12 hr of lumichrome production ranged from 4.2 to 5.2 day^{-1} with no influence of temperature, a_w, or light intensity noted over the range of conditions studied. The findings are similar to the results obtained from the riboflavin degradation data showing that temperature, a_w, or range of light intensity had little influence on the reaction. The second phase of the reaction was not suitable for kinetic analysis because of the apparent change in some samples from lumichrome accumulation to lumichrome degradation.

Lumichrome is a photolysate of riboflavin that forms under neutral or acidic conditions (Metzler, 1960). The occurrence of lumichrome in riboflavin-containing foods has not been well studied, and the formation of lumichrome in enriched macaroni has not been previously reported. No evidence of lumichrome was found in samples that were not exposed to light. The amount of lost riboflavin that was accounted for by lumichrome varied, depending on the experimental conditions but, in general, the amount of riboflavin loss that appeared as lumichrome was about 60%. For example, when stored at 55°C, 0.44 a_w

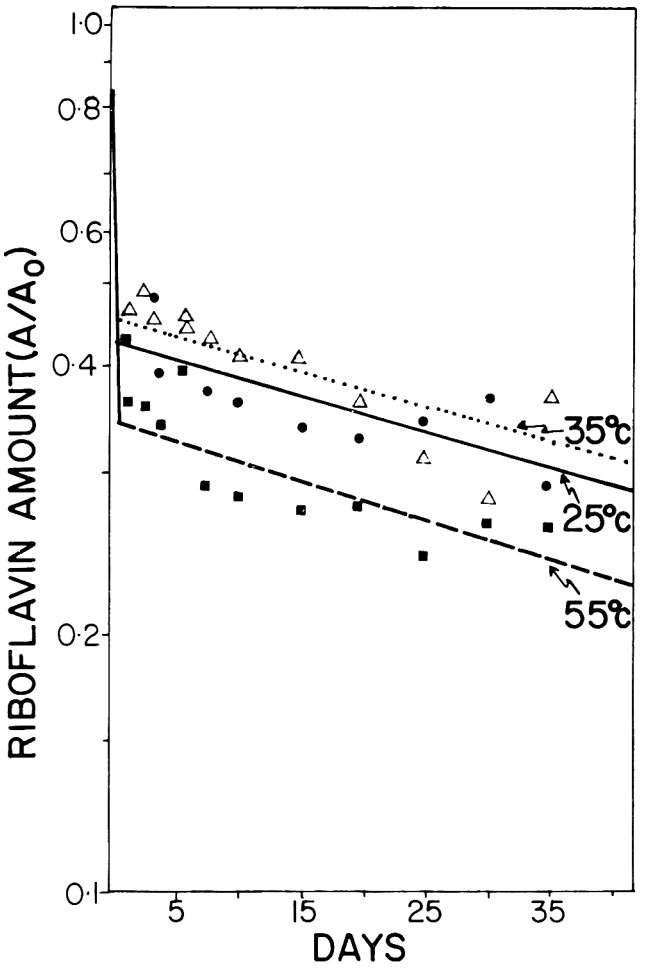


Fig. 5—Retention of riboflavin in enriched macaroni stored at 0.44 a_w, 18.58 lumens/m^2 and 25, 35 and 55° C.

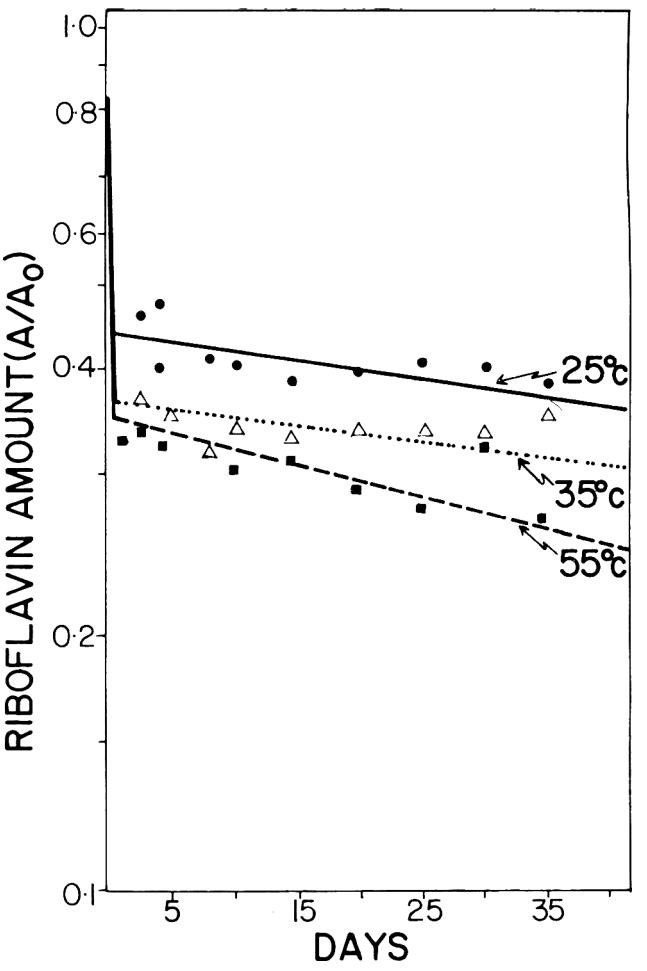


Fig. 6—Retention of riboflavin in enriched macaroni stored at 0.44 a_w, 27.87 lumens/m^2 and 25, 35 and 55° C.

and 27.87 lumens/m^2, for 21 days, the loss of riboflavin from the macaroni was 72%. When the lumichrome level in these samples was compared on a mole basis it represented 42% of the original riboflavin. This suggests that lumichrome is not the only or final riboflavin degradation product. In addition, it appears that with continued irradiation, lumichrome may undergo some degradation. Thus, while lumichrome appears to be the major reaction product of riboflavin in irradiated macaroni, it does not reflect all the riboflavin degradation and could not be used as an indirect measure of the amount of riboflavin deterioration in enriched macaroni.

REFERENCES

Allen, C. and Parks, O.W. 1979. Photodegradation of riboflavin in milks exposed to fluorescent light. J. Dairy Sci. 62: 1377.

Anonymous. 1977. Industrial survey. Macaroni J. 58: 5.

AOAC. 1975. "Official Methods of Analysis." Association of Official Analytical Chemists, Washington, DC.

Chrzanowska, H. 1974. The determination of the maximum storage period for enriched pasta. Biuletyn Centralrego Laboritorium Technologii. Przetoworstw i Przechowalnietwa Zboz w Warsawie. 18:3/4.

Conrad, E. 1975. Applying HPLC to rapid analysis of food nutrients. Fod Prod. Dev. 9(9): 97.

Dennison, D., Kirk, J., Bach, J., Kokoczka, P., and Heldman, D. 1977. Storage stability of thiamin and riboflavin in a dehydrated food system. J. Food Proc. Preser. 1: 43.

Earle, P.L. and Rogers, M.C. 1941. Drying macaroni. Ind. Eng. Chem. 33: 642.

Egberg, D.G. and Potter, R.H. 1975. An improved automated determination of riboflavin in food products. J. Agric. Food Chem. 23(4): 815.

Kamin, H. 1971. "Flavins and Flavoproteins: Proceedings of the Third International Symposium on Flavins and Flavoproteins, Durham, NC. University Park, Press, Baltimore, MD.

Kamman, J.F. Labuza, T.P., and Warthesen, J.J. 1980. Thiamin and riboflavin analysis by high performance liquid chromatography. J. Food Sci. 45: 1497.

Kirk, J.R. 1974. Automated method for the analysis of riboflavin in milk, with application to other selected foods. J. Assoc. Off. Anal. Chem. 57: 1085.

Labuza, T.P. and Kreisman, L. 1978. "Open Shelf Life Dating of Foods." Office of Technology Assessment Contract OTA-C-78-001, Washington, DC.

Metzler, D.E. 1960. "The Enzymes." Academic Press, Inc., New York.

Osborne, D.R. and Voogt, P. 1978. "The Analysis of Nutrients in Foods," p. 210. Academic Press, London.

Parks, O.W. and Allen, C. 1977. Photodegradation of riboflavin to lumichrome in milk exposed to sunlight. J. Dairy Sci. 60: 1038.

Pelletier, O. and Madere, R. 1975. Comparison of automated and manual procedures for determining thiamin and riboflavin in foods. J. Food Sci. 40: 374.

Sattar, A. and deMan, J.M. 1973. Effect of packaging material on light induced quality deterioration of milk. J. Inst. Can. Sci. Technol. Aliment. 6: 170.

Sattar, A. and deMan, J.M. 1977. Light induced degradation of vitamins. I. Kinetic studies on riboflavin decomposition in solution. J. Inst. Can. Sci. Technol. Aliment. 10: 61.

Sebrell, N.H. Jr. and Harris, R.S. 1972. In "The Vitamins," Vol. 5, 2nd ed., Chap. 14. Academic Press,, New York.

Shimizu, S. 1950. Physicochemical properties of riboflavin. J. Ferment. Techn. (Japan) 28: 139 [Chem. Abstr. 47: 1555].

Singh, R.P., Heldman, D.R., and Kirk, J.R. 1975. Kinetic analysis of light induced riboflavin loss in whole milk. J. Food Sci. 40: 164.

Suzuki, A.T., Ohishi, N., and Yagi, K. 1979. Simple procedure for the separation of lumiflavin and lumichrome from photolysates of riboflavin. J. Chromotog. 169: 459.

Taufig, F. 1977. Moisture in macaroni—its causes and effects. Macaroni J. 58(7): 30.

—Continued on page 555

Determination of Neutral Detergent Fiber in Breakfast Cereals: Pentose, Hemicellulose, Cellulose and Lignin Content

R. MONGEAU and R. BRASSARD

ABSTRACT

Cereals are often promoted as important sources of dietary fiber. Ninety one breakfast cereals available in Canada and four unprocessed wheat brans were therefore analyzed for neutral detergent fiber (NDF) after rapid digestion with α-amylase from porcine pancreas. Many breakfast cereals contained less than 5% NDF. Wheat cereals contained 5–30% NDF, oat cereals contained 5% NDF, and corn and rice contained little NDF. Cereal NDF was predominantly hemicellulose (pentosans); pentose sugars liberated under hydrolysis represented up to 15% of processed bran cereals and 20–25% of unprocessed bran.

INTRODUCTION

AMONG DIETARY CHANGES that have occurred this century in the Western countries is a marked decrease in the intake of fiber, particularly from cereals (Heller and Hackler, 1978; Bingham et al., 1979). Fiber intake from fruits and vegetables has increased at the same time as the fiber intake from cereals has decreased (Burkitt, 1976). Although fruits and vegetables produce some of the effects of wheat on the colon (Kelsay et al., 1978), they have a low fiber content and they do not seem to compensate for the decrease in cereal fiber (Hendrikx, 1978). It is essentially cereal fiber that effectively prevents or relieves chronic constipation, the less disputed effect of dietary fiber (Trowell, 1978; Weill and Baumann, 1978) and special value is attributed to pentose-containing polysaccharides or the hemicellulose fraction (Cummings et al., 1978; Bingham et al., 1979).

Although cereals are considered to be important sources of fiber, there is little information concerning the quantity and composition of fiber in manufactured cereal foods. Breakfast cereals were therefore analyzed for the neutral detergent fiber (NDF), hemicellulose, cellulose and lignin and the values were compared. The hemicellulose fraction in NDF was also analyzed for pentose.

MATERIALS & METHODS

Preparation of samples

The cereal products analyzed were obtained from local groceries and supermarkets. A portion of each sample was weighed, freeze-dried and reweighed to determine the dry weight. Approximately 50g of each cereal product was ground with a Wiley Mill to pass through a 20 mesh screen and stored in a screw cap bottle. Before analysis, 5–10g of the stored sample was freeze-dried and reweighed, to measure the uptake of moisture during storage and to calculate the true quantity of dry matter taken for analysis.

Analytical procedures

Duplicate determinations of NDF and its components were performed by the methods of Goering and Van Soest (1970). The modifications from the original methods as well as the rapid procedure using α-amylase from porcine pancreas to remove starch are described below.

Authors Mongeau and Brassard are with the Nutrition Research Division, Food Directorate, Banting Research Centre, Tunney's Pasture, Ottawa, Canada K1A 0L2.

Amylase solution (5% w/v) was prepared by mixing 5 g of α-amylase powder (Sigma A6880) for 15 min with 100 ml of buffer solution pH 7 (61 ml 0.1M Na_2HPO_4 + 39 ml 0.1M NaH_2PO_4), centrifuging 10 min at 3000 rpm and filtering through a coarse sintered glass funnel. After refluxing of the cereal sample with neutral detergent, the contents were filtered through a tared Gooch crucible on the filtering manifold (Goering and Van Soest, 1970) and rinsed with hot distilled water. Next, 10 ml of α-amylase solution and 40 ml of distilled water at 70°C were added to the crucible, giving a mixture around 55°C. This was held for 5 min on the filtering manifold and then suction was applied to the filter which was then washed with hot distilled water. The bottom of the crucible was stoppered (with a rubber stopper #8) and 10 ml of α-amylase solution and 40 ml of distilled water at 70°C were added again to the crucible. The crucible was placed in an oven at 55°C. After 60 min of digestion, the crucible was removed from the oven and its contents were filtered and washed three times with hot distilled water and twice with acetone. The crucible was dried overnight at 100°C and weighed hot. The weight of the crucible was subtracted to obtain the net weight of NDF.

Acid detergent method

The crucible containing NDF was placed in a beaker suitable for refluxing (Goering and Van Soest, 1970). Acid detergent solution (100 ml) was added to the beaker and refluxed for 60 min from the onset of boiling. The external wall of the crucible was carefully washed inside the beaker with hot distilled water, and the crucible was placed on the filtering manifold. The contents of the beaker were filtered, and the residue was washed with hot distilled water and then with acetone. The crucible was dried overnight at 100°C and weighed hot. The difference between NDF and acid detergent residue was used as an estimate of insoluble hemicellulose.

Permanganate method

The crucible containing the acid detergent residue was placed in an enamel pan and treated for 90 min with a single 25-ml portion of permanganate solution (Goering and Van Soest, 1970). Glass rods were used to stir and wet all particles. After the aspiration of the remaining permanganate, the residue was treated with the demineralizing solution and washed with ethanol and then with acetone. The crucible was dried overnight at 100°C and weighed hot. The lignin content was calculated as the loss in weight from the acid detergent residue.

Sulfuric acid method

The residue of the permanganate treatment was treated with 72% sulfuric acid to separate cellulose from cutin. The crucible was placed in an enamel pan and half filled with 72% sulfuric acid. Glass rods were used to stir and wet all particles. Asbestos was not used. The crucible was replenished with sulfuric acid at hourly intervals. After 3 hr, the remaining sulfuric acid was removed by suction and the residue was thoroughly washed with hot distilled water. The crucible was then dried overnight at 100°C and weighed hot. Cellulose was calculated as the loss in weight from the permanganate treatment residue. Further loss of weight upon ashing (4 hr at 525°C) was interpreted as loss of cutin but these low values are not reported here.

Hot weighing procedure

The hot weighing procedure of Goering and Van Soest (1970) was used as described by Mongeau and Brassard (1979).

Measurement of pentose

The neutral detergent fiber was extracted from approximately

0.5g of dry sample, as described above. When necessary, more than one extraction was carried out until 0.1g NDF was obtained. NDF was treated with 20 ml 1N sulfuric acid (2½ hr at 100°C) for hydrolysis of the noncellulosic polysaccharides (Southgate, 1969). Hydrolyzed sugars were extracted with ethanol. The pentose content was measured with the Mejbaum's method, as modified by Albaum and Umbreit (1947). The hexose content was measured by the anthrone/thiourea procedure of Roe (1951) and used to correct the pentose value for interference in the color reaction (100 μg glucose gives a color equivalent to 5 μg arabinose).

RESULTS & DISCUSSION

DIETARY FIBER is generally defined as the plant food polymers (polysaccharides and lignin) which are resistant to hydrolysis by digestive secretions in the human upper gastrointestinal tract, but which serve as substrates for microbial fermentation in the colon (Trowell, 1978; Trowell et al., 1978; Van Soest, 1978). The definition of dietary fiber is related to the fiber hypothesis which claims a positive relationship between ingestion of plant food fiber and health. It is now thought that the pentose-rich sources of fiber such as unrefined cereals are the most effective to reduce the incidence of bowel disorder (Cummings et al., 1978).

The present paper provides data on the fiber content and composition of 91 breakfast cereals. Fig. 1 shows that the majority contained less than 5% NDF and that, at the time of sampling, there was only one breakfast cereal in the 15–25% NDF range. Concentrated bran cereals were thus well apart from the others in their fiber content.

Fig. 2 illustrates the results for 52 simple cereal products (the other 39 were mixed cereals). Puffed wheat cereals excepted, all wheat cereals had more than 5% NDF and half of them had more than 10% NDF. Oat cereals contained a significant amount of NDF but corn and rice cereals did not.

As in almost all sources of plant food fiber (Schaller, 1977; Hellendoorn, 1978; Southgate, 1979), the main component of cereal fiber was hemicellulose which represented about 65% of NDF weight while cellulose represented less than 25% (Fig. 3 and Table 1). Lignin represented the smallest fraction, except in oat fiber: 1g of oat NDF had less than half the cellulose but more than twice the lignin of 1g of wheat NDF. Hemicellulose was estimated by the difference between NDF and ADF (acid detergent fiber); lignin was estimated after the loss of weight following permanganate treatment and cellulose after the loss of weight following 72% sulfuric acid treatment. Anderson and Clydesdale (1980) analyzed the AACC wheat bran with a fractionation procedure and it can be calculated from their data that wheat dietary fiber contains 64–65% hemicellulose, 22–23% cellulose and 7–8% lignin; the proportions of fiber fractions measured in wheat breakfast cereals (Fig. 3) were in full agreement with these later data and with other published values (Southgate, 1979).

Rice and corn cereals had low NDF contents and their hemicellulose/cellulose (HC/C) ratios were not determined. Because of its lower cellulose content (Fig. 3), oats had a higher ($P<0.001$) HC/C ratio than wheat cereals (Table 2). Most types of wheat cereals had an HC/C ratio comparable to that in unprocessed bran. In puffed wheat, however, the ratio was significantly lower ($P<0.01$) than in shredded wheat, wheat germ, and unprocessed bran (Table 2). Puffed wheat cereals were found to contain 61% less hemicellulose ($P<0.001$), 42% less cellulose ($P<0.01$), and 8% less lignin than shredded wheat cereals. This suggests that the lower NDF content of puffed wheat cereals is due to the alteration of the hemicellulose and cellulose fractions during puffing. Although NDF values varied from 6.5–10.7 among the different shredded wheat cereals (Fig. 2), the proportions of hemicellulose, cellulose and lignin remained relatively constant.

Since the NDF method does not recover the soluble polysaccharides which are included in the definition of dietary fiber, the extent of underestimation of total dietary fiber in the present data was verified by measuring the soluble fiber content of different types of cereals with Southgate's method (Southgate, 1976). Table 3 shows that only small amounts were detected. These findings are in general agreement with those of Anderson and Clydesdale (1980) who found about 1% and 0.1% soluble fiber in wheat and corn brans, respectively. Rasper (1979), using the Southgate's method with amylolytic and proteolytic treatments, reported no soluble fiber in rice and negligible amounts in wheat and corn. Thus it seems that breakfast cereals contain little soluble fiber but since the amount recovered depends on the method used, the presence of significant amounts of soluble fiber in some cereals cannot be excluded. *—Text continued on page 553*

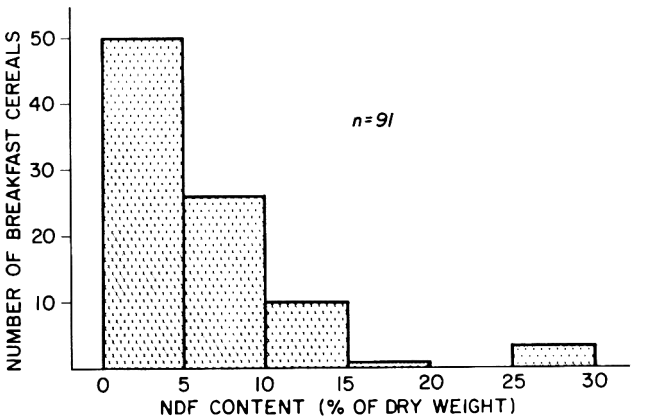


Fig. 1—Distribution of breakfast cereals according to their NDF content.

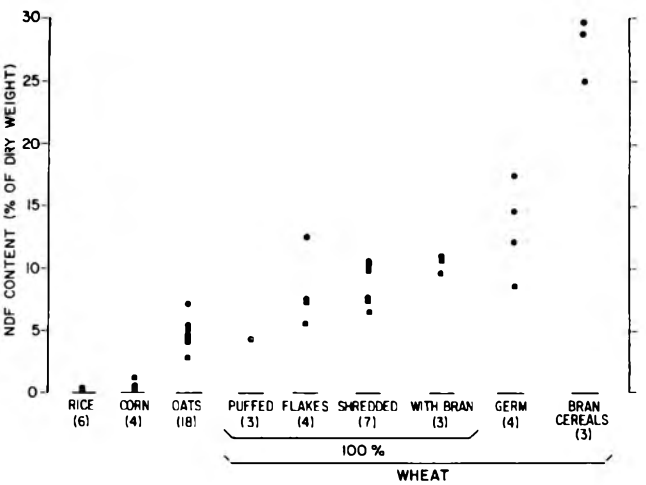


Fig. 2—NDF content of different types of breakfast cereals. The number of cereal products used in each case is shown in parenthesis.

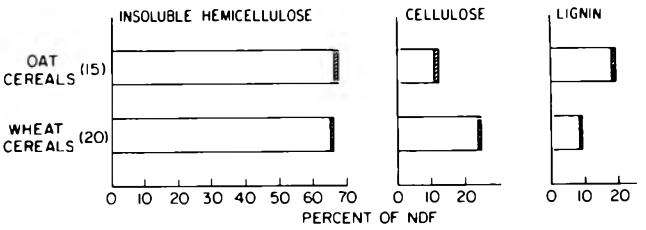


Fig. 3—Proportions of the fiber fractions in NDF of oat and wheat cereals. The number of cereal products used in each case is shown in parenthesis. Hatched areas represent the mean ± S.E.M.

Table 1—Neutral detergent fiber content and composition in breakfast cereals

No.	Name	Dry Weight (%)	Dry weight basis				Fresh weight basis	
			%NDF[a]	%HC[b]	%C[c]	%L[d]	NDF/ portion (g)	Weight of portion (g)
1.	Rice Flakes	99.0	–	–	–	–	–	28
2.	Puffed Rice	92.7	0.1[e]	–	–	–	–	14
3.	Puffed Rice	96.9	0.2	–	–	–	–	14
4.	Frosted Rice	99.4	0.3	–	–	–	0.1	28
5.	Rice Krispies	98.4	0.3	–	–	–	0.1	28
6.	Frosted Flakes	99.4	0.3	–	–	–	0.1	28
7.	Corn Flakes	98.6	0.5	–	–	–	0.1	28
8.	Corn Pops	90.7	0.6	–	–	–	0.2	28
9.	Special K	99.0	0.6	–	–	–	0.2	28
10.	Trix	86.9	0.9	–	–	–	0.2	28
11.	Honey Comb	98.7	0.9	–	–	–	0.2	28
12.	Froot Loops	99.4	0.9	–	–	–	0.3	28
13.	Apple Jack's	98.3	0.9	–	–	–	0.2	28
14.	Grain Team	97.9	0.9	–	–	–	0.2	28
15.	Cocoa Puffs	97.1	1.2	0.6	0.2	0.3	0.3	28
16.	Cap'n Crunch	99.5	1.3	0.7	0.2	0.2	0.4	28
17.	Count Chocula	96.4	1.3	0.5	0.2	0.6	0.4	28
18.	Franken Berry	97.9	1.4	0.8	0.3	0.2	0.4	28
19.	Cream of Wheat, Mix & Eat	90.7	1.5	1.2	–	–	0.4	28
20.	Wheatlets	89.6	1.6	1.1	0.1	–	0.4	28
21.	Sugar Smacks	98.9	1.6	1.0	0.3	0.2	0.4	28
22.	Alpha Bits	98.3	1.7	0.8	0.3	0.4	0.5	28
23.	Boo Berry	97.8	1.7	1.0	0.4	0.3	0.5	28
24.	Golden Honey	99.0	1.7	1.1	0.3	0.2	0.5	28
25.	Lucky Charm	96.9	2.0	1.2	0.3	0.4	0.5	28
26.	Sugar Crisps	97.3	2.1	1.0	0.5	0.3	0.6	28
27.	Cream of Wheat, Quick	91.8	2.4	1.8	0.1	0.3	0.6	28
28.	Cream of Wheat, Regular	93.3	2.4	1.9	0.3	0.1	0.6	28
29.	Product 19	98.9	2.6	1.4	0.4	0.5	0.7	28
30.	Granola Vita Crunch, apple & cinnamon	97.2	3.0	1.8	0.6	0.4	0.8	28
31.	Oats, raisin & spices	92.7	3.1	2.0	0.5	0.5	1.2	43
32.	Cheerios	95.9	3.3	1.9	0.5	0.8	0.9	28
33.	Harvest Crunch	99.2	3.5	2.1	0.8	0.5	1.0	28
34.	Harvest Crunch, raisin & dates	98.5	3.9	2.0	1.0	0.6	1.1	28
35.	Oats, maple & brown sugar	92.2	4.0	2.8	0.3	0.7	1.7	46
36.	Fluffs, Roasted Puffed Wheat	93.3	4.2	2.0	1.2	0.6	0.5	14
37.	Puffed Wheat	93.9	4.2	2.4	1.1	0.6	0.6	14
38.	Oats, cinnamon & spices	92.6	4.3	2.8	0.4	1.0	1.8	46
39.	Alpen	95.7	4.3	2.7	0.9	0.6	1.2	28
40.	Puffed Wheat	96.8	4.4	2.1	1.3	0.8	0.6	14
41.	Harvest Crunch, apple & cinnamon	99.7	4.5	2.5	1.4	0.4	1.3	28
42.	Oatmeal, apple & cinnamon	92.3	4.5	2.7	0.9	0.7	1.8	43
43.	Oats, apple & cinnamon	93.3	4.7	2.9	0.9	0.8	1.4	32
44.	Oatmeal, maple & brown sugar	94.1	4.7	3.0	0.6	0.8	2.1	48
45.	Oatmeal, regular, presweetened	92.2	4.7	3.0	0.5	1.0	1.9	43
46.	Granola Vita Crunch, honey & almonds	98.3	4.8	3.2	0.9	0.5	1.3	28
47.	Crunchy Granola & wheat bran	97.3	4.9	3.3	0.7	0.7	1.3	28
48.	Precooked Oats	91.8	4.9	3.3	0.9	0.7	1.7	37
49.	Oats, sugar & spices	91.8	5.1	3.6	0.5	0.9	1.6	34
50.	Crunchy Granola, honey & almonds	97.4	5.2	3.4	0.8	0.7	1.4	28
51.	Crunchy Granola, fruits & nuts	97.4	5.2	3.4	0.9	0.8	1.4	28
52.	Instant Oats, cook in 1 min.	90.5	5.2	3.3	0.7	1.0	1.3	28
53.	Oats, regular, ready to serve	91.3	5.3	3.6	0.5	1.0	1.5	31
54.	Old Fashioned Rolled Oats	90.8	5.4	3.5	0.6	1.0	1.4	28
55.	Oats, cook in 1 min	90.9	5.4	3.6	0.6	1.1	1.4	28
56.	Granola Vita Crunch, raisins	98.0	5.4	3.6	1.1	0.6	1.5	28
57.	Oats, Quick	90.9	5.4	3.6	0.8	0.9	1.4	28
58.	Quick Oats	90.8	5.5	3.6	0.6	1.0	1.4	28
59.	Old Fashioned Rolled Oats	90.5	5.6	3.8	0.8	0.8	1.4	28
60.	Scotch Oatmeal	92.6	5.6	3.9	0.6	1.1	1.5	28
61.	Pep	98.4	5.8	3.3	1.6	0.6	1.6	28
62.	Naturist Cereal, fruits	94.0	6.3	3.8	1.4	0.9	1.7	28
63.	Buckwheat Maple Flavored Wheat Cereal	99.0	6.4	3.5	1.7	0.9	1.8	28
64.	Naturist Cereal	93.3	6.4	3.8	1.6	0.8	1.7	28
65.	Mini Wheats, brown sugar	96.7	6.5	4.3	1.4	0.6	1.8	28
66.	Grape Nuts Flakes	97.1	6.8	4.2	1.8	0.7	1.8	28
67.	Oatmeal	92.9	7.0	4.7	0.7	1.4	1.8	28
		91.3	7.6	5.3	0.6	1.4	1.9	

(Continued)

Table 1—Continued

No.	Name	Dry Weight (%)	Dry weight basis %NDF[a]	%HC[b]	%C[c]	%L[d]	Fresh weight basis NDF/ portion (g)	Weight of portion (g)
68.	Shreddies	99.1	7.2[e]	4.7	1.7	0.5	2.0	28
		98.8	7.6	5.0	1.9	0.5	2.1	
69.	Wheaties	97.1	7.5	4.7	1.7	0.8	2.0	28
		98.8	7.3	4.9	1.8	0.6	2.0	
70.	Mini Wheats Frosted	95.8	7.0	4.9	1.6	0.5	1.9	28
		96.4	8.5	5.7	2.0	0.7	2.3	
71.	Wheetabix	96.0	8.2	5.1	2.1	0.9	2.5	32
		94.5	7.8	4.9	2.0	0.8	2.4	
72.	Vita B	91.4	8.3	5.7	1.4	1.0	2.1	28
		92.4	8.5	6.0	1.8	0.5	2.2	
73.	Crude Wheat Germ	89.3	8.2	5.6	2.1	0.3	2.1	28
		89.8	9.0	6.2	2.1	0.5	2.3	
74.	Grape Nuts	98.5	8.7	5.6	1.9	1.0	2.4	28
		98.6	8.4	5.6	1.8	0.8	2.3	
75.	Raisin Bran	93.9	9.4	5.7	2.6	0.9	2.5	28
		94.2	8.9	5.4	2.5	0.8	2.4	
76.	Bran Flakes	98.0	10.1	6.1	2.8	1.1	2.8	28
		98.0	9.3	5.8	2.7	0.7	2.6	
77.	Muffets, Malt Flavored	96.8	10.2	6.8	2.2	1.0	2.8	28
		96.5	9.8	6.4	2.3	0.9	2.6	
78.	Shredded Wheat	97.8	10.0	7.0	2.2	0.8	2.7	28
		94.8	10.3	6.9	2.4	0.8	2.7	
79.	Spoon Size Shredded Wheat	98.8	10.2	7.1	2.4	0.6	2.8	28
		97.0	10.7	7.3	2.4	0.9	2.9	
80.	Muffets	96.6	10.6	7.3	2.2	1.0	2.9	28
		94.7	10.8	7.4	2.6	0.6	2.9	
81.	Bran Crunchies	98.2	11.3	7.3	3.1	0.9	3.1	28
		98.8	10.1	6.7	2.4	0.9	2.8	
82.	Red River Cereal	93.2	10.8	7.0	2.2	1.4	2.8	28
		94.1	10.8	7.4	2.2	1.0	2.8	
83.	Bran Flakes	96.5	9.7	6.2	2.6	0.7	2.6	28
		98.3	12.2	7.8	3.4	0.8	3.4	
84.	Wheat Germ, regular	96.4	12.9	8.8	2.7	1.0	3.5	28
		96.6	11.3	7.4	3.1	0.6	3.1	
85.	Wheat Flakes	87.3	10.6	7.2	2.1	0.9	2.6	28
		86.2	14.7	10.1	3.1	1.5	3.5	
86.	Crackling Bran	99.4	14.1	9.0	3.2	1.6	3.9	28
		99.1	13.7	8.8	3.3	1.4	3.8	
87.	Wheat Germ	95.1	13.5	8.9	2.9	1.2	3.6	28
		94.3	15.8	9.9	4.2	1.5	4.2	
88.	Wheat Germ	89.1	17.6	11.9	3.6	1.6	4.4	28
		88.3	17.9	11.3	4.5	1.9	4.4	
89.	Bran Buds	99.0	26.4[e]	18.1	5.9	2.2	7.3	28
		98.6	23.0	14.4	6.4	2.0	6.3	
90.	100% Bran	98.8	28.5	18.9	6.8	2.8	7.9	28
		98.7	29.0	19.8	6.7	2.5	8.0	
91.	All Bran	98.5	29.9	20.2	6.9	2.5	8.2	28
		98.8	29.8	19.3	7.7	2.7	8.2	
92.	Bran, unprocessed	94.8	43.9	26.4	11.4	5.5	5.8	14
		93.0	42.6	27.1	10.6	4.2	5.5	
93.	Bran, unprocessed	90.1	53.7	34.9	13.8	4.7	6.8	14
94.	Bran, unprocessed	90.1	53.7	34.6	13.9	4.7	6.8	14
95.	AACC Bran	91.8	40.9	27.0	9.8	3.7	5.3	14

[a] NDF: neutral detergent fiber
[b] HC: insoluble hemicellulose estimated by the difference between NDF and ADF.
[c] C: cellulose.
[d] L: lignin.
[e] Mean of duplicate analysis: No. 1 to 66, one sampling; No. 67 to 92, two samplings at 6-month interval.

In the measurement of insoluble fiber, the NDF method and Southgate's method gave similar values with unprocessed wheat bran but substantial differences were observed with low fiber products, particularly Corn Flakes (Table 2): only 0.5% was recovered in the NDF method whereas 3.6% was recovered in Southgate's method. Since Paul and Southgate (1978) reported 12% total dietary fiber in Corn Flakes, we investigated it with the completely enzymatic method of Hellendoorn (Hellendoorn et al., 1975): the insoluble fiber residue remaining after pepsin and pancreatin digestions represented about 1% of the dry weight. The relative agreement between Hellendoorn's and the modified NDF method indicates that some material is digested by the pepsin plus pancreatin treatments (thus it is not dietary fiber) or by neutral detergent plus pancreatic α-amylase treatments, but not by the glucoamylase treatment in Southgate's method. The use of the appropriate enzyme preparation appears to be important in the determination of dietary fiber, specially in processed foods. We previously reported that unpurified preparations of

α-amylase from *Bacillus subtilis*, in addition to attacking hemicellulose, were unable to remove some types of artifact fiber easily removed by pancreatic α-amylase (Mongeau and Brassard, 1980). Presence of artifact (including products of the Maillard reaction) in fiber residue is associated with increased amounts of nitrogen in the acid-detergent residue (ADF) (Van Soest, 1965). ADF of two unprocessed brans (no. 92 and 95, Table 1) contained 0.5% and 0.3% nitrogen, respectively; the proportion of nitrogen remained within these values in the ADF of six processed bran products (no. 76, 78, 81, 89, 90, and 91), suggesting that artifact fiber was not present in either ADF or NDF recovered after digestion with the pancreatic α-amylase digestion.

It can be assumed that the food material removed from the NDF residue by digestion with porcine α-amylase would be digested in the human in the upper gastrointestinal tract. The division between dietary fiber and nonfiber material is not always easy because some "digestible" material (e.g. starch) may escape digestion and reach the colon as does dietary fiber. Modified starch and the products of the Maillard reaction are more resistant to endogenous enzymes and are more likely to reach the colon. Their presence in the colon modifies the bacterial environment but it is not known whether they function like dietary fiber. Some products of the Maillard reaction were reported to be the cause of diarrhea and depressed growth in rats (Lee et al., 1977). Moreover, before the products of the Maillard reaction are accepted as being equivalent to dietary fiber, their possible toxicity (Nurstein, 1981) and their anti-nutritional properties (Johnson et al., 1977; Reinhold and Garcia, 1979) should be further examined.

The use of enzymatic incubation with the NDF method usually prolongs the analysis an extra day. We observed that the pancreatic α-amylase preparation (Sigma A6880) can digest most of the residual starch (and some"artifact") within 5 min at 55°C. Further 60 min digestion of the washed residue improved reproducibility. We found that NDF values of various cereals were comparable when measured with this rapid NDF procedure or with the Schaller's modified NDF procedure (AACC #32-20). Twenty-eight of the breakfast cereals listed in Table 1 were among those recently analyzed by Baker and Holden (1981): they obtained for these cereals a mean of 5.54% NDF compared to 5.61% in Table 1. This shows that our rapid NDF method and the AACC-NDF method are in good agreement. The main advantages of the rapid procedure are its simplicity and reproducibility. Although glass wool was not used in the filtering crucible, filtration was easy with all breakfast cereal residues except one: filtration was slow but feasible with Bran Flakes NDF. Iodine tests were performed on various neutral detergent residues (held in refrigerator for at least 2 hr to promote iodine binding) and no colorable starch was observed. Only Corn Flakes NDF showed tiny, probably nonsignificant, dark spots. Thus it seems that the insoluble fiber values of the products reported in Table 1 are close to their true dietary fiber values. Relatively high sensitivity and good reproducibility were obtained, but equally satisfactory results may not be obtained without the use of the hot weighing procedure.

The ranking according to the NDF values (Table 1) should be interpreted with caution since no. 1 to 66 were sampled only once and the extent of variation is not known. On the other hand, no. 67 to 91 were resampled 6 months later and most of the results were well reproduced.

Hemicelluloses from NDF of 30 fiber-rich breakfast cereals were hydrolyzed with 1N sulfuric acid. The completeness of the hydrolysis was verified by using 2N sulfuric acid: pentose, hexose and uronic acid values increased by about 5%. The use of 3N sulfuric acid did not further increase the free sugar values. This suggests that the hydrolysis procedure (Southgate, 1969) prior to pentose analysis was close to completeness. Table 4 shows that pentose constituted about 49% of wheat fiber and about 28% of oat fiber. These values are in good agreement with the literature, from which it can be calculated that pentose

Table 2—Hemicellulose/cellulose (HC/C) ratio in breakfast cereals

	(n)	Mean ± SEM
Oats	(18)	5.61 ± 0.40*
Wheat		
puffed	(3)	1.77 ± 0.15[abc]
flakes	(4)	2.63 ± 0.30
shredded	(7)	2.96 ± 0.06[a]
with bran	(3)	2.30 ± 0.14
germ	(4)	2.75 ± 0.06[b]
bran cereal	(3)	2.73 ± 0.11
unprocessed bran	(4)	2.55 ± 0.10[c]

[abc] Values with the same superscript are significantly different ($P < 0.01$).
* Significantly different ($P < 0.001$) from wheat cereals.

Table 3—Dietary fiber values of different types of cereals by two methods

	(% of dry weight, duplicate analysis)			
	NDF[a]	Southgate's dietary fiber[b]		
	(Insoluble)	Insoluble	Soluble	Total
Rice Krispies	0.3	1.3	0.4	1.7
Corn Flakes	0.5	3.6	0.6	4.2
Oatmeal	5.4	6.2	0.5	6.7
Bran Flakes	9.7	10.7	2.2	12.9
AACC bran	41.0	40.6	1.5	42.1

[a] NDF: neutral detergent fiber after incubation (65 min at 55°C) with α-amylase from porcine pancreas (Sigma, A6880).
[b] According to Southgate (1976). Amyloglucosidase from *Aspergillus niger* (Sigma, A3514) was used for starch digestion (18 hr at 37°C).

Table 4—Pentose[a] content of breakfast cereal fiber

No.	Name	Dry weight basis g/100g NDF[a]	Dry weight basis g/100g sample	Fresh weight basis g/portion[b]
57.	Oats, Quick	28.6	1.5	0.4
67.	Oatmeal	26.1	1.9	0.5
68.	Shreddies	51.8	3.8	1.1
69.	Wheaties	50.4	3.7	1.0
70.	Mini Wheats, Frosted	46.2	3.6	1.0
71.	Wheetabix	54.8	4.4	1.3
72.	Vita B	50.4	4.2	1.1
73.	Crude Wheat Germ	52.7	4.5	1.2
74	Grape Nuts	50.2	4.3	1.2
75.	Raisin Bran	51.1	4.7	1.2
76.	Bran Flakes	49.0	4.8	1.3
77.	Muffets, Malt Flavored	43.0	4.3	1.2
78.	Shredded Wheat	46.2	4.7	1.3
79.	Spoon Size Shredded Wheat	47.4	5.0	1.4
80.	Muffets	46.2	4.9	1.3
81.	Bran Crunchies	49.8	5.3	1.5
82.	Red River Cereal	34.1	3.7	1.0
83.	Bran Flakes	53.6	5.9	1.6
84.	Wheat Germ, regular	47.6	5.8	1.6
85.	Wheat Flakes	46.8	5.9	1.5
86.	Crackling Bran	42.0	5.8	1.6
87.	Wheat Germ	47.8	7.0	1.9
88.	Wheat Germ	43.8	7.8	1.9
89.	Bran Buds	51.8	12.8	3.5
90.	100% Bran	52.8	15.2	4.2
91.	All Bran	50.4	15.1	4.2
92.	Bran, unprocessed	47.2	20.4	2.7
93.	Bran, unprocessed	48.2	25.9	3.3
94.	Bran, unprocessed	46.8	25.1	3.2
95.	AACC Bran	50.8	20.8	2.7

[a] This is the amount of pentose liberated from NDF by hydrolysis with 1N sulfuric acid; the value is corrected for interference of glucose (see Materials and Methods).
[b] See Table 1 for weight of portions.

sugars represent 50% of the wheat fiber (Southgate, 1979) and 21–30% of oat fiber (Southgate, 1978, 1979). Although there are few variations in the pentose content of cereals when expressed per gram NDF, there are wide variations when expressed per 100g sample due to varying fiber contents. Changes in colonic function are closely related to changes in intake of the pentosan-rich polysaccharides (hemicelluloses) of fiber, and a greater consumption of whole wheat cereals and breads has been recommended to supply an extra 3g of pentose daily (Cummings et al., 1978). These authors calculated that 13g of bran daily would provide this amount of pentose; we found that 14g of bran contains 2.7–3.3g pentose as pentosans (Table 4, no. 92–95).

The present data provide information on the amount and composition of insoluble fiber of breakfast cereals. But particle size of fiber is not known and large particles have been reported to be essential to produce a beneficial effect in the colon (Frexinos and Louis, 1978; Brodribb and Groves, 1978; Van Soest et al., 1978; Heller et al., 1980). A further report will provide other important characteristics of cereal fiber.

REFERENCE

Albaum, H.G. and Umbreit, W.W. 1947. Differentiation between ribose-3-phosphate and ribose-5-phosphate by means of the orcinol-pentose reaction. J. Biol. Chem. 167: 369.

Anderson, N.E. and Clydesdale, F.M. 1980. An analysis of the dietary fiber content of a standard wheat bran. J. Food Sci. 45: 336.

Baker, D. and Holden, J.M. 1981. Fiber in breakfast cereals. J. Food Sci. 46: 396.

Bingham, S., Cummings, J.H., and McNeil, N.I 1979. Intakes and sources of dietary fiber in the British population. Amer. J. Clin. Nutr. 32: 1313.

Brodribb, A.J.M. and Groves, C. 1978. Effect of bran particle size on stool weight. Gut 19: 60.

Burkitt, D.P. 1976. Some mechanical effects of fiber-depleted diets. In "Dietary Fibre." Proceedings of the Miles Symposium, 76, p. 26, Ed. W.W. Hawkins. Miles Laboratories, Ltd.

Cummings, J.H., Southgate, D.A.T., Branch, W., Houston, H., Jenkins, D.J.A. and James, W.P.T. 1978. Colonic response to dietary fiber from carrot, cabbage, apple, bran and guar gum. Lancet 1(8054): 5.

Frexinos, J. and Louis, A. 1978. Effet sur le poids des selles de trois produits contenant des fibres alimentaires. Gastroenterol. Clin. Biol. 2: 1055.

Goering, H.K. and Van Soest, P.J. 1970. Forage Fiber Analyses. U.S. Department of Agriculture, Handbook No. 379: 1.

Hellendoorn, E.W. 1978. Fermentation as the principal cause of the physiological activity of indigestible food residue. In "Topics in Dietary Fiber Research," p. 127, Ed. G.A. Spiller and R.J. Amen. Plenum Press, New York and London.

Hellendoorn, E.W., Noordhoff, M.G., and Slagman, J. 1975. Enzymatic determination of the indigestible residue (dietary fiber) content of human food. J. Sci. Food Agric. 26: 1461.

Heller, S.N. and Hackler, L.R. 1978. Changes in the crude fiber content of the American diet. Amer. J. Clin. Nutr. 31: 1510.

Heller, S.N., Hackler, L.R., Rivers, J.M., Van Soest, P.J., Roe, D.A., Lewis, B.A., and Robertson, J. 1980. Dietary fiber: the effect of particle size of wheat bran on colonic function in young adult men. Amer. J. Clin. Nutr. 33: 1734.

Hendrikx, M.E. 1978. Practical dietary research design and applications for southwestern American Indians. In "Topics in Dietary Fiber Research," p. 169, Ed. G.A. Spiller and R.J. Amen. Plenum Press, New York and London.

Johnson, G.H., Baker, D.H., and Perkins, E.G. 1977. Nutritional implications of the Maillard reaction: the availability of fructose-phenylalanine to the chick. J. Nutr. 107: 1659.

Kelsay, J.L., Behall, K.M., and Prather, E.S. 1978. Effect of fiber from fruits and vegetables on metabolic response of human subjects. Amer. J. Clin. Nutr. 31: 1149.

Lee, C.M., Chichester, C.O., and Lee, T.C. 1977. Effect of Maillard reaction products on disaccharidase activities in the rat. J. Agr. Food Chem. 25: 775.

Mongeau, R. and Brassard, R. 1979. Determination of neutral detergent fiber, hemicellulose, cellulose and lignin in breads. Cereal Chem. 56: 437.

Mongeau, R. and Brassard, R. 1980. Rapid digestion of starch and artifact fibre in the measurement of neutral detergent fibre of cereal products. Getreide Mehl und Brot 34: 125.

Nurstein, H.E. 1981. Recent developments in studies of the Maillard reaction. Food Chem. 6: 263.

Paul, A.A. and Southgate, D.A.T. 1978. "The Composition of Foods," Fourth ed. Elsevier/North-Holland Inc., New York.

Rasper, V.F. 1979. Chemical and physical properties of dietary cereal fiber. Food Technol. 40(1): 44.

Reinhold, J.G. and Garcia, J.S. 1979. Fiber of the maize tortilla. Amer. J. Clin. Nutr. 32: 1326.

Roe, J.H. 1951. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 212: 335.

Schaller, D. 1977. Analysis of dietary fiber. Food Prod. Dev. 11(9): 70.

Southgate, D.A.T. 1969. Determination of carbohydrates in foods. 2. Unavailable carbohydrates. J. Sci. Fd. Agric. 20: 331.

Southgate, D.A.T. 1976. The analysis of dietary fiber. In "Fiber in Human Nutrition," Ed. G.A. Spiller and R.J. Amen. Plenum Press. New York and London.

Southgate, D.A.T. 1978. Dietary fiber: Analysis and food sources. Amer. J. Clin. Nutr. 31: S107.

Southgate, D.A.T. 1979. The definition, analysis and properties of dietary fiber. In "Dietary Fiber: Current Developments of Importance to Health," Ed. K.W. Heaton. Food & Nutrition Press, Inc., Westport, CT.

Trowell, H. 1978. The development of the concept of dietary fiber in human nutrition. Amer. J. Clin. Nutr. 31: S3.

Trowell, H., Godding, E., Spiller, G., and Briggs, G. 1978. Fiber bibliographies and terminology. Amer. J. Clin. Nutr. 31: 1489.

Van Soest, P.J. 1965. Use of detergents in analysis of fibrous feeds. 3. Study of effects of heating and drying on yield of fiber and lignin in forages. J. AOAC 48: 785.

Van Soest, P.J. 1978. Component analysis of fiber in food. Amer. J. Clin. Nutr. 31: S75.

Van Soest, P.J., Robertson, J.B., Roe, D.A., Rivers, J., Lewis, B.A., and Hackler, L.R. 1978. The role of dietary fiber in human nutrition. Proc. Cornell Nutrition Conference, p. 5. Cornell University, Ithaca, NY.

Weill, J.P. and Baumann, R. 1978. Les fibres alimentaires: mythe ou réalité? Cahier de Nutrition et Diététique 13: 47.

Ms received 4/20/81; revised 10/14/81; accepted 10/16/81.

RIBOFLAVIN PHOTOCHEMICAL DEGRADATION . . . From page 549

Treadwell, G.E., Cairns, W.L., and Metzler, D.E. 1968. Photochemical degradation of flavins. 5. Chromatographic studies of the products of photolysis of riboflavin. J. Chromatography 35: 376.

United States Senate Select Committee on Nutrition and Human Needs. 1977. "Dietary Goals For The United States." Government Printing Office, Washington, DC.

United States Standard of Identity fo Enriched Macaroni Products, Code of Federal Regulations, Title 21, Food Drug Admin. Part 16, Sec. 16. 9-16. 12, 1955.

Van de Weerdhof, T., Wiersum, M.L., and Reissenweber, H. 1973. Application of liquid chromatography in food analysis. J. Chromatog: 83: 455.

Williams, D.C., Baker, D.R., and Schmidt, J.A. 1973. Analysis of water-soluble vitamins by high-speed ion-exchange chromatography. J. Chromatog. Sci. 11: 618.

Wills, R.B.H., Shaw, C.G., and Day, W.R. 1977. Analysis of water-soluble vitamins by high pressure liquid chromatography. J. Chromatog. Sci. 15: 262.

Ms received 6/15/81; revised 10/14/81; accepted 10/16/81.

Presented at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, 1980.

This project was supported in part by the University of Minnesota Agricultural Experiment Station Project Nos. 18-87 and 18-78, and Grant No. PFR-7910370 from the National Science Foundation. Scientific Journal Series Paper No. 11,807, Minnesota Agricultural Experiment Station.

Effect of Dietary Fiber Constituents on the In Vitro Digestibility of Casein

J. C. ACTON, L. BREYER and L. D. SATTERLEE

ABSTRACT

The in vitro digestibility of casein was substantially decreased by food type gums in the following order: karaya > ghatti > tragacanth > guar > locust bean. Extent of reduction of protein digestibility appeared to be related to the structure of the gum (degree of branching and extent of ionization). The fiber constituents, holocellulose, lignin, apple pectin, and the residues from protease predigested wheat bran and great northern bean, when present, significantly ($P < 0.05$) reduced casein digestibility. Gel filtration of the soluble portion from the casein hydrolysates containing pectin and bran or bean residues showed the presence of peptide fractions of larger molecular weight than those in a hydrolysate from the casein control. Results supported the hypothesis that dietary fiber constituents may reduce protein digestibility and increase nitrogen excretion through ionic interaction, matrix restriction, and modification of filtration characteristics by the fiber components tested.

INTRODUCTION

DIETARY FIBER has been defined as the complex of plant cell wall polysaccharides and lignins that are not hydrolyzed by the enzymes secreted into the human digestive tract (Trowell, 1974). Fiber constituents primarily include cellulose, hemicellulose, lignin, gums and other mucilages. Epidemiological evidence relating fiber intakes of various population groups to clinical manifestations of certain disease conditions has been of interest to nutritionists and food scientists alike (excellent reviews are in Spiller and Amen, 1975; 1976; Burkitt and Trowell, 1975; Spiller and Kay, 1980). Positive and negative physiological effects of the interaction of fiber components with specific nutrients of foods and the effects of fiber on gastrointestinal functions were recently reviewed by Kelsay (1978) and Anderson and Chen (1979).

Several studies with animals and humans have shown that increasing the dietary fiber content of diets affects nitrogen metabolism. In rat feeding trials, using from 0–30% cellulose, Meyer (1956) reported no significant change in endogenous nitrogen while the total nitrogen excretion significantly increased at the 15–30% cellulose levels. The presence of 10% "indigestible polysaccharides" in casein diets fed to rats was reported by Harmuth-Hoene and Schwerdtfeger (1979) to significantly increase fecal nitrogen excretion and significantly decrease the digestibility of casein. Nomani et al. (1979) reported similar results for four of six fiber materials fed at 2.1% of the diet at marginal intakes of protein and an energy level near the requirement for rat growth. In a study on the in vivo rat digestibility of nine cooked starches, Fleming and Vose (1979) found many instances where starch significantly lowered casein digestibility, when compared to a wheat starch-casein control diet. The lowest casein digestibility occurred for diets containing wrinkled field pea starch, which also possessed the lowest starch digestibility and the highest fiber content.

Low and high fiber diets in the human study of Kelsay et al. (1978) also demonstrated that a significant increase in fecal nitrogen excretion and a significant decrease in apparent protein digestibility occurred in subjects on a high fiber diet. Decreases in protein digestibility and increases of fecal nitrogen excretion as dietary fiber content increases have been noted in other studies with human subjects (McCance and Widdowson, 1947; Southgate and Durin, 1970; Walker, 1975). While some dietary fiber materials reduce the transit time through the small intestine, the effects on complex interactions between the different functions of the small intestine and the availability and absorption of nutrients has not been clearly established (Losowsky, 1979). In some populations, fiber-containing cereal and legume foodstuffs form a large portion of the diet and serve as the primary proteinaceous dietary constituent. In addition, with protein intake at a marginal level and at less than desirable quality (compared to animal source proteins), any decrease of protein digestibility through fiber interaction would affect overall nitrogen utilization.

In vitro testing of the possible interference with enzymatic activity (Schneeman, 1978) and protein hydrolysis (Harmuth-Hoene and Schwerdtfeger, 1979) have been inconclusive. Hsu et al. (1977) developed an in vitro multienzyme procedure to predict apparent protein digestibilities which correlated significantly with in vivo values from rat trials. The procedure has subsequently been used in computing protein efficiency ratios (Satterlee et al., 1979). This study was conducted to determine the effect of various fiber components on the in vitro protein digestibility assay. In addition, hydrolysates of a reference protein, ANRC casein, were evaluated to ascertain whether the size of the peptides generated were being affected by the presence of the fiber constituents, i.e., using another approach to ascertain if fiber affected proteolysis.

MATERIALS & METHODS

Sources of materials

De-lignified holocellulose from alfalfa and a lignin preparation (Indulin AT, Westvaco) were obtained from Dr. Jon Story, Dept. of Foods & Nutrition, at Purdue Univ. The lignin preparation was washed twice with glass distilled water, recovered by centrifugation, dried and finely powdered. The gums (karaya, ghatti, guar, tragacanth, carrageenan Type I, and locust bean) were purchased from Sigma Chemicals. Apple pectin was prepared by Speas Company (Kansas City, MO). The purity and methoxyl content of the pectin material was unknown and was used as obtained.

Great northern beans (cooked) and hard red winter wheat bran were pepsin digested prior to use using the following procedures. Fifteen grams of dry beans were conventionally cooked, drained, then macerated with mortar and pestle. The macerate was blended with 500 ml H_2O for 10 min, then made to 1L with additional water. The pH of the cooked bean preparation was adjusted to 2.0 with concentrated HCl and 150 mg of pepsin was added. The mixture was placed in a slow shaker bath and held at 37–38°C.

The work was conducted while Dr. Acton was a Visiting Professor with the Food Protein Research Group, Dept. of Food Science & Technology, Univ. of Nebraska-Lincoln, Lincoln, NE 68583. His present address is Dept. of Food Science, Clemson Univ., Clemson, SC 29631. Authors Breyer and Satterlee are with the Dept. of Food Science & Technology, Univ. of Nebraska, Lincoln, NE 68583.

After 72 hr, the pH was adjusted to 7.5–7.6 and held at 4°C for 72 hr. The pepsin-digested sample was then freeze-dried. Wheat bran was prepared using the same procedures described for the dry beans, but with the elimination of the inital cooking step.

The purpose of the above pepsin treatment of the bean and bran materials was to hydrolyze digestibile protein which would contribute to the pH reduction used in the digestibility procedure for casein. The proteolytic enzyme mixture of the in vitro assay was not used for predigestion due to the quantity required compared to the pepsin predigestion. No attempt was made to remove the food source peptides prior to subsequent gel filtration of digested casein-food material hydrolysates. Approximately 18% of the total peptides in the casein-bean hydrolysate and 10% of the total peptides in the casein-bran hydrolysate were potentially from the previously pepsin-treated bean and bran sources, respectively.

Acid detergent fiber

Acid detergent fiber content of the pepsin digested bean and bran residues were determined by the method of Van Soest (1973).

Digestibility procedure

ANRC sodium caseinate served as the reference protein for determining how fiber affects in vitro protein digestibility.

The in vitro digestion was conducted following the multienzyme method of Hsu et al. (1977) as improved by Satterlee et al. (1979). The enzymatic assay consists of a 37°C hydrolysis by trypsin, chymotrypsin and peptidase, followed by a 55°C hydrolysis by bacterial protease from *Streptomyces griseus*. Casein-fiber mixtures were prepared at fiber weight-to-protein weight ratios of 0.32, 0.64, and 0.96. Upon combining the casein and fiber in the dry form, both substances were hydrated for at least 2 hr prior to beginning the 4-enzyme digestion assay. Four to six determinations were made at each weight ratio. Results from the assay were expressed as the percent decrease in the digestibility from that of the casein control. Each fiber-containing sample was paired with the control and statistically analyzed by Student's t-test.

Gel filtration

In vitro enzymic hydrolysates of the selected fiber-casein combinations having the fiber weight-to-protein weight ratio of 0.96 were analyzed by gel filtration chromatography. Immediately after hydrolysis, the hydrolysates were placed in screw cap tubes, frozen at −20°C, and subsequently freeze-dried. The freeze-dried samples were taken up in eluting buffer to provide approximately 20 mg original casein protein/mL Samples were centrifuged at 27,000 x *g* for 30 min to remove insolubles and the supernatant filtered through an 0.8 μm filter (Millipore). One ml of the soluble portion of the hydrolysate was loaded onto a Sephadex G-50 column (0.9 x 60 cm) and eluted with 0.1M NaCl in 0.05M Tris-HCl buffer, pH 8.0. The effluent from the column was monitored using a 280 nm UV detector. Effluent fractions collected from the gel filtration of select casein hydrolysates containing pectin, gum ghatti, and predigested great northern bean were also analyzed for total carbohydrate (glucose equivalents) by the phenol-sulfuric acid procedure of Dubois et al. (1956) and peptides by the method of Lowry et al. (1951). Blue dextran (2 x 10^6 Daltons), myoglobin (17,600 Daltons), cytochrome C (12,380 Daltons), insulin (5,600 Daltons) and vitamin B_{12} (1,355 Daltons) were used as standards to calibrate the Sephadex G-50 column.

RESULTS

Casein digestibility

The in vitro protein digestibility of casein without addition of fiber was 90.0 ± 0.7%. In the presence of the various fiber constituents, a wide range of protein digestibilities for casein were noted for the various food gums (Fig. 1). Guar, locust bean and tragacanth gums had only a negligible to slight (0–1.2%) decrease (NS, $P > 0.05$) on the enzymatic hydrolysis of casein. Karaya, carrageenan and ghatti significantly ($P < 0.05$) decreased the in vitro digestibility of casein when present at the 0.32 ratio, and caused even greater reduction in the digestibility when present at the two higher levels. Due to the extreme viscosity of the carrageenan, the digestion had to be limited to the 0.32 ratio. Based on maximum inhibition of casein digestibility at the fiber ratios of 0.64 and 0.96, the reduction in digestibility by gums ranked as follows: karaya > ghatti > tragacanth > guar > locust bean (Fig. 1). Casein digestibility reductions greater than 1.48% were significant ($P < 0.05$) using the t-statistic for the in vitro assay.

Of the plant fibers and food residues studied (Table 1), holocellulose showed a stepwide reduction in casein digestibility to a maximum 2.14%, intermediate in effect to gums ghatti and tragacanth (Fig. 1). Apple pectin, lignin and the predigested bran and great northern bean residues significantly ($P < 0.05$) reduced casein digestibility, from 2.0–2.4% at the 0.32 ratio, to 5.3–5.5% at the 0.96 ratio. Within each ratio, the magnitude of the reduction in in vitro protein digestibility was the same for the pectin, lignin, and bran and bean residues. Acid detergent fiber values for the red wheat bran and great northern bean residues were 10.2% and 8.7%, respectively.

Gel filtration of casein hydrolysates

The elution patterns for several hydrolysates including the casein control and casein-fiber mixtures from gel filtration on Sephadex G-50 are shown in Fig. 2 (A, B and C). With minor exceptions, holocellulose (Fig. 2A) and lignin and karaya gum (Fig. 2B) had elution profiles similar to that of the casein control. The 280 nm absorbance profiles of the pectin and ghatti gum (Fig. 2A) hydrolysates were similar to the casein control with respect to the major fraction which had an elution volume, V_e of 35–37 ml, but showed other absorbing fractions near the void volume, V_o of 13.4 ml for ghatti gum and at the V_e of 18–23 ml for pectin. The major fraction of the casein hydrolysates was substantially altered in the presence of the bran and bean residues (Fig. 2C), with the V_e shifted to 28–30 ml from the V_e of 34–38 for the casein control.

–Continued on next page

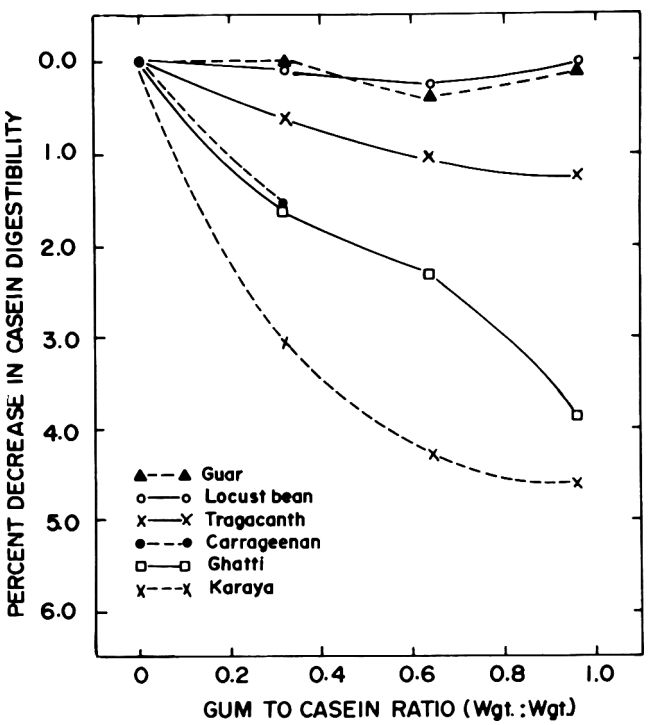


Fig. 1–Percent reduction of the in vitro digestibility of ANRC casein by various concentrations of food gums.

Fractions from the gel filtration of casein hydrolysates containing pectin, ghatti and bean residue were analyzed for carbohydrate and protein contents (Fig. 3A, B and C, respectively). For the pectin-casein hydrolysate (Fig. 3A), the material eluted between V_e = 13–26 ml contained both carbohydrate and partially digested (larger) casein peptides. The component eluting at the V_o from the ghatti-casein hydrolysate (Fig. 3B) was due to carbohydrate, whereas the peptides eluting at a V_e of 34 ml, were similar in V_e for peptides from the casein control. Very minor quantities of carbohydrate were noted in the bean residue-casein hydrolysate fractions (Fig. 3C). It is likely that the phenol-sulfuric acid method (Dubois et al., 1956) did not accurately measure the complex bean carbohydrates, or that they were removed by the centrifugation step. The major casein-peptide fraction of the bean-casein hydrolysate had a V_e of 28–30 ml and was clearly made up of larger units than was typical for those obtained from a hydrolyzed casein control.

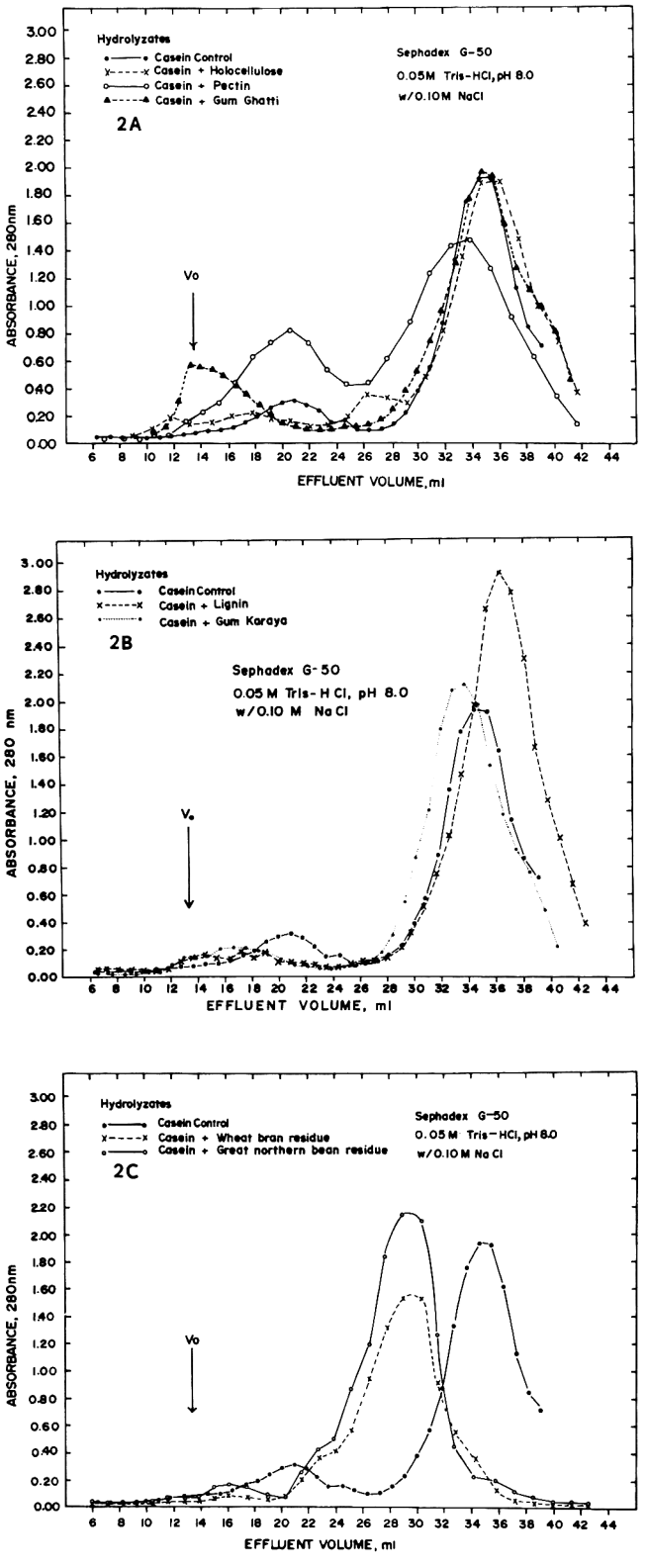


Fig. 2—Gel filtration (Sephadex G-50) of the soluble portion of enzymic hydrolysates from (A) casein control, and holo-cellulose-, pectin-, and gum ghatti-casein mixtures; (B) casein control, and lignin- and gum karaya-casein mixtures; and (C) casein control, and predigested wheat bran- and great northern bean residue-casein mixtures.

Based on the comparison of V_e for the casein control and the peptide fractions illustrated in Fig. 3A, B and C for casein mixtures with pectin, ghatti gum and the bean residue, and the casein control (Fig. 2A), approximate molecular weights for the peptides were calculated (Table 2). Very few differences were noted for the molecular weights of the small peptides (<4,000 Daltons), except for those present in the hydrolysate containing the bean residue, which were of a slightly higher molecular weight. The pectin-casein mixture showed the presence of several minor fractions (Fig. 3A) in the intermediate (4,000–12,000 Daltons) and large (>12,000 Daltons) molecular weight ranges, which were unique to this hydrolysate. The major peptide fractions of the bean residue-casein hydrolysate (Fig. 3C) were of approximately 9,300 and 2,980 Daltons, whereas the approximate molecular weight of the major casein peptide fractions for the casein control hydrolysate and those containing pectin and ghatti gum were 1,050 and 1,230 Daltons, respectively.

DISCUSSION

THE IN VITRO PROTEIN digestibility for casein (90.1%) is in agreement with the findings of Hsu et al. (1977) and of Satterlee et al. (1979). In vitro protein digestibilities obtained with this method are similar to the in vivo apparent digestibilities for a wide range of protein sources (Hsu et al., 1977).

For purposes of discussion of the digestibility results of the gum-casein mixtures, the general physical and chemical properties of the gums (degree of branching and presence of ionizable groups) referred to are those given by Whistler (1969). Guar and locust bean gums had negligible effects on casein digestibility (Fig. 1). Both polysaccharides are neutral and predominantly linear (single galactose unit branches) galactomannans of seed origin with no acidic or sulfonated end substituent groups. Tragacanth, a shrub exudate, is primarily a galacturonic acid polymer with some branching with hexoses and pentoses. The acid nature of

Table 1—Decrease in the in vitro digestibility of casein digested in the presence of various fiber constituents

Fiber constituent	Percent decrease in In Vitro protein digestibility[a]		
	Fiber:Protein ratio (wt:wt)		
	0.32:1	0.64:1	0.96:1
Apple pectin	2.42	4.08	5.41
Holocellulose	0.94	1.28	2.14
Lignin	2.03	3.83	5.42
Wheat bran (predigested)	2.14	3.94	5.30
Great northern bean (predigested)	2.36	3.94	5.52

[a] Value is % digestibility of control ANRC casein — % digestibility of ANRC casein plus fiber component.

the exposed uronic acid groups may be responsible for tragacanth's slight interference with the enzymatic hydrolysis of casein. Karaya and ghatti gums are tree exudates and while differing in saccharide composition, are highly branched polymers. Ghatti has a low content of glucuronic acid residues (approximately 3%) whereas karaya contains approximately 37% glucuronic acid. From the in vitro casein digestibilities obtained in the presence of these gums (Fig. 1), it is possible to suggest that the greater the degree of polymer branching, and/or the greater the content and exposure of ionizable uronic acid groups present in these fiber components, the greater will be the inhibition of casein hydrolysis. Although carrageenan was tested only at the 0.32 ratio to casein, its inhibition (Harmuth-Hoene and Schwerdtfeger, 1979) may be attributed to a high content of sulfate groups and its possible interaction with the protein and its peptides thereby inhibiting complete hydrolysis.

As hydrocolloids, food grade gums may form gel matrices and/or impart viscosity to the aqueous system through water absorption and colloidal interaction. Thus the physical structure of a fiber component may also limit substrate-enzyme interaction with the proteins and peptides, a characteristic possessed by some of the gums. From a practical viewpoint, the low levels of gums used in most formulated food products would not be expected to significantly inhibit protein digestion and amino acid absorption.

Of the plant fibers and food materials compared, holocellulose (consisting of cellulose and some hemicelluloses) showed the least detrimental effect on casein digestibility, when compared to the other fiber sources (Table 1). Cellulose is generally considered nonreactive due to its linear structure, insolubility and lack of electrostatic side groups. The nature of the hemicellulose contained in the preparation used in this study was not known. However, the fiber matrix slightly retarded the substrate-enzyme reaction when the holocellulose was tested at the higher levels (Table 1). Pectin, a slightly branched galacturonic acid-containing polysaccharide did substantially reduce the level of casein hydrolysis at all levels tested. In addition, large molecular weight casein peptides were present in the pectin-containing hydrolysates (Fig. 3A and Table 2). It is possible that pectin's negatively charged acidic residues (in the pH 6.0–8.0 range) interfered or complexed with the enzymes and/or substrates, thus reducing the extent of casein hydrolysis. In comparison to pectin, gum ghatti which is highly branched but void of acidic residues, interfered with casein hydrolysis (Fig. 1) and still yielded only small molecular weight casein peptides (Fig. 3B and Table 2). This finding supports the conclusion that gum ghatti's interference with casein hydrolysis was due to matrix restriction rather than the ionic interference as was suggested for pectin's action.

Table 2–Approximate molecular weights (Daltons) of peptide fractions in the soluble portion from casein hydrolysates containing various fiber components

Sample hydrolysate	Peptide fraction molecular weight range[a]		
	Large	Intermediate	Small
Casein-No fiber	13,040	–	1050
Casein-Gum ghatti	–	–	1230
Casein-Apple pectin	19,060	(a) 11,490	1230
		(b) 4,260	
Casein-Great northern bean (predigested)	–	9,300	2980

[a] Range designation: Large = >12,000 Daltons; Intermediate = 4000–12,000 Daltons; Small = <4000 Daltons.

The chemical complexity of lignins was reviewed by Gordon (1978). Although a noncarbohydrate component of dietary fiber, lignin may hinder enzymatic hydrolysis of substrates both physically and by the hydrogen bonding of its numerous internal ether and ester linkages to polar proteins and peptides in the hydrolysate. Lignin has been shown to actively bind bile salts in vitro (Story and Kritchevsky, 1976). No further explanation for the significant reduction of casein digestibility by lignin (Table 1) can be given. Gordon (1978) estimated that the lignin intake by humans is probably less than 2 to 3 g/day.

–Continued on next page

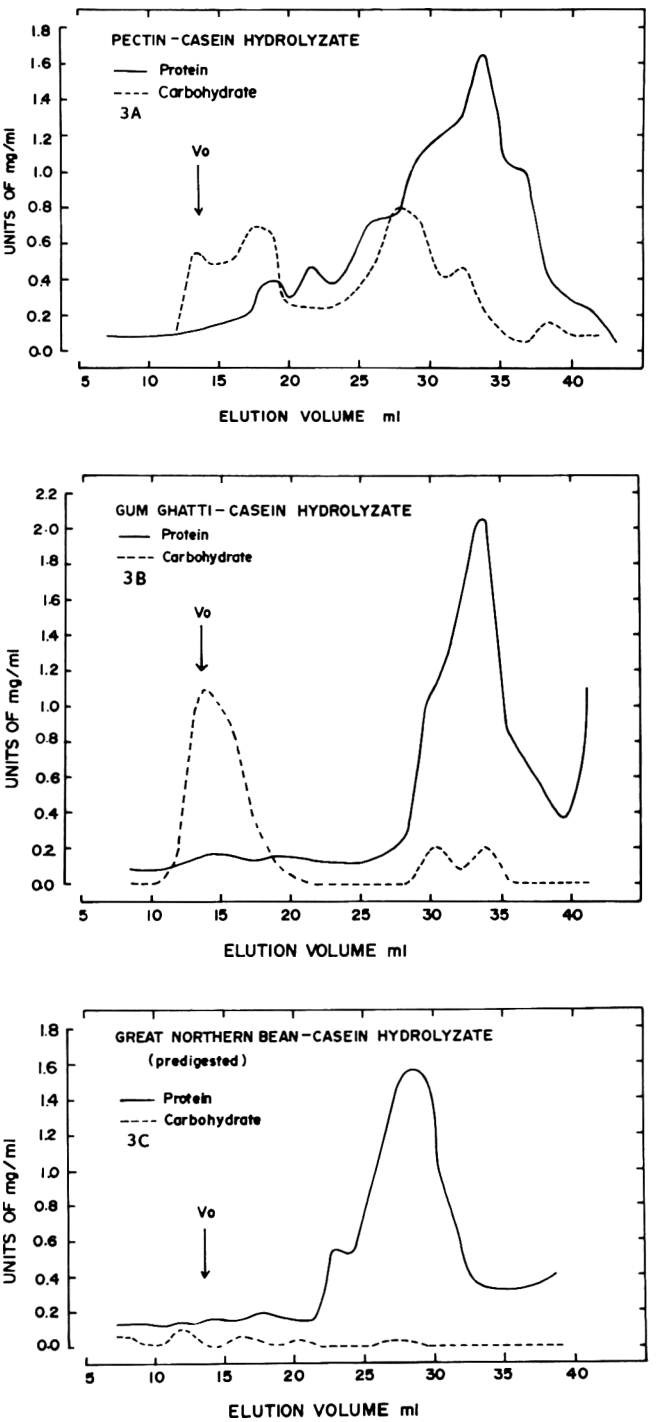


Fig. 3–Carbohydrate and protein contents of the effluent from Sephadex G-50 chromatography of (A) pectin-casein hydrolysate; (B) gum ghatti-casein hydrolysate; and (C) predigested great northern bean residue-casein hydrolysate.

The wheat bran and great northern bean residues obtained by pepsin pretreatment yielded similar reductions of casein digestibility when present during hydrolysis (Table 1). The casein-food material peptide patterns obtained from gel filtration of these hydrolysates were also similar (Fig. 2C). Even though both hydrolysates contained some peptides from the original food material, the profiles show obvious shifts from the expected lower molecular weight peptides of the referenced control casein hydrolysate. The fiber components present in the food materials were not defined; however, acid detergent fiber values for each indicated a slightly higher content of cellulose plus lignin for the bran (10.2%) as compared to the bean (8.7%). The mode of interference with casein digestibility by these food preparations, in the manner tested, will require greater characterization of the specific fiber constituents present. The major casein peptide fraction in the casein-bean residue hydrolysate had an approximate molecular weight of 2,980 Daltons as compared to 1,050 Daltons for the peptide fraction from the casein control (Table 2). A minor peptide fraction in the casein-bean residue hydrolysate was noted to have a molecular weight of 9,300 Daltons.

All samples in this study were fully hydrated prior to addition of the enzymes to prevent possible absorption of the enzymes into the fiber matrix, as was described by Schneeman (1978). Gel filtration of the soluble portion of each hydrolysate does not preclude the fact that some peptide components from casein hydrolysis remained with the insoluble residue removed upon centrifugation.

Studies by Rasper (1979), McConnell et al. (1974), and Childs and Abajian (1976) on various fibrous residues and potential fiber sources show that most function as weak cation exchange resins. Eastwood (1973) proposed that dietary fiber may act in the digestive tract as a chromatography column with adsorptive, ion exchange, and possibly gel filtration properties. The results of the current study with in vitro protein digestion technique lend support to the belief that some fiber materials have ionic properties, fiber matrix absorption and restrictive filtration characteristics which affect protein digestion. Restrictive filtration characteristics may be viewed as the degree of movement into and out of the fiber matrix by variable sized peptides to the exclusion of larger molecular weight proteins, whereas matrix absorption indicates physical and/or chemical binding to the fiber constituent. The reduction in casein digestibility reported here, generally agrees with in vivo studies which show decreases in the apparent digestibility of casein when certain fiber components are added to casein based animal diets.

REFERENCES

Anderson, J.W. and Chen, W.L. 1979. Plant fiber: Carbohydrate and lipid metabolism. Am. J. Clin. Nutr. 32: 346.

Burkitt, D.P. and Trowell, H.C. 1975. "Refined Carbohydrate Foods and Disease: Some Implications of Dietary Fiber." Academic Press, New York.

Childs, E. and Abajian, A. 1976. Physico-chemical characterization of peanut hull as a potential fiber additive. J. Food Sci. 41: 1235.

Dubois, M., Giles, K.A., Hamilton, J.K., Rebers, P.A., and Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Eastwood, M.A. 1973. Vegetable fiber: Its physical properties. Proc. Nutr. Soc. 32: 137.

Fleming, S.E. and Vose, J.R. 1979. Digestibility of raw and cooked starches from legume seeds using the laboratory rat. J. Nutr. 109: 2067.

Gordon, A.J. 1978. The chemical structure of lignin and quantitative and qualitative methods of analysis in foodstuffs. In "Topics in Dietary Fiber Research," E. Spiller, G.A., p. 59. Plenum Publishing Co., New York.

Harmuth-Hoene, A.E. and Schwerdtfeger, E. 1979. Effect of indigestible polysaccharides on protein digestibility and nitrogen retention in growing rats. Nutr. Metab. 23: 399.

Hsu, H.W., Vavak, D.L., Satterlee, l.D., and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Kelsay, J.L. 1978. A review of research on effects of fiber intake on man. Am. J. Clin. Nutr. 31: 142.

Kelsay, J.L. Behall, K.M., and Prather, E.S. 1978. Effect of fiber from fruits and vegetables on metabolic responses of human subjects. I. Bowel transit time, number of defecations, fecal weight, urinary excretions of energy and nitrogen and apparent digestibilities of energy, nitrogen, and fat. Am. J. Clin. Nutr. 31: 1149.

Losowsky, M.S. 1979. Effects of dietary fiber on intestinal absorption. In "Dietary Fiber: Current Developments of Importance to Health," Ed. Heaton, K.W., p. 124. Technomic Publishing Co., Inc., Westport, CT.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent J. Biol. Chem. 193: 265.

McConnell, A.A., Eastwood, M.A., and Mitchell, W.D. 1974. Physical characteristics of vegetable foodstuffs that could influence bowel function. J. Sci. Fd. Agric. 25: 1457.

McCance, R.A. and Widdowson, E.M. 1947. The digestibility of English and Canadian wheats with special reference to the digestibility of wheat protein by man. J. Hyg. 45: 59.

Meyer, J.H. 1956. Influence of dietary fiber on metabolic and endogenous nitrogen excretion. J. Nutr. 58: 407.

Nomani, M.Z.A., Fashandi, E.F., Davis, G.K., and Bradac, C.J. 1979. Influence of dietary fiber on the growth and protein metabolism of the rat. J. Food Sci. 44: 745.

Rasper, V.F. 1979. Chemical and physical properties of dietary cereal fiber. Food Technol. 33: 40.

Satterlee, L.D., Marshall, H.F., and Tennyson, J.M. 1979. Measuring protein quality. J. Am. Oil Chem. Soc. 56: 103.

Scheeman, B.O. 1978. Effect of plant fiber on lipase, trypsin and chymotrypsin activity. J. Food Sci. 43: 634.

Southgate, D.A.T. and Durin, J.V.G.A. 1970. Calorie conversion factors. An experimental reassessment of the factors used in the calculationof the energy value of human diets. Brit. J. Nutr. 24: 517.

Spiller, G.A. and Amen, R.J. 1975. Dietary fiber in human nutrition. Crit. Rev. in Food Sci. Nutr. 39: 69.

Spiller, G.A. and Amen, R.J. (Ed.). 1976. "Fiber in Human Nutrition." Plenum Publishing Co., New York.

Spiller, G.A. and Kay, R.M. (Ed.). 1980. "Medical Aspects of Dietary Fiber." Plenum Publishing Co., New York.

Story, J.A. and Kritchevsky, D. 1976. Dietary fiber and lipid metabolism. In "Fiber in Human Nutrition," Ed. Spiller, G.A. and Amen, R.J. (Ed.), p. 171. Plenum Press, New York.

Trowell, H. 1974. Definitions of fiber. Lancet 1: 503.

Van Soest, P.J. 1973. Collaborative study of acid detergent fiber and lignin. J. AOAC. 56: 781.

Walker, A.R.P. 1975. Effect of high crude fiber intake on transit time and the absorption of nutrients in South African Negro school children. Am. J. Clin. Nutr. 28: 1161.

Whistler, R.L. 1969. Pectins and gums. In "Carbohydrates and Their Roles," Ed. Schultz, H.W., Cain, R.F., and Wrolstad, R.W. (Ed.), p. 73. AVI Publishing Co., Inc., Westport, CT.

Ms received 6/25/81; revised 9/18/81; accepted 10/14/81.

Published as Paper No. 6614, Journal Series, Nebraska Agricultural Experiment Station. Research reported was funded in part under Project No. 16-028 and by the FPRG grant.

Taste Thresholds and Hedonic Responses of Panels Representing Three Nationalities

LOREN L. DRUZ and RUTH E. BALDWIN

ABSTRACT

Taste thresholds for sodium chloride, sucrose, citric acid and caffeine in aqueous solutions did not differ among panels from Nigeria, Korea, and the United States. The Nigerians and Koreans liked tomato juice more than the Americans, and preferred it sweetened. The Koreans liked applesauce better than the other two nationalities, but did not differentiate among the sweetened applesauce, that containing sodium chloride and the control. Americans liked the control, and Nigerians liked the sweetened applesauce best. Frequency of consuming groups of foods by panelists was related to the trend toward liking tomato juice with sweet, sour, salty, and bitter, and applesauce with sweet and salty taste substances added, but there was no significant relationship between hedonic responses and thresholds.

INTRODUCTION

RAPIDLY DEVELOPING COUNTRIES, such as Nigeria and Korea, offer tremendous profit potential to the international marketer (Colamosca, 1977). These markets, however, are characterized by economic uncertainty and by diverse cultural factors influencing food acceptance. Pangborn (1967) suggested that eating patterns of the cultural environment may subjugate taste and olfactory sensitivity to such an extent as to invalidate them as factors influencing regulation of food intake. Also, Jerome (1977), in discussing preferences for a sweet taste in adults, stated that the physical, social and technological environment, including marketing and distribution, are more closely related to preference than early stimulation of the taste buds.

Moskowitz et al. (1975) imputed a preference among Indian laborers for bitter and sour tastes to the dominance of sour taste in their usual diet. Indian medical students, not consuming the predominantly sour diet, perceived sweet as pleasant as did Americans. Kelty and Mayer (1971) also noted that strong acceptance and rejection responses to the primary tastes influence dietary choices. However, acceptance and rejection are determined, in part, by intensity of the taste. As a sweet stimulus increases in magnitude, its hedonic tone increases toward pleasantness, reaches a maximum, and declines toward neutraility or unpleasantness. Intensity may parallel hedonic response with some tastes such as the bitterness of quinine sulfate (Moskowitz, 1977). Food dislikes have been linked to sensitivity to bitter (Fischer et al., 1961), and the high acceptance of coffee by Yucatan inhabitants has been attributed to their diminished sensitivity to the bitter taste as compared to Americans (Davis, 1978).

Despite the complexity of factors influencing food acceptance, information on sensitivity and hedonic response to primary tastes among diverse cultural groups can be invaluable to the international marketer. This study was designed to examine the sensitivity of American, Nigerian and Korean subjects to primary tastes, their hedonic responses to tomato juice and applesauce with differing levels of primary taste substances added, and the composition of their usual diets.

Authors Druz and Baldwin are affiliated with the Dept. of Food Science & Nutrition, Univ. of Missouri-Columbia, Columbia, MO 65211.

MATERIALS & METHODS

SENSORY TESTS were conducted with eight panelists, each from the United States, Nigeria, and Korea. Panelists were rewarded for participation in the project with a small financial remuneration. Nigerian and Korean panelists were selected with the assistance of the Center for International Studies at the University of Missouri-Columbia. Americans who expressed interest in the project, as well as students from food science classes at the University were enlisted for participation. Panel size was limited by the availability of Nigerian and Korean subjects.

Survey of food habits of panelists

Demographic data for each panelist was obtained by written questionnaire. Background information on food consumption before and after coming to the United States was obtained from Nigerian and Korean panelists by interview following a pre-set line of questioning, and American subjects were asked about their current eating practices.

Panel procedures

Four practice sessions were conducted to orient each group of panelists to the process and procedures for sensory analysis. Panelists were unaware of the particular taste series being tested at one time, but were familiarized with the primary tastes and with the taste of the double-distilled water used for preparation of solutions and for rinsing the palate before tasting each sample. Panelists were instructed to expectorate both samples and rinse water. All tests were conducted, mid-morning, Monday through Friday, in an air-conditioned (24°C) taste panel facility, and panelists were seated in individual booths. Communication via a two-way light switch in each booth minimized verbal interaction between panelists and server.

Threshold determinations for primary taste in aqueous solutions

Aqueous solutions of sucrose, citric acid, caffeine, and sodium chloride, as well as glass double-distilled water, were prepared 24 hr before testing. The solutions were stored at room temperature (24°C) until used. The six concentrations tested for each primary taste substance are listed in Table 1.

Ten-ml samples were poured 1 hr prior to serving. Panelists were presented two samples at a time. Each was coded with a 3-digit random number. One contained redistilled water and the other was the test solution. The order of serving pairs and samples within pairs was randomized. Additionally, the order of presentation for each primary taste series was randomized for each panelist. Panelists were asked to identify the sample with the most intense taste within each pair. All six pairs of samples from each of the four primary taste series were evaluated at each of the four panel sessions.

Hedonic scoring of tomato juice and applesauce

Tomato juice and applesauce were selected for this study because the primary taste substances could be dispersed uniformly in them. In orientation and practice sessions, it was determined that both foods were acceptable to panelists.

Sufficient quantities of tomato juice and applesauce for the study were obtained at a local market. Sucrose, citric acid, caffeine, and sodium chloride were added to these products 24 hr before testing and stored in a refrigerator (4°C). Concentrations of primary

taste substances added are listed on Fig. 1. Samples were removed from refrigeration 3 hr before panels began and achieved a temperature of 24°C before serving. Fifteen-ml samples of tomato juice were poured into 57-ml opaque odorless plastic cups, each bearing a 3-digit random code. Twenty-gram samples of applesauce were portioned with a small scoop (No. 60) into 85-ml randomly coded plastic souffle cups. Each sample of applesauce was accompanied by a clean plastic spoon for tasting. The samples of tomato juice and applesauce were evaluated on a 5-point hedonic scale. Order of serving tomato juice and applesauce samples was randomized for each panelist for each of the four replications.

Statistical analyses

Descriptive terms were assigned numerical values so that analysis of variance (Snedecor and Cochran, 1967) could be applied to the data for both frequency of consuming foods and hedonic scores. The term denoting the most frequent consumption, "daily", was given a value of 5, and that describing the least frequent consumption, "never," was given a value of 1. Similarly, the phrase, "like very much," was assigned a value of 5, and "dislike very much," was given a value of 1. Differences ($P < 0.05$) among means were located by applying Duncan's (1955) New Multiple Range Test. Coefficients of determination (Huntsberger, 1961) were computed between individual panels and frequency of consuming specific food groups, thresholds, and mean hedonic responses.

To determine taste thresholds, a linear regression of positive responses (Snedecor and Cochran, 1967) was calculated for each panel for each primary taste. The threshold was designated as the concentration at which 50% of the judges were able to detect ($P = 0.05$) the presence of the primary taste (Amerine et al., 1965).

RESULTS & DISCUSSION

THE MAJORITY of all three panels were from urban areas in their respective countries. For the American, Nigerian, and Korean panels, the mean age was 21.3, 26.5, and 36.6 yr, respectively. In addition, the members of the Korean panel tended, on the average, to have had more years of formal education than Nigerian or American panelists.

Food consumption patterns

American panelists consumed food groups with a dominant sweet taste (desserts, candies, and soft drinks) more often than either Nigerians or Koreans. Frequency of consumption was similar among the three panels for sour and bitter food groups including fermented milk products, coffee, and tea. Koreans ate salty foods more often than the Nigerians, who included this group of foods in their diets more often than Americans.

Nigerians mentioned the following typical foods as being frequently consumed in their native country: ogi, a fermented corn meal porridge; agidi, a semi-solid fermented corn meal product; Ovaltine, and evaporated milk. Sungyung, rice tea; kim-chee, pickled vegetables, and twenchang-ku, fermented bean soup, were the frequently consumed native foods of the Koreans when in their own country. Americans reported pizza, hamburgers, and submarine sandwiches as frequently consumed foods.

Both the Nigerians and Koreans altered their food habits after coming to the United States. Nigerians increased their consumption of sweet foods, cheese, and processed foods and reduced their intake of fish and fermented grains. Koreans increased their intake of meat, corn and wheat, cheese, processed fruits and vegetables, and desserts and reduced their consumption of fish, soy, and rice.

Thresholds for primary tastes in solution

Sensitivities of the three panels to the primary taste substances in aqueous solution were similar (Table 2). Statistically significant ($P < 0.05$) differences were found neither among thresholds nor the slopes of the regression lines plotted for each panel. This is in contrast to the findings of Johansson et al. (1973) who reported a difference in the threshold for salt among different nationalities. In their investigation, peoples from the Netherlands had lower thresholds for this primary taste substance than peoples from Sweden, the United States, and Poland among whom there was no significant difference.

Hedonic responses to tomato juice and applesauce with added primary tastes

In general, the Nigerian and Korean panels liked tomato juice more than the American panel. Analysis of variance (Snedecor and Cochran, 1967) and separation of means by Duncan's (1955) New Multiple Range test revealed the following statistically significant ($P < 0.05$) differences. The Nigerian and Korean panels preferred sweetened (2% sucrose) tomato juice, whereas the Americans liked the control tomato juice better than tomato juice with sucrose, citric acid, caffeine or NaCl added. Americans rated tomato juice with a sour taste (0.2% citric acid) second best, while the Nigerians and Koreans liked this treatment least. All panels liked tomato juice with the low concentrations of the primary taste substances added better than samples with higher concentrations of the same additive (Fig. 1).

Data grouped according to nationality indicated that the Korean panel liked applesauce more than the Nigerians, who in turn liked this product better than the Americans. The Americans preferred the control sample of applesauce while the Nigerians liked this product best when it contained 8% sucrose. The Korean panel rated the control, the sweetened applesauce, and the sample containing 0.2% NaCl as most liked and did not differentiate among these treatments (Fig. 1).

Interrelationships

Coefficients of determination (r^2) revealed that dietary history, as reflected by frequency of food group consumption, was closely related to affective responses to tomato juice with sweet ($r^2 = 0.99$), sour ($r^2 = 0.96$), bitter ($r^2 = 0.94$), and salty ($r^2 = 0.73$) substances added and to applesauce with sweet ($r^2 = 0.88$) and salty ($r^2 = 0.94$) taste substances added. Other r^2 values were low indicating little relationship between frequency of food group consumption and hedonic response to applesauce with added sour ($r^2 = 0.09$) or added bitter ($r^2 = 0.43$) taste substances. Only one strong relationship between a threshold for a primary taste and food consumption was apparent. Thresholds for caffeine were directly associated with frequency of consuming foods with a prominent bitter taste ($r^2 = 0.81$). This supports the findings of Davis (1978) that peoples who customarily ingest large quantities of bitter tasting products, such as coffee, have low sensitivities to this primary taste.

Table 1—Six concentrations of aqueous solutions of four primary taste substances

Taste substances	Concentrations (% w/v)					
Sucrose	0.3	0.15	0.075	0.0375	0.01875	0.009375
Citric acid	0.0128	0.0064	0.0032	0.0016	0.0008	0.0004
Caffeine	0.008	0.004	0.002	0.001	0.0005	0.00025
Sodium chloride	0.01	0.005	0.0025	0.00125	0.000625	0.0003125

IMPLICATIONS

ALTHOUGH THIS STUDY was conducted with a limited number of subjects, all of whom currently reside in the United States, the data suggest that food processors competing in culturally diverse markets can delineate food preference and formulate products to maximize the potential for success. Affective responses, not sensory thresholds to primary tastes, are applicable. The data from this study suggest that both tomato juice and applesauce would have a greater acceptance in Nigeria and Korea if sweetened to a higher level than is currently customary in the United States. If unable to assess affective responses, the firm entering international markets could substantially reduce

—Continued on page 569

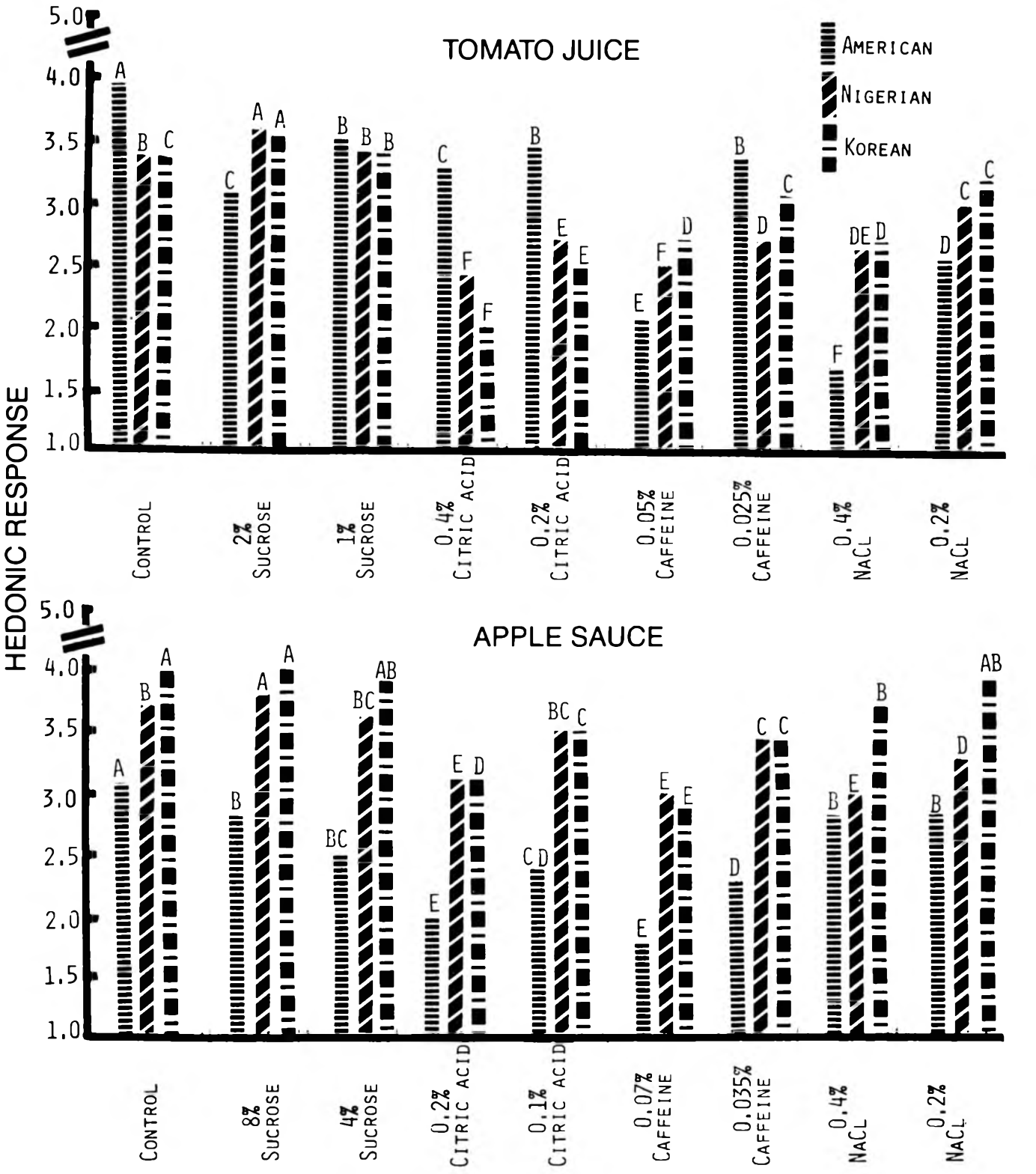


Fig. 1—Mean hedonic scores of panels representing three nationalities: (1 = dislike very much, to 5 = like very much. Where letters above bars differ within a nationality, means differ significantly, $P < 0.05$).

Quality Profiles of Rice: A Tentative Scheme for Classification

K. R. BHATTACHARYA, C. M. SOWBHAGYA, and Y. M. INDUDHARA SWAMY

ABSTRACT

Based on a study of 177 samples, rice varieties could be tentatively classified into eight quality types primarily on the basis of total and hot-water-insoluble amylose contents and viscogram pattern. Rice of type I (high total and insoluble amylose) cooked very hard and flaky, while that of type VIII (waxy, negligible amylose) cooked very soft and sticky. Gelatinization temperature (GT) could be indexed by either alkali score or water-uptake ratio or soluble-amylose ratio. Equilibrium moisture attained by rice upon soaking was a combined function of the amylose content, the GT, and kernel chalkiness. The viscogram pattern was largely, but not wholly, a function of the amylose. Protein content was not related to other properties.

INTRODUCTION

DESPITE CONSIDERABLE PROGRESS in the last few decades, much still remains to be understood about the physicochemical basis of rice grain quality (Juliano, 1979). In an earlier study with 32 varieties (Bhattacharya et al., 1978), we showed the importance of a new factor—the insoluble amylose content—as a key determinant of rice quality, and additionally observed that the experimental samples could be classified into a few groups with distinct quality profiles.

In a continuation of these studies, a much larger lot (177 samples, 169 varieties), representing a broad cross-section of world's rice (but with emphasis on India), has now been analyzed. The importance of various properties as indices or determinants of rice quality as well as their interrelations have been tested. Further, a tentative scheme for classification of world's rice on the basis of quality has been drawn up (this scheme was briefly reported in a note: Bhattacharya et al., 1979b).

MATERIALS & METHODS

Rice

A brief description of the samples is given in Table 1. A majority of them (106 samples, 100 varieties) represented popular traditional (tall) rice of various Indian states, collected from the respective state experimental farm as detailed elsewhere (Bhattacharya et al., 1980). The remaining 71 samples (69 varieties) comprised of various other Indian and foreign rices as explained in the Table, and were procured either from the Central Rice Research Institute, Cuttack, or the University of Agricultural Sciences Experiment Station at Mandya.

The paddy samples (1–2 kg each) were thoroughly dried in air, fumigated, and then stored in cloth bags in large metal drums in the laboratory at room temperature (24–34°C). As stored, their moisture contents lay in the range 11–13% (wet basis). The bulk of the analyses were carried out between 0.75 and 2.0 yr after their harvest, but certain tests (see later) were conducted much later.

Paddy was milled in the laboratory using a McGill or a Satake sheller and a McGill miller No. 3 by standard methods (to 8–10% degree of milling by weight). Rice was ground for amylose and viscograph tests in a Buhler disc grinder (MLI 204) and then in a Raymond hammer mill to roughly 65 mesh.

Analytical methods

Total (Sowbhagya and Bhattacharya, 1971) and hot-water-insoluble (Bhattacharya et al., 1972) amylose contents were determined in undefatted flour in all the samples. They were also determined in defatted flour in about 25 selected samples representing the entire range. The undefatted-flour values in the samples other than these 25 were then converted to defatted-flour values by reading from a defatted- vs undefatted-value graph prepared from the 25 samples. Alkali score and type (Bhattacharya, 1979; Bhattacharya and Sowbhagya, 1972, 1980); equilibrium moisture content attained by whole-grain milled rice when soaked in water at room temperature (EMC-S) (Indudhara Swamy et al., 1971); ratio of apparent

Table 1—Identification of experimental rice samples

Broad group	Amylose class		Number of Samples	Number of Varieties
Popular traditional (tall) Indian	High		79	73
	Intermediate:	scented	12	12
		nonscented	9[a]	9
	Low		4[a]	4
	Waxy		2	2
			106	100
Other traditional (tall) Indian	High		2	2
	Intermediate:	scented	13	12
	Waxy		8[b]	8
			23	22
Modern (semidwarf) Indian	High		28	27
	Intermediate		2	2
	Low		1	1
			31	30
American long grain	Intermediate		4	4
	Low		1	1
			5	5
Bulu	Intermediate		4	4
			4	4
Japanese type	Low: Ponlai		4	4
	Japonica		2	2
	Indica x *japonica*		2	2
			8	8
		Total	177	169

[a] All from northeast and northwest hilly regions of India
[b] Three of these are exotic collections from northeast hilly regions of India, and one (CR HP8) is a high-protein variety developed by CRRI, Cuttack.

Authors Bhattacharya, Sowbhagya, and Indudhara Swamy are affiliated with the Discipline of Rice & Pulse Technology, Central Food Technological Research Institute, Mysore-570013, India.

water uptake at 80°C to that at 96°C (W'_{80}/W'_{96}) (Bhattacharya and Sowbhagya, 1971); the ratio of the amylose content soluble in water at 65° to that at 96°C (sA_{65}/sA_{96}) (Bhattacharya et al., 1972); and stickiness of cooked rice as measured by the percent retention of rice on a sieve under specified conditions and its consistency (apparent viscosity, hardness) as measured by back extrusion in the Haake consistometer (Manohar Kumar et al., 1976; Bhattacharya et al., 1978) were determined as described earlier. Gelatinization temperatures (GT) of the samples were calculated from the 10%-slurry viscograms as suggested by Juliano et al. (1964b). Protein content (in milled rice) was determined approximately by the biuret method of Parial et al. (1970).

Brabender viscograms were initially run for all the samples with 10% iso-starch slurry (14% moisture basis) as already described (Bhattacharya et al., 1978). But these data did not seem to provide much useful information. Subsequently 44 selected samples were tested for their Brabender viscograms at a number of slurry concentrations (in the range 5–16%, dry basis) each, and the samples were then divided into 'viscogram BD_r types' I to VIII by noting the pattern of their 'relative breakdown' [BD_r = breakdown/(breakdown + setback)] values and other viscosity ratios against the corresponding peak viscosity values; these data have been fully described earlier (Bhattacharya and Sowbhagya, 1979). The BD_r types of the remaining samples were then determined by comparing their BD_r and peak viscosity values as obtained in the 10%-slurry viscograms with the above standard curves, after applying appropriate correction for the difference in age, as also fully explained in the above paper. To study the correlation of the viscogram BD_r type with other properties, the BD_r types were assigned ranks as follows:

BD_r type	Rank
I	1
II	2
III	3
IV, V	4
VI, VII	5
VIII	6

This ranking was done because the BD_r values at a peak viscosity of 1000 BU broadly followed the above order (Bhattacharya and Sowbhagya, 1979). Mixed types were given intermediate ranks (e.g. type II.III = rank 2½.

Chalkiness scores of the samples were determined to test their correlation with EMC-S. In an earlier exploratory work (Bhattacharya et al., 1979a) it was observed that chalky kernels in 5 out of 7 varieties had indeed higher EMC-S than the corresponding non-chalky kernels, but the size of chalky area in kernels of a given variety had only a slight effect on the EMC-S. Hence a modified score card for kernel chalkiness with a narrow range of scores was used as follows:

% Kernel area chalky	Score
Nil	0
0–20	1
> 20	2

Duplicate 100 randomly selected milled whole grains were individually assigned scores as per the above and the mean aggregate score was calculated.

Since the large number of samples was analyzed at different times and many had aged considerably by then, the insoluble amylose contents and EMC-S values of the samples were all converted to an approximate age of 6 months by correcting them with the help of the age-trend curves described earlier (Indudhara Swamy et al., 1978). The other parameters did not need to be so converted as they were largely independent of grain age.

The texture (stickiness and consistency) of cooked rice was determined in only 45 selected samples, since the determination is very laborious. Unfortunately the tests were run at varying times, as a result of which the data were not strictly comparable. Hence these data have not been presented here. However, a perceptible trend could be discerned in the relation between the physicochemical parameters of the samples and their cooked-rice texture, which moreover was in agreement with the trend observed earlier (Bhattacharya et al., 1978) with 32 samples. Hence this broad trend alone has been presented in the following results.

RESULTS & DISCUSSION

Quality classification of rice

Amylose content is now well recognized as the most important determinant of rice quality (Juliano et al., 1965, Juliano, 1979). We have shown that the hot-water-insoluble amylose content is another key parameter (Manohar Kumar et al., 1976; Bhattacharya et al., 1978). The viscogram BD_r type too is a very effective indicator of quality (Bhattacharya and Sowbhagya, 1979). The alkali degradation type of rice and the equilibrium moisture content attained by it upon soaking in water at room temperature (EMC-S) are two other properties which seem to be related to rice quality (Bhattacharya et al., 1978).

Based on the above factors determined in the 177 samples, the samples could be divided into eight distinct groups. There were four major amylose-based classes: high-, intermediate- and low-amylose, and waxy. Earlier work (Bhattacharya et al., 1978) showed that there were three clear sub-classes in the high-amylose class, based on differences in their hot-water-insoluble amylose contents. This was again amply confirmed with the present samples. Hence these formed the first three types. Next came the intermediate-amylose class. Here again three sub-divisions became apparent, although the water-insoluble amylose content, which was quite low, did not appreciably vary among these samples. One sub-class was the scented rice: the 24 scented varieties (25 samples), collected from different parts of the country, had a surprisingly narrow range of properties so that they could be considered a distinct type. They also had a distinct viscogram type (Bhattacharya and Sowbhagya, 1979). Bulu rices again seemed a type by themselves, with, among other properties, characteristic and unusually high BD_r values. The remaining intermediate-amylose varieties formed the third sub-class or type among this broad class. The low-amylose and waxy rices formed the remaining two types, giving eight quality types in all.

The range of values of various properties of the 177 samples divided into eight groups as above, along with their means and standard deviations, are shown in Table 2. On this basis, it appears that rice can be tentatively divided into eight quality types with the characteristics as shown in Table 3. The overall trends of change of relative breakdown, stickiness and consistency among the types are shown by arrows in the Table.

The number and percentages of samples showing values outside the ranges defined in Table 3 are shown in Table 4, and the exceptional values as such are reproduced in Table 5.

It is clear from Tables 4 and 5 that the exceptions were neither too large in number nor too wide in magnitude to mar the tentative classification proposed in Table 3. The bulk of the exceptions in the viscogram type represented at most a departure by one type from the expected type. The significant number of exceptions in alkali type suggests that while this parameter is *generally* characteristic of the rice quality type, there are other unknown causative factors behind it. The EMC-S too is only *generally* characteristic of the rice type, for, as discussed later, it is a function of certain other properties. The alkali score (i.e., the gelatinization temperature) too is only *generally* characteristic of the rice type, being an independent variable with no definite influence on rice quality (Juliano et al., 1965). Yet somehow, perhaps due to genetic association, its values were remarkably typical of the rice types. The exceptions in total and insoluble amylose contents were quite insignificant.

Further work is necessary to determine the precise range of texture values of cooked rice of the individual groups. No doubt the gradation of stickiness and consistency among Groups I, II, III, VI and VII were already confirmed

in our earlier work (Bhattacharya et al., 1978). That there would be a further gradation to Group VIII (waxy rice) is obvious. But the precise values and positions of Groups IV and V need to be further confirmed.

Indices of gelatinization temperature

The gelatinization temperature (GT) of rice is one of its important parameters. But it is laborious to determine it in routine work by the standard birefringence-end point or viscograph techniques (Beachell and Stansel, 1963). Hence the more easily determinable parameters, alkali score, water uptake ratio (W'_{80}/W'_{96}) and soluble-amylose ratio (sA_{65}/sA_{96}) – which are already known to be well correlated with GT (Bhattacharya et al., 1978) – of the samples were regressed against their gelatinization temperature as determined from their viscograms. All four parameters

Table 2–Ranges and mean values of the various properties of the rice samples[a]

Quality type	No. of samples	Protein (% d.b.)	Amylose content (% d.b.)		Alkali score	EMC-S (% – w.b.)
			Total	Insoluble		
1	2	3	4	5	6	7
I High-amylose A	10	6.3 – 11.8 8.42 ± 1.50	26.3 – 29.2 28.07 ± 0.75	15.1 – 17.8 17.11 ± 0.90	4 – 8 7.00 ± 1.24	28.3 – 30.2 29.19 ± 0.66
II High-amylose B	38	6.3 – 12.6 8.83 ± 1.64	26.4 – 30.0 28.22 ± 0.84	11.9 – 15.1 13.70 ± 0.65	1/2 – 6 2.79 ± 1.16	27.2 – 29.8 28.30 ± 0.60
III High-amylose C	61	6.1 – 13.1 8.50 ± 1.55	25.6 – 30.8 28.44 ± 1.17	9.4 – 13.0 11.45 ± 0.88	1/2 – 8 3.09 ± 1.83	26.3 – 31.5 28.15 ± 1.03
IV Intermediate amylose A (scented)	25	7.2 – 14.3 10.03 ± 1.84	20.7 – 27.0 24.18 ± 1.55	7.6 – 10.5 9.07 ± 0.81	3/4 – 4 3/4 2.39 ± 1.22	28.1 – 30.8 29.21 ± 0.61
V Intermediate-amylose B	10	7.3 – 11.9 9.18 ± 1.35	22.7 – 25.6 24.15 ± 0.82	8.7 – 11.1 9.64 ± 0.74	3 3/4 – 8 6.48 ± 1.56	30.2 – 32.5 31.15 ± 0.68
VI Intermediate-amylose C (Bulu)	9[b]	6.3 – 9.3 7.84 ± 1.04	23.0 – 25.6 24.19 ± 0.96	7.6 – 11.6 9.84 ± 1.54	1/4 – 4 2.00 ± 1.68	27.8 – 29.8 28.54 ± 0.78
VII Low-amylose	14	7.4 – 12.6 8.77 ± 1.75	15.1 – 21.3 18.60 ± 1.96	7.1 – 9.1 8.26 ± 0.60	0 – 8 4.88 ± 1.77	30.4 – 33.1 31.59 ± 0.64
VIII Waxy	10	7.5 – 12.1 9.20 ± 1.51	4.4 – 8.1 6.39 ± 1.48	3.0 – 5.6 4.43 ± 0.77	3 – 7 5.30 ± 1.40	34.5 – 37.0 35.48 ± 0.76
Overall	177	6.1 – 14.3 8.85 ± 1.66	4.4 – 30.8 25.25 ± 5.70	3.0 – 17.8 11.13 ± 2.88	0 – 8 3.55 ± 2.07	26.3 – 37.0 29.27 ± 2.03

[a] The first row against each quality type gives the ranges of the various properties, the second row gives the means and standard deviations.
[b] There were four true bulu rices (Baok, Beak Ganggas, Benong 130, Sukanandi). Four American long-grain rices (Rexoro, Blue Bonnet 50, Blue Belle, Belle Patna) and Intan were included in this group, for all their properties were similar. However, the cooked-rice texture of the latter 5 varieties were not tested.

Table 3–Quality classification of rice

Property \ Quality type	I High amylose A	II High amylose B	III High amylose C	IV Intermediate amylose A (scented)	V Intermediate amylose B	VI Intermediate amylose C (bulu)	VII Low amylose	VIII Waxy
Example	IR8 IR22 Jaya	GEB24 Co32 IR20	T141 SR26B Adt8	Basmati370 T9 Br9	Kuki Abor Red Tengo	Benongl30 Sukanandi Baok	Tainan3 Norin8 Phoudum	Asm44 Purple puttu
Total amylose, % db	>26	>26	>26	22 – 26	22 – 26	22 – 26	15 – 22	<5
Insoluble amylose, % db	>15	12.5–15	⩽12.5	7 – 10	7 – 10	7 – 10	7 – 9	–
Alkali type	B, Mixed B	A, B_1	A, B_1	Mixed C	Mixed C	Mixed C	C	D
Mean Alkali score	7	2½	3	2½	6½	2	6	5½
EMC-S, % wb	28 – 30	27 – 29	27 – 29	28 – 30	30 – 32	28 – 30	30.5 – 33	34 – 37
Relative breakdown (viscogram	Low →							High
Stickiness (cooked rice)	Low →							High
Consistency (cooked rice)	High →							Low

were very highly significantly correlated (Fig. 1 and 2), the three regression equations against GT(y) and the corresponding correlation coefficients (r) being as follows:

(1) Against alkali score of Bhattacharya and Sowbhagya (1972):

$y = 74.54 - 1.40\ x$ (excluding waxy varieties, n = 157)
or $74.80 - 1.57\ x$ (including waxy varieties, n = 165)
$r = -0.848^{***}$ (excluding waxy varieties)
or -0.806^{***} (including waxy varieties)

(2) Against W'_{80}/W'_{96}:

$y = 77.89 - 0.28\ x$ (excluding *japonica* varieties, n = 133)
or $77.03 - 0.24\ x$ (including *japonica* varieties, n = 144)
$r = -0.854^{***}$ (excluding *japonica* varieties)
or -0.791^{***} (including *japonica* varieties)

(3) Against sA_{65}/sA_{96}:

$y = 75.36 - 0.99\ x$ (excluding waxy varieties, n = 157)
$r = -0.795^{***}$

Clearly, any of the above three parameters can be used as a measure of the GT of rice. The alkali score, being the easiest to determine and one of the most widely used tests, would perhaps be the first choice.

Interrelation among various rice properties

The large number of samples provided an excellent opportunity to test the interrelation between various rice properties. The statistical correlations among the various properties are shown in Table 6. Since waxy varieties have certain peculiar features (negligible amylose, low GT, high EMC-S, fully chalky kernels, type VIII viscograms) they could lead to some fortuitous associations. Hence correlations were calculated both including and excluding the waxy samples.

The equilibrium moisture content attained by rice upon soaking in water at room temperture (EMC-S) was seen to have a good type specificity (Table 3). The parameter was earlier shown to be related to amylose content and usually to kernel chalkiness, but its relation to GT was felt unertain (Bhattacharya et al., 1979a). In fact Kongseree and Juliano (1972) concluded that EMC-S was independent of GT. However, the data in Table 6 clearly show that the EMC-S (y_1) is very highly significantly related inversely to total (x_1) and insoluble (x_2) amylose contents and directly to alkali score (x_3) (i.e., inversely to GT) and kernel chalkiness index (x_4) (although the correlation with chalkiness index is markedly reduced when the waxy samples are excluded). The insoluble amylose can be disregarded, being in the present context only a reflection of the total amylose. The relevant multiple regression equation was

$$y_1 = 34.837 - 0.279\ x_1 + 0.263\ x_3 + 0.734\ x_4$$
$$R = 0.941^{***}\ R^2 = 0.925\ (n = 165)$$

Clearly the fit is almost exact, for the three parameters to-

Table 4—Samples showing exceptional values of various properties

Group	Total no. of samples	Percentage of samples in each group showing exceptional values of					
		Total amylose	Insoluble amylose	Alkali score	Alkali type	EMC-S	BD_r type
I	10	0	0	10 (1)[a]	40 (4)[a]	0	20 (2)[a]
II	38	0	3 (1)[a]	3 (1)	10 (4)	13 (5)	34 (13)
III	61	2 (1)[a]	3 (2)	10 (6)	13 (8)	18 (11)	28 (17)
IV	25	12 (3)	8 (2)	0	16 (4)	4 (1)	20 (5)
V	10	0	30 (3)	10 (1)	10 (1)	10 (1)	40 (4)
VI	9	0	44 (4)	0	0	0	22 (2)
VII	14	0	0	0	14 (2)	0	14 (2)
VIII	10	0	0	0	10 (1)	0	40 (4)
Overall	177	2.3 (4)	6.8 (12)	5.1 (9)	13.6 (24)	10.2 (18)	27.7 (49)

[a] Figures in parentheses give the actual number of samples showing exceptional values.

Table 5—Values of properties given by exceptional samples

Group	Total amylose (% d.b.)	Insoluble amylose (% d.b.)	Alkali score	Alkali type	EMC-S (% w.b.)	BD_r type
I	–	–	4	A(4 no.)	–	II(2 no.)
II	–	11.9	5	A(BB_1), AB, BB_1, B	29.1, 29.3, 29.4(2), 29.8	I.II(2), II.III(6), III(4), III.IV.V
III	25.6	12.8, 13.0	5¼, 5¾, 7½, 8(3)	A(B)(2), A(BB_1C), ABC(2), A(C), B(B_1), C	26.3, 26.6, 29.4(4), 29.6, 30.5, 31.0, 31.2, 31.5	II.III(4), III.V(10), V(3)
IV	21.5, 26.5, 27.0	10.3, 10.5,	–	AB_1(2), A(2)	30.8	II, II.IV, III.IV(2), IV.V
V	–	10.1, 10.4, 11.1	4	A	32.5	III.V, V.VII(3)
VI	–	11.2(2), 11.3, 11.6	–	–	–	V.VI(2)
VII	–	–	–	AC, BC	–	VII.VIII(2)
VIII	–	–	–	CD	–	V.VII(2), VII(2)

gether account for over 92% of the variations in EMC-S. It is therefore also evident that EMC-S is not a fundamental variable; its type specificity (Table 3) is largely a reflection of that of amylose and alkali score.

The viscogram BD_r type, on the basis of data reported earlier (Bhattacharya and Sowbhagya, 1979) and the trend observed here, is clearly a key index of rice quality. It is seen from Table 6 that the BD_r rank (y_2) was very highly significantly negatively correlated with total (x_1) and insoluble (x_2) amylose contents but was not correlated with alkaki score (i.e., GT), chalkiness index or protein content. It was correlated with EMC-S, but this can be perhaps disregarded being possibly a reflection of the amylose content. The multiple regression equation was

$$y_2 = 7.555 - 0.024\, x_1 - 0.325\, x_2$$
$$R = 0.881^{***}\ R^2 = 0.777$$

The total and insoluble amylose contents account for about 78% of the variations in BD_r type, the remaining 22% of the variations being due to as yet unknown factors. This will explain the exceptions in this property observed in Tables 4 and 5, as also seen earlier (Bhattacharya and Sowbhagya, 1979). Its importance as an independent rice quality factor is thus further emphasized.

Alkali score was very highly significantly negatively correlated with amylose content, although to a small extent ($r = -0.311^{***}$ for $n = 165$). The correlation between amylose and GT has been controversial. Halick and Kelly (1959) and Beachell and Stansel (1963) observed in limited samples the two parameters to be unrelated. Juliano et al. (1964a) found them significantly correlated in 19 samples but not when Taichung (Native) 1 and Century Patna 231 were included. In another study, Juliano et al. (1964b) found that the two parameters were unrelated in 51 nonwaxy samples but were highly correlated if four waxy varieties were included. In the present study, the two parameters were highly significantly correlated even excluding the waxy samples ($r = -0.229^{**}$, $n = 145$). These results with such a large number of samples would finally show that while the GT was largely an independent variable, it was definitely affected (positively) by the amylose content to a small extent.

Insoluble amylose content was very highly significantly correlated to total amylose ($r = 0.774^{***}$ for $n = 165$), its variation being accounted for by the latter to the extent of about 60% ($r^2 = 0.599$). The two parameters were rather independent of each other in the first three quality groups but were otherwise interrelated in the overall sense (Table 3).

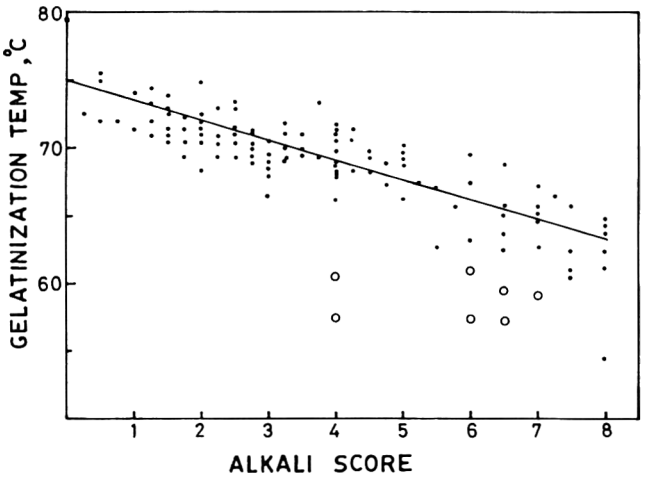


Fig. 1—Correlation between alkali score (of Bhattacharya and Sowbhagya, 1972) and gelatinization temperature of rice. Open circles (O) represent waxy varieties.

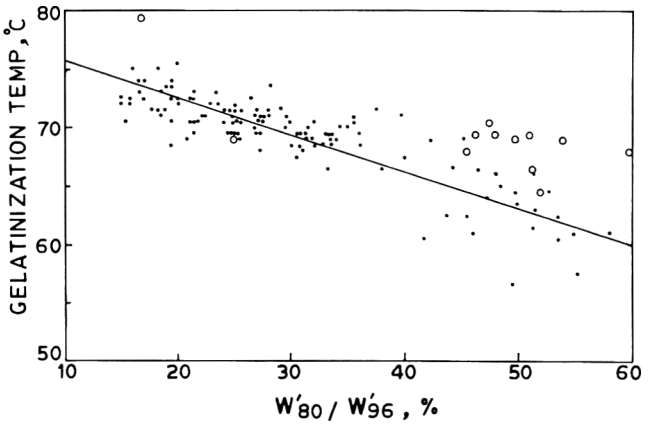


Fig. 2—Correlation between water-uptake ratio and gelatinization temperature of rice. Open circles (O) represent japonica varieties.

Table 6—Correlation coefficients between various rice properties

	Total amylose	Insoluble amylose	Alkali score	EMC-S	Chalkiness index	Vicsogram rank	Protein
Total amylose A[a]		.774***	−.311***	−.905***	−.291***	−.725***	−.108
B		.660***	−.229**	−.752***	.211**	−.710***	−.172*
Insoluble amylose A			−.041	−.645***	—	−.879***	−.099
B			.089	−.393**	.151	−.856***	−.117
Alkali score A				.544***	.260***	.086	−.026
B				.594***	.112	.019	−.064
EMC-S A					.503***	.624***	.053
B					.217**	.497***	.017
Chalkiness index A						—	—
B						−.117	−.136
Viscogram rank A							.049
B							.042
Protein A							
B							

[a] A, n = 165 (excluding 12 samples for which viscogram data were not available); B, n = 145 (excluding all 10 waxy samples, and 22 other samples for which viscogram and/or chalkiness index data were not available).

Protein content was not correlated with any other property except probably showing a small negative correlation with amylose. This could be because, as protein content increased, percentage of starch, and hence amylose, decreased.

The total amylose content and chalkiness index showed an interesting relation. They were negatively correlated to a small extent in the lot as a whole ($r = -0.291^{***}$, n = 165), but the correlation became positive when the waxy samples, which are fully chalky, were excluded ($r = 0.211^{**}$, n = 145). That this small but very significant positive correlation was not due to the influence of Group I high-amylose modern dwarf *indica* samples, which usually have chalky kernels, was shown by the fact that the correlation remained of the same order when both the Group I and Group VIII (waxy) samples were excluded ($r = 0.221^{**}$, n = 148). These results suggest that a high amylose content tends to make the rice kernel somewhat prone to chalkiness.

The influence of waxy samples have been seen in many cases above. In another instance, the alkali score was positively correlated with chalkiness index in the lot as a whole ($r = 0.260^{***}$, n = 165), but the correlation became insignificant when the waxy samples,which are fully chalky and usually give high alkali score, were excluded ($r = 0.112^{NS}$, n = 145).

The last factor which seemed to have a definite relation to rice quality was the alkali degradation type (Table 3). However, its correlation with other properties could not be calculated as it could not be assigned any quantitative values or ranks.

REFERENCES

Beachell, H.M. and Stansel, J.W. 1963. Selecting rice for specific cooking characteristics in a breeding program. Internat. Rice Comm. Newslet. Special issue: 25.

Bhattacharya, K.R. 1979. A note on alkali test of rice using a petri dish. Inernat. Rice Res. Newslet. 4(2): 4.

Bhattacharya, K.R. and Sowbhagya, C.M. 1971. Water uptake by rice during cooking. Cereal Sci. Today 16: 420.

Bhattacharya, K.R. and Sowbhagya, C.M. 1972. An improved alkali reaction test for rice quality. J. Food Technol. 7: 323.

Bhattacharya, K.R. and Sowbhagya, C.M. 1979. Pasting behavior of rice: A new method of viscography. J. Food Sci. 44: 797.

Bhattacharya, K.R. and Sowbhagya, C.M. 1980. On the alkali degradation type of rice kernels. J. Sci. Food Agric. 31: 615.

Bhattacharya, K.R., Sowbhagya, C.M., and Indudhara Swamy, Y.M. 1972. Interrelationship between certain physicochemical properties of rice. J. Food Sci. 37: 733.

Bhattacharya, K.R., Sowbhagya, C.M., and Indudhara Swamy, Y.M. 1978. Importance of insoluble amylose as a determinant of rice quality. J. Sci. Food Agric. 19: 359.

Bhattacharya, K.R., Indudhara Swamy, Y.M., and Sowbhagya, C.M. 1979a. Varietal difference in equilibrium moisture content of rice and effect of kernel chalkiness. J. Food Sci. Technol. 16: 214.

Bhattacharya, K.R., Sowbhagya, C.M., and Indudhara Swamy, Y.M. 1979b. Quality classification of rice. Internat. Rice Res. Newslet. 4(4): 7.

Bhattacharya, K.R., Sowbhagya, C.M., and Indudhara Swamy, Y.M. 1980. Quality of Indian rice. J. Food Sci. Technol. 17: 189.

Halick, J.V. and Kelly, V.J. 1959. Gelatinization and pasting characteristics of rice varieties as related to cooking behavior. Cereal Chem. 36: 91.

Indudhara Swamy, Y.M., Ali, S.Z., and Bhattacharya, K.R. 1971. Hydration of raw and parboiled rice and paddy at room temperature. J. Food Sci. Technol. 8: 20.

Indudhara Swamy, Y.M., Sowbhagya, C.M., and Bhattacharya, K.R. 1978. Changes in the physicochemical properties of rice with aging. J. Sci. Food Agric. 29: 627.

Juliano, B.O. 1979. The chemical basis of rice grain quality. In 'Proceedings of the workshop on chemical aspects of rice grain quality,' International Rice Research Institute, Los Banos, Laguna, Philippines, p. 69.

Juliano, B.O., Bautista, G.M., Lugay, J.C., and Reyes, A.C. 1964a. Studies on the physicochemical properties of rice. J. Agric. Food Chem. 12: 131.

Juliano, B.O., Cagampang, G.B., Cruz, L.J., and Santiago, R.G. 1964b. Some Physicochemical properties of rice in southeast Asia. Cereal Chem. 41: 275.

Juliano, B.O., Oñate, L.U., and del Mundo, A.M. 1965. Relation of starch composition, protein content and gelatinization temperature to cooking and eating qualities of milled rice. Food Technol. 19: 1006.

Kongseree, N. and Juliano, B.O. 1972. Physicochemical properties of rice grain and starch from lines differing in amylose content and gelatinization temperature. J. Agric. Food Chem. 20: 714.

Manohar Kumar, B., Upadhyay, J.K., and Bhattacharya, K.R. 1976. Objective tests for the stickiness of cooked rice. J. Texture Studies 7: 271.

Parial, L.C., Rooney, L.W., and Webb, B.D. 1970. Use of dye-binding and biuret techniques for estimating protein in brown and milled rice. Cereal Chem. 47: 38.

Sowbhagya, C.M. and Bhattacharya, K.R. 1971. A simplified colorimetric method for determination of amylose content in rice. Stärke 23: 53.

Ms received 5/30/81; revised 8/31/81; accepted 9/5/81.

We are greatly indebted to Mr. B.S. Ramesh for carrying out the large number of statistical analyses.

TASTE THRESHOLDS . . . From page 563

Table 2—Thresholds of American, Nigerian, and Korean panels[a] for selected sweet, sour, bitter, and salty compounds

Taste substances	Nationality		
	American	Nigerian	Korean
	% w/v	% w/v	% w/v
Sucrose	0.243	0.248	0.242
Citric acid	0.004	0.004	0.004
Caffeine	0.006	0.006	0.009
NaCl	0.007	0.006	0.008

[a] N = 8 panelists, 4 replicatlons.

the risk of misformulation by surveying frequency of consumption of groups of food products with tastes similar to their product to be introduced.

REFERENCES

Amerine, M.A., Pangborn, R.M., and Roessler, E.B. 1965. "Principles of Sensory Evaluation of Food," p. 255. Academic Press, New York, NY.

Colamosca, A. 1977. Building business with Africa. Dun's Review. 5: 92.

Davis, R.G. 1978. Increased bitter taste detection thresholds in Yucatan inhabitants related to coffee as a dietary source of niacin. Chem. Senses & Flavour. 3: 423.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1.

Fischer, R., Griffin, F., England, S., and Garn, S.M. 1961. Taste thresholds and food dislikes. Nature 191: 1328.

Huntsburger, D.V. 1961. "Elements of Statistical Inference," p. 200. Allyn and Bacon, Inc., Boston, MA.

Jerome, N.W. 1977. Taste experience and the development of a dietary prefrence for sweet in humans: Ethnic and cultural variations in early taste experience. In "Taste and Development," Ed. Weiffenbach, J.M. U.S. Dept. of Health, Education, and Welfare, Bethesda, MD. DHEW Pub. No. (NIH) 77-1068, p. 235.

Johansson, B., Drake, B., Pangborn, R.M., Barylkopikielna, N., and Koster, E. 1973. "Difference taste thresholds for sodium chloride among young adults: An interlaboratory study", J. Food Sci. 38: 524.

Kelty, M.F. and Mayer, J. 1971. Rapid determination of taste threshold: A group procedure. J. Clin. Nutr. 24: 177.

Moskowitz, H. 1977. Intensity and hedonic functions for chemosensory stimuli. In "The Chemical Senses and Nutrition." Ed. Kare, M. and Maller, O., p. 71. Academic Press, Inc., New York, NY.

Moskowitz, H., Kumaraiah, B., Sharma, R., Jacobs, H., and Sharma, S. 1975. Cross-cultural differences in simple taste preferences. Science 190: 1217.

Pangborn, R.M. 1967. Some aspects of chemoreception in human nutrition. In "The Chemical Senses and Nutrition." Ed. Kare, M. and Maller, O., p. 45. John Hopkins Press, Baltimore, MD.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed, p. 135, 258, 432. The Iowa State University Press, Ames, IA.

Ms received 7/3/81; revised 10/14/81; accepted 10/16/81.

Contribution from the Missouri Agricultural Experiment Station. Journa' Series No. 8656.

A Microbial Investigation of Selected Spices, Herbs, and Additives in South Africa

R. BAXTER and W. H. HOLZAPFEL

ABSTRACT

A total of thirty-six spices, herbs and food additives from various sources was investigated. High total viable numbers of sporeformers were present of which a considerable percentage was proteolytic and/or amylolytic. The total number of colony forming units (c.f.u.) varied from several hundred to several million per gram, depending on the source, the manufacturing process, age and type of condiment. Exceptionally high contamination (i.e. > 10^6 c.f.u./g) was found in black pepper, coriander, paprika, mace, pimento and white pepper from source A. No significant numbers of potentially pathogenic organisms were found except for *B. cereus*, moulds and faecal streptococci. The presence of *B. cereus* was confirmed in 7 out of 19 samples from source A. Enterococci were isolated and identified from paprika, white pepper, black pepper, pimento, onion powder and salamita. The need for spice cleanliness, commercial sterilization and subsequent proper storage of condiments is stressed.

INTRODUCTION

SPICES AND HERBS may contain large numbers of microorganisms (Frazier and Westhoff, 1978; Guarino and Peppler, 1976). These include members of the genus *Bacillus*, whereas anaerobic sporeformers, thermophilic anaerobes and aerobes are found occasionally, sometimes in moderate numbers. Enterococci and members of the family *Enterobacteriaceae* occur occasionally and a variety of moulds and yeasts may be found. *Clostridium perfringens* spores are present at low levels. Pathogens such as *Salmonella*, *Shigella* and coagulase-positive staphylococci are rarely found in spices. Powers (1976) were concerned about the large numbers of *B. cereus* present in 53% of the spice samples they analyzed.

Some spices, because of the marked bactericidal effect of their essential oil content, harbour fewer bacteria. These include cloves, mustard seed, onion, garlic and all spice (pimento) (Frazier and Westhoff, 1978). The total number of viable aerobic organisms vary considerably depending on the spice source (Julseth and Deibel, 1974), and numbers may range from several thousand to several million per gram (Pruthi, 1980). This has been confirmed by most studies undertaken. Spices are grown and harvested in areas of the world where often sanitary practices are poor and, moreover, are grown in warm, humid areas where the growth of a wide variety of fungi and bacteria is readily supported (Julseth and Deibel, 1974).

Uncleaned spices may be grossly contaminated (Krishnaswamy et al., 1971) and contamination of foods by spice carriers has been reported to lead to food spoilage and can even lead to food poisoning (Krishnaswamy et al., 1971; Powers et al., 1976; Goepfert et al., 1972). Sporeformers may lead to food spoilage when they survive the cooking process and multiply under favorable conditions (Powers et al., 1976; Beuchat et al., 1980).

Fumigation of spices with ethylene oxide (Vajdi and Pereira, 1973; Gerhardt, 1969) can effectively reduce the microbial population by up to 90%. However, the use of fumigation is prohibited in certain countries because of the possible human health hazard constituted by fumigation by-products (e.g. ethylene chlorhydrin). The present study was initiated as means of determining the microbial flora of a number of spices and food additives that may be used in the preparation of shelf stable products.

Authors Baxter and Holzapfel are affiliated with the Dept. of Microbiology and Plant Pathology, Faculty of Agriculture, Univ. of Pretoria, 0002 Pretoria, Republic of South Africa.

PROCEDURE

Samples

Thirty-six spices, herbs and food additives from various sources (A, B and C) were selected for microbial analysis. Samples from source A were obtained from two different wholesalers and the paprika from a local producer. Spices from source B were purchased directly from the factory, and had been subjected to ethylene oxide fumigation. The milk powder was locally manufactured. Samples from source C were flavoring materials consisting of essential oils on carrier bases.

Preparation of samples

Twenty grams of each sample were weighed into sterile plastic Stomacher bags to which 180 ml sterile peptone/NaCl solution (Merck) were added. These were homogenized in a Colworth Stomacher 400 for approximately 2 min. Duplicate serial dilutions were prepared in sterile peptone/NaCl solution (Merck).

Microbial counts and colony isolation

Total viable counts were determined on STD 1 agar (Merck) after incubation at 30°C for 48 hr.

Enterobacteriaceae were counted on Brilliant-Green Phenol-red lactose agar (BPL) (Merck) and incubated at 30°C for 48 hr, and on DHL Agar according to Sakazaki (Merck). DHL was incubated at 30°C for 24 hr and colonies were then counted and marked. After an additional 24 hr at room temperature further colonies were counted as "pseudomonads." A cytochrome-oxidase test was performed on representative colonies and isolations were made of several randomly selected "typical" *Enterobacteriaceae* colonies. This was also done on BPL plates.

Brochothrix thermosphacta was enumerated on Streptomycin-sulphate-Thallous acetate-Actidione medium (STAA) according to Gardner (1966).

Counts of lactobacilli, leuconostocs and pediococci (LLP-group) were made on Lactobacillus-selective-agar (Merck). The plates were incubated aerobically for up to 5 days at 30°C.

Enterococci (Group D Streptococci) were determined by surface-plating on Citrate-Azide-Tween-Carbonate agar (CATC agar) (Merck) and counted after 48 hr at 30°C. Only typical deep red colonies were counted. Several isolations were made at random. Baird-Parker medium (BP) (Baird-Parker, 1962; Merck) was used for the enumeration of possible coagulase positive staphylococci. Random isolations were made and identified.

Bacillus cereus-selective-agar (Merck) was used for the counting of *Bacillus cereus*. "Typical" colonies were counted and of these representatives were isolated at random and speciated according to Wolf and Barker (1968) and Buchanan and Gibbons (1974). Cognizance was taken of the method used by Powers et al. (1976) to distinguish between *B. cereus*, *B. thuringiensis*, *B. mycoides* and *B. anthracis*, and applied similarly.

The presence of *Salmonella* spp. was determined qualitatively. The methods of Georgala and Boothroyd (1970) were modified in order to detect any possible salmonella. Enrichment was achieved by suspending 20g of sample into 180 ml each of selenite and tetra-

thionate broth respectively, followed by incubation at 37°C. After 6, 12 and 18 hr enrichment cultures were streaked onto Bismuth-Sulphite Agar (BSA) according to Wilson and Blair (Merck).

Yeasts and moulds were enumerated on Potato-Dextrose-Agar (PDA) (Merck). The pH of the medium was adjusted to 3.7 after sterilization by means of a sterile 10% solution of tartaric acid. The plates were incubated at 25°C for 3–5 days.

Clostridium perfringens-selective-Agar according to Hauschild et al. (1974) was used for detection of *Cl. perfringens* and incubated anaerobically at 37°C in an anaerobic jar or a Forma Scientific anaerobic incubator. Isolated colonies were kept on nutrient agar slants and identified by standard biochemical tests.

Initial identification tests for Gram negative rods were performed on SIMS and TSI media (Merck), followed by the use of the API 20E identification system.

Aerobic sporeformers, proteolytic, amylolytic, and anaerobic thermophiles were counted by using the methods of Julseth and Deibel (1974).

RESULTS & DISCUSSION

CONSIDERABLE VARIATIONS were observed in total numbers of aerobic bacteria, not only between samples from the same source, but especially between samples from different sources (Tables 1 and 2; Fig. 1 and 2). The total aerobic counts varied between 200 organisms per gram for cardamom and several million per gram in the case of black pepper (Fig. 2).

Fig. 1 indicates that the predominant type of organism in the samples from source A were aerobic sporeformers, and these organisms accounted for between 50% and 95% of all organisms found in the samples examined. Table 1 shows that the aerobic sporeformers in black pepper, white pepper, paprika, marjoram, coriander, pimento and onion powder conisted largely of proteolytic organisms. Amylolytic organisms occurred in significant numbers in black and white pepper, pimento, onion powder and cinnamon. Few anaerobic thermophiles were present and results were recorded as <100 colony forming units (c.f.u.) for all samples investigated. The trend was thus that samples with high numbers of c.f.u. (marjoram, salamita, black pepper, parika, soy meal, coriander, mace, white pepper) also showed corresponding high numbers per gram of proteolytic and/or amylolytic organisms. Only some samples (i.e. cardamom, soy meal, paprika and majoram) showed low counts of amylolytic organisms (Table 1).

Samples from source A contained large numbers of microorganisms, as compared to sources B and C. Little information on the preparation of source A condiments could be gathered. All samples from source A showed significant numbers of *B. cereus* "type" colonies on *B. cereus*-selective agar. Several typical and other *Bacillus* colonies were isolated at random and identified (Table 3). Organisms of *Bacillus* Group 1 were the major species present. *B. cereus* was confirmed in soy meal, paprika, pimento, white pepper and marjoram by biochemical tests. The representative colonies from other spices were very similar in appearance and morphology to those confirmed by biochemical tests, and it was thus assumed that *B. cereus* was present in most spices (Fig. 1), often in large numbers. However, since isolates were only made at random, the presence of *B. cereus* were not confirmed in spices like mace and coriander (see Table 3 and Fig. 1). A comprehensive study of this problem has been completed by Powers et al. (1976).

B. cereus has been recognized as the etiological agent in food poisoning outbreaks in Europe as far back as 1906 and in Hungary it was ranked as the third most common cause of food poisoning during the period 1960–1966 (Goepfert et al., 1972). It was noted that the high incidence of *B. cereus* food poisoning in meat was attributed to the Hungarian custom of highly seasoning meat dishes with spices which often contained large numbers of aerobic sporeformers (Goepfert et al., 1972).

Soy meal, "flavor enhancer," paprika, pimento, white pepper, rosemary and majoram show significant numbers of both lactose positive and lactose negative *Enterobacteriaceae*. This is in contrast with reports (Julseth and Deibel, 1974; Powers et al., 1975) where relatively low numbers of *Enterobacteriaceae* are mentioned. Several Gram negative bacteria were found in paprika, including *Salmonella* spp.

Table 1—Aerobic sporeformers from spices, herbs and food additives: Source A (numbers per gram)[a]

Sample[b]	Total count	*B. cereus*[c]		Proteolytic	Amylolytic
		Total	Confirmed		
Cardamom	60	<100	<100	30	<100
Cinnamon	2.2×10^5	<100	nil	1.9×10^4	1.4×10^5
Cloves	4.8×10^4	<100	nil		
Coriander	9.0×10^6	3.0×10^5	nil	8.8×10^5	3.5×10^5
Ginger	3.2×10^5	<1000	nil		
Mace	4.8×10^6	1.1×10^6	nil	2.1×10^4	3.6×10^3
Marjoram	5.0×10^5	5.0×10^5	1.0×10^5	4.0×10^5	<100
Nutmeg	5.0×10^3	1.0×10^3	nil		
Onion Powder	3.5×10^5	<100	<10	2.9×10^5	1.49×10^5
Paprika	6.2×10^6	3.0×10^3	1.0×10^3	2.7×10^6	<100
B/Pepper	6.0×10^7	3.0×10^3	nil	1.35×10^7	1.17×10^7
W/Pepper	1.9×10^6	6.0×10^3	2.0×10^3	3.7×10^5	4.4×10^5
Pimento	1.1×10^6	1.0×10^2	1.0×10^2	4.8×10^5	2.4×10^5
Rosemary	1.9×10^4	<1000	nil	1.8×10^3	6.5×10^3
Thyme	1.2×10^5	<1000	nil		
Food additives					
Egg powder	1.0×10^3	<10	nil		
Flavor enhancer	4.5×10^4	3.0×10^3	nil	4.5×10^3	3.0×10^3
Salamita	4.9×10^5	5.0×10^3	nil	4.2×10^5	1.7×10^5
Soy meal	5.1×10^4	3.0×10^4	1.0×10^4	3.0×10^4	<100

[a] All samples investigated showed <100 c.f.u./g for anaerobic thermophiles.

[b] Samples from source A were purchased with few exceptions from one wholesaler. Although it was indicated that these products were subjected to fumigation, the results in general do not confirm this claim. A second wholesaler supplied "flavor enhancer," salamita (spice mixture for salami production) and egg powder. Ground paprika was produced locally and did not receive any fumigation treatment.

[c] The 'typical' *B. cereus* colony has a pink coloring (i.e. mannitol negative), is rough and dry and surrounded by a ring of dense, white precipitate. Colonies surrounded by a yellow zone are definitely not *B. cereus* as these are mannitol positive organisms. Some *B. cereus* "type" colonies were subjected to further tests, the results of which may be found in Table 3. Several other Bacillus isolates were made and identified since in most cases, 90% of colonies in media appear to be *Bacillus* spp.

(Table 5). The major genus detected in the samples was *Enterobacter*. *E. coli* was isolated from rosemary, indicating possible faecal contamination at some stage by rodents or other sources. This report however shows that enteric pathogenic organisms were not present in significant numbers since none was isolated after enrichment techniques. Cytochrome-oxidase positive *Pseudomonadaceae* were found in all spices, but no quantitative tests were performed to determine their numbers.

No coagulase positive staphylococci were isolated, although considerable numbers of saprophytic, lysostaphin-sensitive staphylococci were present in soy meal, "flavor enhancer," mace, paprika and majoram (Fig. 2).

Faecal streptococci was isolated from white and black pepper, salamita, pimento and paprika (Table 4), with considerable numbers present in onion powder, white pepper and paprika (Fig. 2).

No clostridia, *Brochothrix thermosphacta* or *Lactobacillaceae* could be detected on selective media.

Several samples from source A showed high mould counts whilst paprika contained high numbers of viable yeasts. Despite its microbicidal properties (Farbood et al., 1976; Shelef et al., 1980) rosemary spice had relatively high mould and aerobic spore populations (Fig. 2). The study of moulds in spices and herbs is of significance because of the potential production of mycotoxins (Llewellyn et al., 1981). Work by Flannigan and Hui (1976) showed moulds associated with spices and herbs to be predominantly *Aspergillus* spp. of which 7 out of 24 strains produced aflatoxins *in vitro*. Whole ginger and white pepper supported the growth of *A. flavus* and the production of aflatoxins reported that of 29 different spices tested, cloves and allspice (pimento) completely inhibited fungal growth, whereas other spices (e.g. thyme, sage, majoram) inhibited only the toxin production. For these experiments, spice essential oils were extracted which at higher concentrations were more effective in their fungicidal and fungistatic action. The bacteriostatic and bactericidal action of spices has been

Table 2—Microbial population of spices from source B

Spice	Total count/gram[a]
Coriander	2.6×10^4
Cloves[b]	5.3×10^3
Milk powder	3.8×10^3
Pimento	1.6×10^7
Nutmeg[b]	9.6×10^3
W/Pepper	2.1×10^5

[a] Most organisms on the plates appeared to be *Bacillus* spp. type colonies. No *Enterobacteriaceae, Clostridium* spp. or *Bacillus cereus* were found; in addtion no *Salmonella* spp. were isolated even after enrichment.
[b] The plates exhibited typical inhibition of bacterial growth by the spices Cloves and Nutmeg at 10^{-1} and 10^{-2} dilutions.

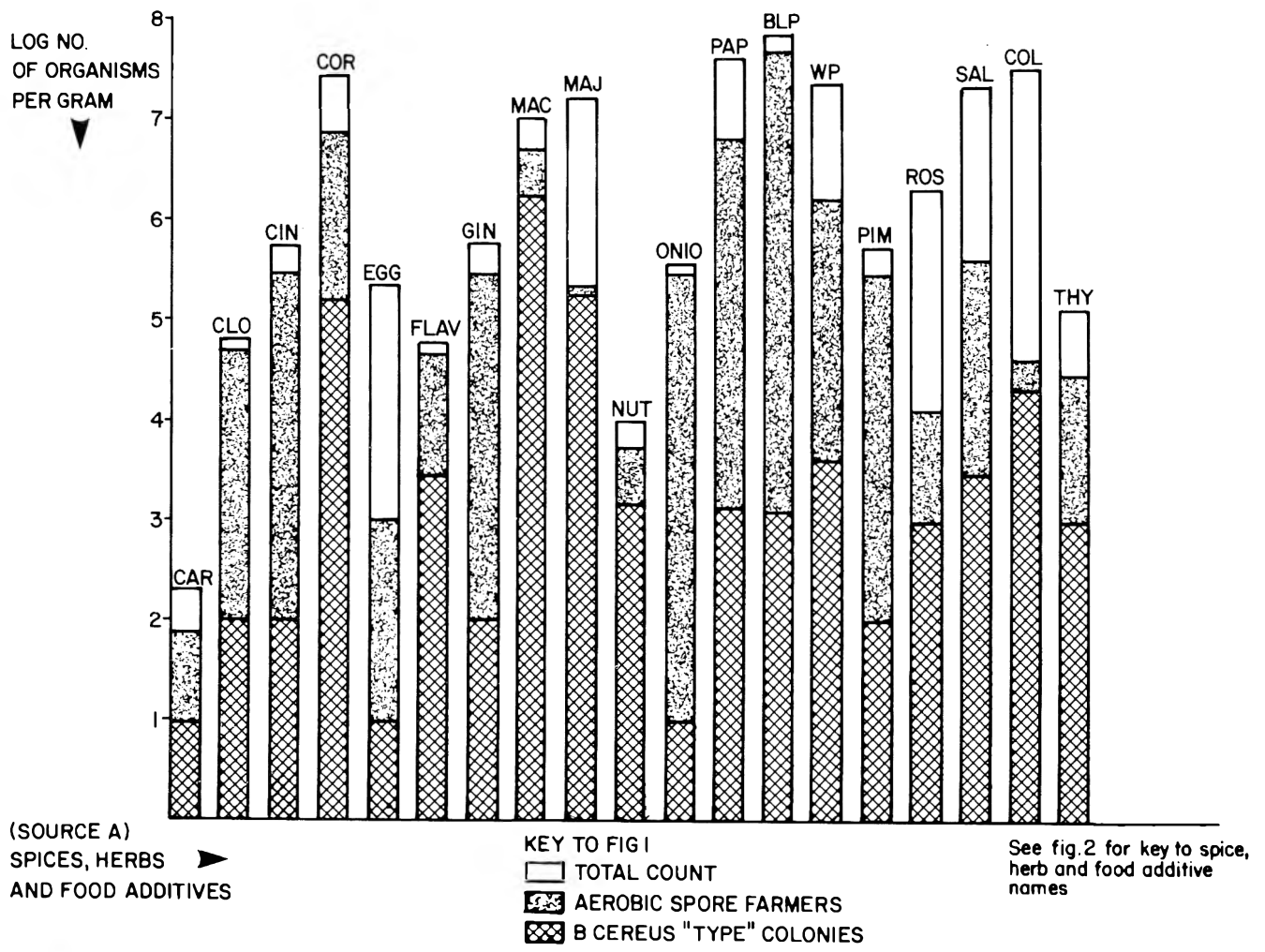


Fig. 1—Relationship between the total number of aerobic organisms, aerobic sporeformers and B. cereus "type" colonies in spices from source A.

widely reported (Farbood et al., 1976; Shelef et al., 1980; Frazier and Westhoff, 1978; Llewellyn et al., 1981).

Results with spices (flavoring materials) from source C reflect near to commercial sterility. No *Bacillus cereus, Clostridium* spp., coagulase positive staphylococci, *Enterobacteriaceae,* moulds or yeasts were found in these specially prepared spices. The method of preparation involves the extraction of the spice essential oil (flavor mass) which is then placed on a dextrose or rust meal carrier base. Oils of pimento, cloves, cinnamon, coriander, mace, nutmeg, onion powder, black pepper, white pepper, rosemary and thyme on suitable bases (Source C spices) showed total viable numbers of <1000 per gram in comparison with ginger with <3000 per gram.

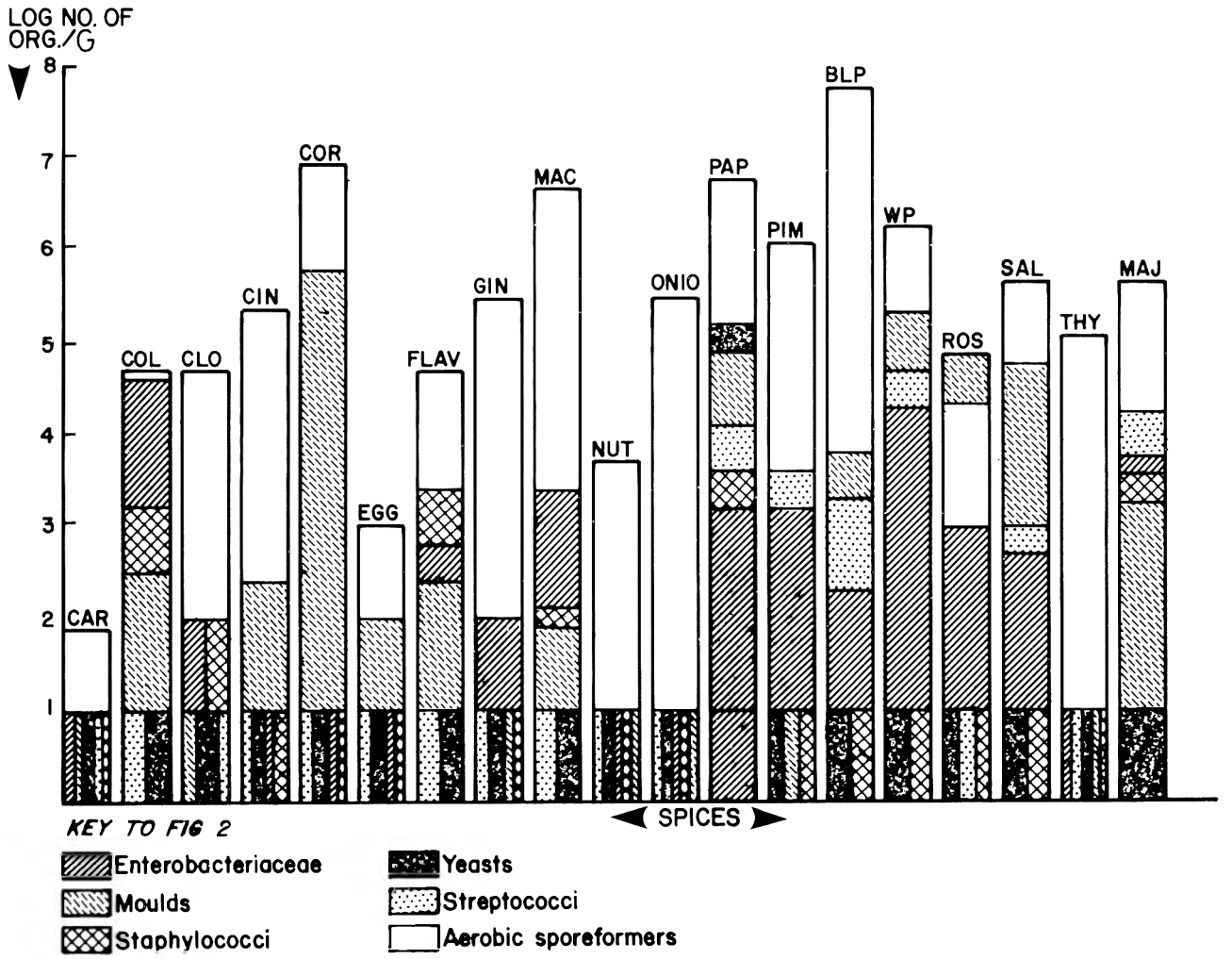
Spices from source B, which were freshly cleaned and ground, also yielded lower total counts than the samples from Source A. The inhibitory effect of some spices from Source B was clearly seen on STD 1 agar where fewer organisms were detected on the 10^{-1} and 10^{-2} dilutions than on the 10^{-3} dilution. These findings of a selective inhibitory action by spices and herbs are confirmed by several authors (Frazier and Westhoff, 1978; Guarino and Peppler, 1976; Shelef et al., 1980).

The presence of aerobic sporeformers and moulds is important since their survival or the presence of their toxins

Table 3—Bacillus spp. isolated from different samples[a]

Source	*B. subtilis*	*B. brevis*	*B. firmus*	*B. sphaericus*	*B. cereus*	*B. licheniformis*	*B. polymyxa*	*B. anthracis*[b]	*B. coagulans*	*B. megaterium*	*Total*
Soy meal	1	1			1						3
Marjoram			1		1					1	3
Mace	1	1		2							4
Onion powder			3		1	1	1				6
W/Pepper					1	2			1		4
Paprika					1	1					2
Pimento					1			1			2
Coriander										1	1
Total	2	2	4	2	6	4	1	1	1	2	25

[a] *Bacillus* isolates were speciated according to Wolf and Barker (1968) and Buchanan and Gibbons (1974).
[b] This isolate was distinguished from *B. cereus* on basis of its non-motility.



ROS Rosemary; THY Thyme; CLO Cloves; CIN Cinnamon; NUT Nutmeg; GIN Ginger; CAR Cardamom; EGG Egg-powder; WP White Pepper; BLP Black Pepper; SAL Salamita; FLAV Flavour enhancer; COL Soy meal; PAP Paprika; COR Coriander; ONIO Onion powder; MAC Mace; MAJ Majoram, PIM Pimento (all spice);

Fig. 2—Range of population numbers of different organisms in selected spices from source A.

Table 4—Organisms[a] isolated from CATC Agar plates

Isolate no.	Source	Gelatinase	Tellurite-reduction	Fermentation of: Glycerol	Lactose	Maltose	Mannitol	Sorbitol	Arabinose	Raffinose	Sucrose	Identification
S1, S2, S6, S7	S1-Paprika, S2-Pimento, S6-Salamita, S7-B/Pepper	–	+	+	+	+	+	+	–	–	+	*S. faecalis*
S3	Onion powder	±	+	+	+	+	+	+	–	–	+	*S. zymogenes* or *liquefaciens*
S4	Paprika	–	–	+	+	+	+	–	+	–	+	*S. faecium*
S5	W/Pepper	±	+	+	+	+	+	+	–	+	+	*S. faecalis* var. *liquefaciens*

[a] Fermentative on Hugh-Leifsons glucose. Organisms isolated from CATC were all deep red colonies and catalase negative, Gram positive cocci in pairs and chains. Other Group D species were present as light pink colonies, but were not counted.

Table 5—Identification of some Gram negative rods isolated from spices, herbs and food additives

Source	*Serratia rubidea*	*Enterobacter agglomerans*	*Enterobacter cloacae*	*Klebsiella pneumoniae*	*Klebsiella ozaenae*	*Escherichia coli*	*Salmonella spp.*[a]	*Pseudomonas spp.*[b]	*Total*
Soy meal		1						4	5
Paprika		4	4	2			2	1	13
Cardamom			1					1	2
W/Pepper			1		1			1	3
B/Pepper							1	1	2
Rosemary						1		2	3
Pimento				1	1			1	3
Onion powder	1							1	2
Salamita				1				1	2
Total	1	5	6	4	2	1	3	13	35

[a] Pseudomonas and Salmonella species were not identified further.
[b] Isolations were made from BPL and DHL plates showing significant numbers of Enterobacteriaceae colonies. Several isolates were also taken from selenite enrichment broth and also of cytochrome oxidase positive colonies on BPL and DHL plates.

after the cooking process may result in food poisoning or deterioration of the product in which spices have been used. This is of vital importance for the microbial quality and safety of the experimental shelf stable products under investigation, considering the mild heat treatment (72°C to 80°C core temperature for 10 to 30 min) of the final product. The need for clean or preferably commercially sterile spices, herbs and food additives is emphasised by these results, especially in view of the presence of high numbers of viable endospore forming bacteria in samples from source A. Safe methods for commercial cleaning and sterilization of condiments are vital.

According to Weber (1980) ethylene oxide treatment appears to be the best method available. The by-products of this process are said to be consumed in negligible amounts by the general public. Investigations on the use and effectivity of gamma-irradiation for the sterilization of spices are being conducted presently as a co-operative project between the University of Pretoria and the Atomic Energy Board. A decisive factor in the practical use of commercial sterilization processes will be the economical viability (Vadji and Pereira, 1973; Gerhardt, 1969; Powers et al., 1975).

REFERENCES

Baird-Paker, A.C. 1962. An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bact. 25: 12.

Buchanan, R.E. and Gibbons, N.E. (Ed.). 1974. "Bergey's Manual of Determinative Bacteriology," 8th edition. Williams and Wilkins, Baltimore, MD.

Beuchat, L.W., Ann Ma-Lin, C.F., and Carpenter, J.A., 1980. Growth of Bacillus cereus in media containing plant seed materials and ingredients used in Chinese cookery. J. Appl. Bact. 48: 397.

Farbood, M.I., MacNeil, J.H. and Ostovar, K. 1976. Effect of rosemary spice on the growth of micro-organisms in meats. J. Milk Food Technol. 39(10): 675.

Flannigan, B. and Hui, S.C. 1976. The occurence of aflatoxin producing strains of Aspergillus flavus in the mould floras of ground spices. J. Appl. Bact. 41: 411.

Frazier, W.C. and Westhoff, D.C. 1978. "Food Microbiology," 3rd ed. McGraw Hill, New York.

Gardner, G.A. 1966. A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. J. Appl. Bact. 29: 45.

Georgala, D.L. and Boothroyd, M. 1970. Methods for the detection of salmonellas in meat and poultry. In "Isolation Methods for Microbiologists," Shapton, D.A. and Gould, G.W., Ed. Series No. 3 p. 29. Academic Press, London.

Gerhardt, U. 1969. Sterilization of spices. Gordian 69 (1631): 427.

Goepfert, J.M., Spira, W.M. and Kim, H.U., 1972. Bacillus cereus: food poisoning organism. A review. J. Milk Food Technol. 35: 213.

Guarino, P.A. and Peppler, H.J. 1976. Spices and condiments. In "Compendium of Methods for the Microbiological Examination of Foods," Chapt. 46. Ed. M.L. Speck. American Public Health Association, Washington, DC.

Hauschild, A.H.W., Hilsheimer, R.and Griffith, D.W. 1974. Enumeration of faecal Clostridium perfringens spores in egg-yolk free tryptose-sulphite-cycloserine agar. Appl. Microbiol. 27(3): 527.

Hitokoto, H., Morozumi, S., Wauka, T., Sakai, S., and Kurata, H. 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl. & Environ. Microbiol. 39(4): 818—822.

Julseth, R.M. & Deibel, R.H., 1974. Microbial profile of selected spices and herbs at import. J. Milk Food Technol. 37(8): 414.

Krishnaswamy, M.A., Patel, J.D. and Pathasarathy, N., 1971. Enumeration of micro-organisms in spices and spice mixture. J. Food Sci. & Technol. 8: 191.

Llewellyn, J.C., Burkett, M.L. and Eadie, T. 1981. Potential mold growth, aflatoxin production and antimycotic activity of selected spices and herbs. J. Assoc. Off. Anal. Chem. 64(4): 955p.

Powers, E.M., Latt, T.G. and Brown, T., 1976. Incidence and levels of Bacillus cereus in processed spices. J. Milk Food Technol. 39(10): 668.

Powers, E.M., Lawyer, R. and Masuoka, Y. 1975. Microbiology of processed spices. J. Milk Food Technol. 38(11): 683—687.

Pruthi, J.S. 1980. "Spices and Condiments: Chemistry, Microbiology, Technology. Adv. Food Res., Supp. No. 4. Academic Press, New York.

—Continued on page 578

Analysis of Phytic Acid in Foods by HPLC

A. L. CAMIRE and F. M. CLYDESDALE

ABSTRACT

A quantitative HPLC method for the analysis of phytic acid in foods was developed based on the precipitation of phytic acid with ferric chloride followed by conversion to sodium phytate before injection onto a C_{18} reversed-phase column. Standard food grade wheat bran samples were analyzed by the method of standard addition and recovery of phytic acid ranged from 99–103%. 3% H_2SO_4 was found to be as effective as 3% TCA in the extraction of phytic acid. AAS was shown to be potentially valuable as a metal specific detector for the HPLC of phytate-metal complexes.

INTRODUCTION

PHYTIC ACID is not ubiquitous in the plant kingdom. The presence and concentration of phytate in food depends largely on the part of the plant consumed and its stage of maturity at harvest (O'Dell et al., 1972). The phytate concentration in whole grain cereals and oilseeds has been reported to be approximately 1%. This becomes significant from a nutritional point of view when one considers that phytate intakes by adults in Iran and other Middle Eastern countries of 2–5g/day are not uncommon (Reinhold, 1975).

The great concern over the presence of phytic acid in certain foods stems from the fact that phytic acid complexes with certain dietary minerals potentially decreases their bioavailability. Several excellent reviews deal with the nutritional implications of phytic acid and its chemistry (Cheryan, 1980; Erdman, 1979).

Since phytic acid does not have a characteristic absorption spectrum and because there are no specific reagents that identify phytate, phytic acid determination has been an analytical problem for over 30 years. Precipitation methods have been subdivided into "direct" methods in which the ferric phytate is removed and determined as phosphorus or inositol (McCance and Widdowson, 1935) and "indirect" methods in which an excess of ferric chloride is added to precipitate the phytate and the iron in the ferric phytate precipitate is determined while the phytate concentration is calculated from these results using a theoretical Fe:P ratio of 4:6.

This investigation reports an HPLC method for the analysis of phytic acid in foods combining the column/mobile phase conditions established by Tanjendjaja et al. (1980) along with a preparatory procedure which results in improved analytical performance.

MATERIALS & METHODS

Food samples

Standard food grade soft white and hard red wheat brans were obtained from the American Association of Cereal Chemists. Refined corn bran was obtained courtesy of Illinois Cereal Mills, Inc. (Paris, IL). Defatted soy flour was obtained courtesty of Cargill Incorporated (1010–10th Ave. S.W., Cedar Rapids, IA). Carrots, split peas, broccoli, brown rice, parsnips, and lettuce were obtained at the local supermarket.

Equipment

The HPLC was carried out with Laboratory Data Control (LDC) (P.O. Box 10235 Riviera Beach, FL) equipment consisting of a Constametric II pump for solvent delivery, Spectromonitor II Model 1202 UV-VIS detector, Model 1107 RefractoMonitor (RI) detector and a model 7120 Reodyne syringe loading sample injector with a 20 μl loop. The analytical column was 25 cm X 4.6 cm ID containing Spherisorb ODS C_{18} 10μ packing. A Perkin Elmer model 372 double beam atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) was used for the analysis of metals.

Extracting solvents and reagents

All chemicals used were reagent grade or better. Solvents were made up fresh using distilled water. Sodium phytate was obtained from Sigma Chemical Co. (St. Louis, MO) and was found to contain 13.18% moisture.

Operating conditions for the chromatography

Phytic acid was detected by RI on elution from the reversed-phase column. 0.005M sodium acetate was used as a mobile phase at a flow rate of 0.5 ml/min. A 20 μl sample loop was used for all analyses. It was found that a 5 cm precolumn containing silica was necessary to protect the analytical column from deterioration due to the strongly basic sample solutions injected.

The feasibility of atomic absorption spectrophotometry (AAS) as a detector for the analysis of phytate-metal complexes was evaluated using the instrumentation shown in Fig. 1. The liquid chromatograph was interfaced to the AAS by means of a drilled out 1/16-inch stainless steel "tee" one side of which was connected to the output of the RI detector. The other portion of the "tee" was connected to a separate pulseless pump which was set for 4 ml/min of mobile phase. The combination of these two inputs produced a flow

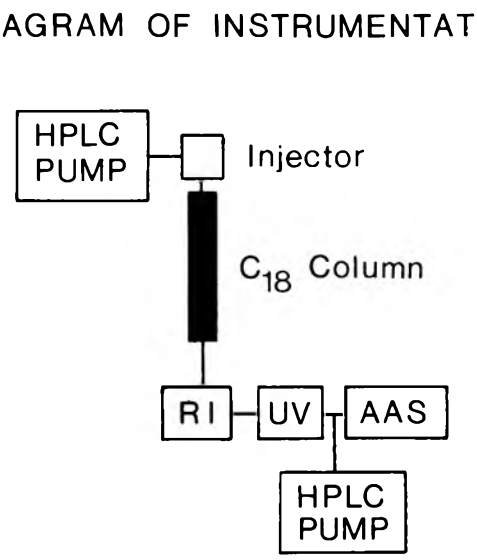


Fig. 1—Instrumentation used to test the feasibility of AAS as a metal specific detector for liquid chromatography.

Authors Camire and Clydesdale are affiliated with the Dept. of Food Science & Nutrition, Massachusetts Agricultural Experiment Station, Univ. of Massachusetts, Amherst, MA 01003.

outlet from the third portion of the "tee" into the AAS aspirator of 4.5 ml/min, the flow rate necessary for maximum sensitivity of the AAS. Synthetic monoferric phytate was prepared according to the procedure of Lipschitz et al. (1979) to serve as a standard compound for injection.

Procedure

The fresh vegetables were first freeze-dried and then ground to a powder in a Micro Mill. The wheat brans, corn bran, and soy flour were used as is, and the rice and split peas were similarly ground in the Micro Mill.

Each sample was then treated as follows:

1. Weigh a sample estimated to contain 25–100 mg phytic acid into a 125 ml Erlenmeyer flask. For example, for soft white wheat bran a 1.000g sample was utilized.
2. Extract with 25 ml of 3% H_2SO_4 for 30 min on a shaker bath at medium speed at room temperature.
3. Filter the slurry through fast filter paper (Whatman #41 and rinse by using a fine jet stream from a squeeze bottle, with a small volume of extracting solvent.
4. Transfer the filtrate to 50 ml screw top centrifuge tubes and place in a boiling water bath (BWB) for 2–5 min (to aid in the precipitation of ferric phytate) before addition of 3 ml of a $FeCl_3$ solution containing 6 mg ferric iron per ml in 3% H_2SO_4. For samples high in inorganic phosphate it might be advisable to incubate the ferric iron precipitate in 0.5M HCl for 2 hr at room temperature with occasional stirring (Ellis et al., 1976).

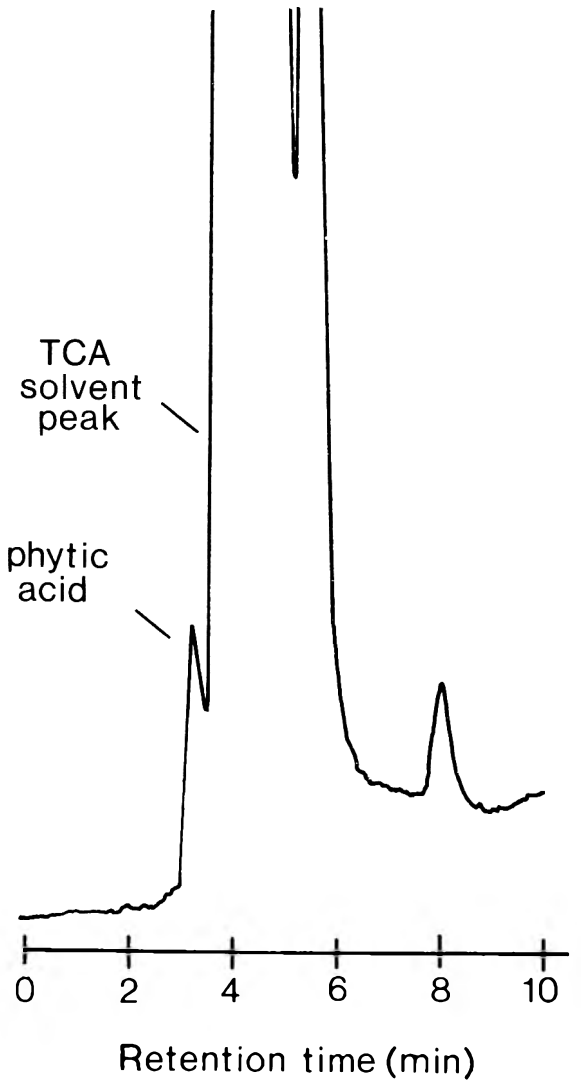


Fig. 2—Chromatogram of a 3% TCA extract of soft white wheat bran using the method of Tanjendjaja et al. (1980).

5. Heat the tubes in a BWB for 45 min to allow for complete precipitation of the ferric phytate complex.
6. Centrifuge at 2,500 rpm for 10 min and discard the supernatant.
7. Wash the precipitate once with 30 ml of distilled water, centrifuge, and discard the supernatant.
8. Add 3 ml of 1.5N NaOH and a few ml of distilled water to the contents of the tubes. Break up the precipitate with the aid of a glass rod and then sonicate to completely disperse the precipitate.
9. Bring the volume to approximately 30 ml with distilled water and heat in a BWB for 30 min to precipitate the ferric hydroxide.
10. Centrifuge the cooled samples and quantitatively transfer the supernatant to 50 ml volumetric flasks. Rinse the precipitate once with approximately 10 ml of distilled water, centrifuge, and add this to the volumetric flask.
11. Run a series of standards of sodium phytate before injection of the unknowns.

Preparation of the standard curve

A stock solution containing 10 mg/ml of sodium phytate in distilled water was prepared. Serial dilutions were made to contain from 50 mg/100 ml to 500 mg/100 ml which corresponds to 10–100 μg of sodium phytate injected into the HPLC using a 20 μl sample loop. A detection limit of 5 μg sodium phytate was obtained and the curve was linear up to 100 μg of sodium phytate injected.

Linear regression was performed on the data points using a Hewlett Packerd programmable pocket calculator and unknown sample values were obtained in this manner. Results for sodium phytate content were corrected for 13.18% moisture present in the standard sodium phytate and values for phytic acid were obtained from the sodium phytate values by assuming sodium phytate to be 29.95% sodium (Vohra et al., 1965).

Determination of percent recovery

Percent recovery was evaluated by using the internal standard technique. Soft white and hard red wheat brans as obtained from the American Association of Cereal Chemists were used in the analysis. Duplicate 1.000g samples of wheat brans were analyzed for endogenous phytic acid content by the proposed procedure. Then 25 and 50 mg of sodium phytate were added as internal standards to another set of duplicate 1.000g samples and analyzed after adding up to 5 ml of $FeCl_3$ solution (made to contain 6 mg Fe^{+++}/ml in 3% H_2SO_4) to precipitate the greater amount of phytic acid present. The minimum amount of ferric iron needed to precipitate the added sodium phytate was determined using a 4:6 molar ratio of iron to phosphorus and assuming sodium phytate to be 20% phosphorus.

Evaluation of a "direct injection" method

Duplicate samples of soft white wheat bran were extracted with 3% TCA for 1/2 hr on a shaker bath at room temperature. Samples were then centrifuged at 10,000 rpm for 20 min before injection into the HPLC following the procedure established by Tanjendjaja et al. (1980).

Evaluation of extracting solvents

Several extracting solvents (3% H_2SO_4, 3% TCA, 1% TCA, 3% CH_3COOH, 1% HCl, 1M NH_4 acetate, pH 1.0 buffer, and H_2O) all reagent grade or better, were evaluated for efficacy of extraction in soft white wheat bran by using the proposed methodology.

RESULTS & DISCUSSION

A TYPICAL CHROMATOGRAM of a 3% TCA extract of wheat bran analyzed using the HPLC method of Tanjendjaja et al. (1980) is shon in Fig. 2. The procedure involves a "direct injection" of the 3% TCA extract following a centrifugation step. As can be seen in Fig. 2 the solvent peak interferes with this analysis and we have found that the results are not quantitative. We attribute this to the presence of endogenous iron in wheat bran which may precipitate some of the phytate during extraction thereby resulting in a low estimate of phytate concentration by this method. Other extracting solvents such as 3% H_2SO_4

and 1% HCl completely mask the sodium phytate peak making analysis impossible.

Fig. 3 shows a chromatogram of a 3% H_2SO_4 extract of soft white wheat bran that has been analyzed by the proposed methodology. For this figure (3) it is evident that the preparatory step used in this procedure resolved some of the chromatographic and quantitative problems seen with the method of Tanjendjaja et al. (1980) as shown in Fig. 2. The advantages of this method over the non-chromatographic methods are the elimination of the use of the theoretical 4:6 ratio of Fe:P in calculations, the elimination of the necessity for many time-consuming washing and centrifugation steps used in the "indirect method," relative speed and simplicity, and the "directness" of analysis for sodium phytate. However, the disadvantages of this method are that an external standards is used for quantitation and that there is a need for improved column/mobile phase conditions for the chromatography so that phytic acid is retained to a greater degree on the column.

The use of an NH_2-bonded phase column with a mobile phase of acetonitrile: water (60:40) as described by Tanjendjaja et al. (1980) resulted in the same problems with retention. We also investigated the use of paired-ion chromatography using a C_{18} column with 0.005M tetrabutylammonium bromide in 0.025M K_2HPO_4 and KH_2PO_4 as the mobile phase. We were not successful but recommend that this approach be investigated further.

A wide variety of foodstuffs were chosen to be analyzed by the proposed methodology and a comparison of results for hard red wheat bran, soft white wheat bran and defatted soy flour was made with the method of Wheeler and Ferrel (1971) as shown in Table 1. Results of the proposed methodology are the average of two determinations expressed on a dry weight basis. Defatted soy flour was found to contain 2.2% phytic acid on a dry weight basis compared to hard red and soft white wheat brans which contained 6.6 and 5.0% phytic acid respectively. Similar values were found by Morris and Ellis (1980) who reported that soft wheat bran and hard wheat bran contained 5.0% and 4.0% phytic acid respectively on an as used basis. Baker et al. (1981) reported full fat soy flour to contain 1.3% phytic acid. A comparison of values obtained using the method of Wheeler and Ferrel (1971) (Table 1) shows lower values for the wheat bran samples. This difference may be due in part to the use of different extracting solvents. Wheeler and Ferrel (1971) used 3% TCA while the proposed methodology used 3% H_2SO_4.

Several extracting solvents were evaluated to determine their relative effectiveness in the extraction of phytic acid from soft white wheat bran and the results are summarized in Table 2. Three percent H_2SO_4 was found to extract more phytic acid than 3% TCA; however, the results were not significant with a sample size of two. Three percent TCA was found to be a more effective solvent than 1% TCA. Three percent acetic acid, 1M ammonium acetate, 1% HCl, and a pH 1.0 acetate buffer were all less effective than 3% H_2SO_4 in the extraction of phytic acid from soft wheat bran. Wheeler and Ferrl (1971) reported 3% TCA to be more effective than 3% H_2SO_4 in the extraction of phytic acid from wheat protein concentrate. Reddy and Salunkhe (1981) reported water to remove 88% of the phytic acid from black gram cotolyedons. Our results using wheat bran show water to be the least effective extracting solvent, removing only a trace of phytic acid.

Twenty-five and fifty mg of standard sodium phytate were added to duplicate 1.000g samples of soft white and hard red wheat brans in order to determine percent recovery. The results are shown in Table 3. Recovery of phytic acid in the wheat bran samples ranged from 99–103%. It is realized that the use of "spiked" samples has some limitations but it does not give some indication of recovery.

In our study using AAS as a metal specific detector for liquid chromatography a detection limit for Fe in monoferric phytate of 10 μg was obtained. However, in attempting to analyze a food extract for phytate-metal complexes we were not successful since phytic acid is not retained long enough on the reversed-phase column and therefore any

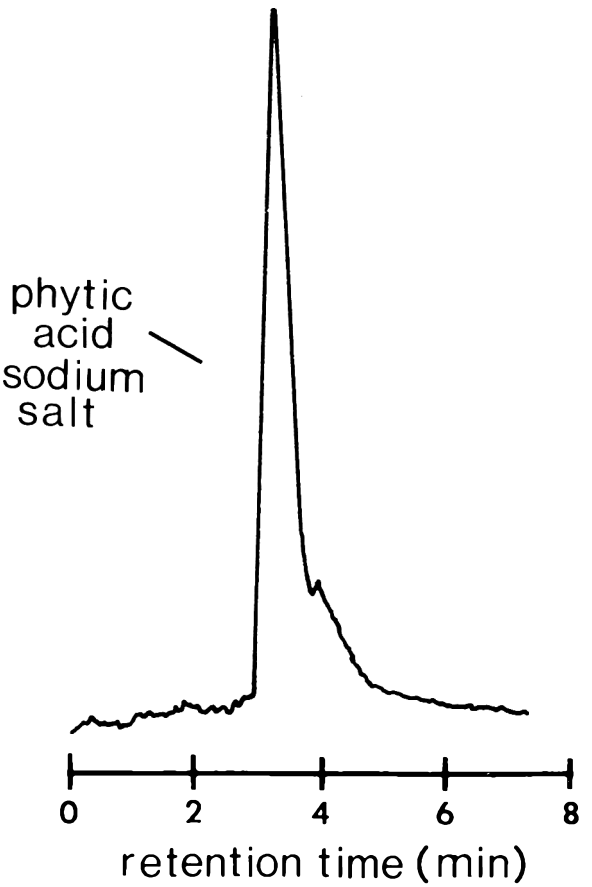


Fig. 3—Chromatogram of a 3% H_2SO_4 extract of soft white wheat bran using the proposed methodology.

Table 1—Phytic acid content of selected foodstuffs

Foodstuff	Phytic acid (mg/g)[a]	Phytic acid (mg/g)[b]
Hard red wheat bran	66.81 ± 1.85	53.71 ± 1.71
Soft white wheat bran	50.27 ± 1.45	46.62 ± 0.46
Soy flour (defatted)	22.45 ± 1.15	23.53 ± 0.48
Refined corn bran	15.77 ± 2.51	
Parsnips	8.18 ± 0.18	
Carrots	5.15 ± 0.58	
Split peas	16.79 ± 1.02	
Brown rice (parboiled)	15.55 ± 1.92	
Broccoli	N.D.	
Lettuce	N.D.	

[a] Each value represents the average of two determinations based on the proposed methodology and is expressed on a dry weight basis.
[b] Each value represents the average of three determinations based on the method of Wheeler and Ferrel (1971) and is expressed on a dry weight basis.
N.D. = None detectable

Table 2—Comparison of extracting solvents for the extraction of phytic acid from soft white wheat bran

Extracting solvent	pH	Phytic acid (mg/g)[a]
3% H_2SO_4	0.8	50.59 ± 1.72
3% TCA	1.0	47.98 ± 0.96
1% TCA	1.1	36.99 ± 0.31
3% CH_3COOH	3.1	33.80 ± 0.84
1% HCl	0.7	41.45 ± 1.37
1M NH_4 acetate	6.9	29.34 ± 1.26
pH 1.0 buffer	0.9	38.25 ± 1.17
H_2O	6.7	trace

Table 3—Percent recovery of added sodium phytate to wheat brans

Sample	Phytic acid (mg/g) found	Sodium phytate[a] mg added	Total phytic acid found	
			mg/g	% of total present
Soft White Wheat Bran	50.27 ± 2.39	25.0	65.80 ± 1.28	99.5
		50.0	80.30 ± 0.66	100.5
Hard Red Wheat Bran	66.81 ± 3.04	25.0	80.36 ± 0.64	102
		50.0	94.81 ± 1.37	103

[a] Sodium phytate used was found to contain 13.18% moisture and is assumed to be 29.95% sodium.

metals in solution would coelute with phytic acid. Morris and Ellis (1976) have been able to achieve retention of a phytate-metal complex which they have identified as monoferric phytate using a BioGel P-4 gel filtration column. With the recent introduction of HPLC gel columns it may be possible that similar analyses of phytate metal complexes in foods can be done in a matter of minutes rather than hours using AAS as a metal specific detector for HPLC.

In summary, the results of this study have shown HPLC to be a fast reliable method for the analysis of phytic acid in foods. The values reported for the phytic acid content of selected foodstuffs are generally higher than the values reported in the literature. Finally, AAS is potentially valuable as a metal specific detector for the HPLC of phytate-metal complexes once improve chromatographic conditions are established.

REFERENCES

Baker, E.C., Mustakas, G.C., Erdman, J.W. Jr., and Black, L.T. 1981. The preparation of soy products with different levels of native phytate for zinc bioavailability studies. JAOCS 58: 541.
Cheryan, M. 1980. Phytic acid interactions in food systems. CRC In Food Science & Nutrition. 13(4): 297.
Ellis, R., Morris, E.R., and Philpot, C. 1976. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal. Biochem. 77: 536.
Erdman, J.W. Jr. 1979. Oilseed phytates: nutritional implications. J. Am. Oil Chem. Soc. 56: 736.
Lipschitz, D.A., Simpson, K.M., Cook, J.D., and Morris, E.R. 1979. Absorption of monoferric phytate by dogs. J. Nutr. 109: 1154.
McCance, R.A. and Widdowson, E.M. 1935. Phytin in human nutrition. Biochem. J. 29: 2694.
Morris, E.R. and Ellis, R. 1976. Isolation of monoferric phytate from wheat bran and its bioloigcal value as an iron source to the rat. J. Nutr. 106: 753.
Morris, E.R. and Ellis, R. 1980. Bioavailability to rats of iron and zinc in wheat bran: response to low-phytate bran and effect of the phytate/zinc molar ratio. J. Nutr. 110: 2000.
O'Dell, B.L., DeBoland, A.R., and Koirtyohann, S.R. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food Chem. 20: 718.
Reddy, N.R. and Salunkhe, D.K. 1981. Interactions between phytate, protein, and minerals in whey fractions of black gram. J. Food Sci. 46: 564.
Reinhold, J.G. 1975. Phytate destruction by heast fermentation in while wheat meals. J. Amer. Diet. Assoc. 66: 38.
Tanjendjaja, B., Buckle, K.A., and Wootton, M. 1980. Analysis of phytic acid by high-performance liquid chromatography. J. Chrom. 197: 274.
Vohra, P., Gray, G.A., and Kratzer, F.H. 1965. Phytic acid-metal complexes. Proc. Soc. Exp. Biol. Med. 120: 447.
Wheeler, E.L. and Ferrel, R.E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 48: 312.
Ms received 7/3/81; revised 10/14/81; accepted 10/16/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, Georgia, June 7—10, 1981.

Paper No. 2085, Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst. This research was supported in part from Experiment Station Project No. NE 116.

SPICES/HERBS/FOOD ADDITIVES—MICROBIALS . . . From page 574

Shelef, L.A., Naglik, O.A. and Bogen, D.W. 1980. Sensitivity of some common food-borne bacteria to the spices sage, rosemary and all spice. J. Food Sci. 45: 1043.
Vadji, M. and Pereira, R.R. 1973. Comparative effects of ethylene-oxide, gamma-irradiation and micro-wave treatments on selected spices. J. Food Sci. 38: 893.
Weber, F.E. 1980. Controlling micro-organisms in spices. Cereal Foods World. 25(6): 319.
Wolf, J. and Barker, A.N. 1968. The genus Bacillus: Aids to the identification of its species. In Identification methods for Microbiologists," Series No. 2, Part B, Gibbs, B.M. and Shapton, D.A. Academic Press, London, New York.
Ms received 6/22/81; revised 9/11/81; accepted 10/15/81.

Valuable assistance and support for this investigation was rendered by Dr. S. de Jong and Mr. L. Facius.

Media Comparison for the Enumeration and Recovery of *Clostridium Sporogenes* P.A. 3679 Spores

D. A. POLVINO and D. T. BERNARD

ABSTRACT

The efficiency of trypticase peptone agar (TPA), yeast extract agar (YEA), and pork pea infusion agar (PPIA) for enumerating heat activated *Clostridium sporogenes:* P.A. 3679 spores was tested using a pour plate procedure. All three media types gave reproducible results between test runs, however TPA and YEA gave higher counts than PPIA. The three media types listed above plus pork infusion agar (PIA) and T-Best agar (TBA) were evaluated for recovery of heat stressed P.A. 3679 spores using a similar pour plate procedure. Andersen's pork pea infusion gave the highest counts, although the variation found between tests indicates that the pour plate procedure may not be adequate for recovery of heat stressed spores.

INTRODUCTION

IN ORDER TO PRODUCE shelf-stable or commercially sterile low acid canned foods, a thermal process is applied which is adequate to eliminate mesophilic endospore-forming organisms of the genus *Clostridium*. Time/temperature parameters needed to achieve commercial sterility are determined by methods similar to those outlined in the *Laboratory Manual for Food Canners and Processors* (1968). Important aspects of this procedure are the enumeration of spore suspensions to be used in these determinations, and the recovery of viable but heat injured spores.

Counts must be made prior to any thermal death time (TDT) work in order to obtain correct inoculum levels and to facilitate the calculation of decimal reduction times (D values). The medium used must be adequate to support ougrowth of heat activated spores in order to achieve accuracy in any counting procedure. In addition, the adequacy of the medium used for recovering viable but heat stressed spores is a primary concern due to the potential for underestimation of D values if the medium does not provide a proper environment for germination and outgrowth.

Infusion extracts have been widely used to recover and enumerate test organisms in the food industry, but the preparation of infusion media is very time consuming and expensive. Therefore, efforts have been made by researchers to find alternatives that would give reliable, reproducible results. Our objective in this investigation was to evaluate some of the more recently developed synthetic media as well as a few of the more classic infusion media in a two phase testing regimen. The first phase consisted of a series of experiments to determine the reliability of enumeration procedures by evaluating both media type and potential sources of variation using a pour plate procedure and heat activated spores. The second phase was an evaluation of recovery media for two levels of heat stressed spores using a similar procedures.

METHODS & MATERIALS

Test organism

A single *C. sporogenes*, P.A. 3679 stock spore suspension was used in all experiments. This spore crop was harvested 8-1-73 from a culture medium containing 5% tryptone (Difco), 0.5% peptone (Difco), 1.0% yeast extract (Difco), 0.2% sucrose and 0.1% thioglycollate and stored in sterile distilled water at 4°C. The method of culturing and harvesting was similar to that outlined in the *Laboratory Manual for Food Canners and Processors* (1968).

Culture media

Pork pea infusion agar was prepared according to the formula of Andersen (1951); yeast extract agar was prepared by the formula of Pflug et al. (1979); the pork infusion agar was prepared according to Stumbo (1965) and T-Best agar was made by the formula of Wheaton and Pratt (1961). The formula for trypticase peptone agar is as follows: trypticase (BBL) 5%, peptone (Bacto) 0.5%, sodium thioglycollate 0.1%, agar 1.5%, and distilled water 1000 ml. Sodium bicarbonate was added as a separately sterilized solution after autoclaving to achieve a final concentration of 0.14%. Since all media were prepared on the day of use, dry sodium thioglycollate was added to each before autoclaving. For media formulations that did not stipulate the use of thioglycollate or bicarbonate, additions consistent with TPA were incorporated.

Heat activation tests

For each test 1.0 ml of a spore suspension containing approximately 1.0×10^8 spores was added to a 20 x 150 mm screw cap test tube containing 9 ml of Sorenson's M-15 phosphate buffer (pH 7.0), and the tube heated for 13 min in an 80°C constant temperature bath. At the end of the heating interval, the tube was cooled in tap water. The heat activated spores were diluted in sterile, distilled water to obtain approximately 30 to 300 colony forming units per ml. Two analysts each inoculated duplicate 15 x 100 mm petri dishes with 1 ml inoculum for each media type. Approximately 20 ml of test medium was poured per plate; when the plates had solidified they were overlayed with 2% agar. plates were inverted after the overlay had set and placed into GasPak anaerobic jars (BBL) with hydrogen/carbon dioxide generators and incubated at 30°C for 48±2hr. Colonies were counted using a darkfield Quebec colony counter and recorded as the average of two duplicates for each medium per analyst. The media used in the heat activation study were pork pea infusion, yeast extract and trypticase peptone agars.

Heat stress tests

For each run in the second phase of testing, 0.5 ml of the stock spore suspension was added to 4.5 ml of Sorenson's phosphate buffer in a 20 x 150 mm screw cap test tube for a total of 5.0 ml containing approximately 1.0×10^7 spores per ml. Two ml of this suspension were transfered into each of two thermal death time (TDT) tubes made from Pyrex brand, standard wall glass tubing cut into 5 to 6 inch lengths and flame sealed at one end. After filling, the open end was flame sealed, and the tubes totally immersed in an oil bath preheated to 121±1°C. An initial come-up time (C.U.T.) of 100 sec was allowed for the tube contents to reach 121°C before timing was started. Previous studies with this oil bath had determined that the lethality equivalent of the 100 second come-up time is approximately 0.33 min at 121°C for a TDT tube containing one to two ml of an aqueous medium. At the end of 1 min plus C.U.T. the first tube was removed and cooled immediately in cold tap water. After 2 min plus C.U.T. the second tube was removed and cooled in the same manner.

The TDT tubes containing the heat injured spore suspensions were opened aseptically and diluted to obtain approximately 30–300 colony forming units per plate based on the heating time and temperature parameters. Each analyst used inocula derived from separately heated TDT tubes and subsequent dilutions. Thus the

Authors Polvino and Bernard are affiliated with the Washington Research Laboratory, National Food Processors Association, 1133 20th Street, N.W., Washington, DC 20036.

counts resulting from each heated TDT tube were analyzed as a separate test run. All other aspects of the inoculation, plating and enumeration procedure were the same as for phase one with the exception that 0.1% thioglycollate was added to the overlay agar to enhance anaerobiosis. The media used were the same three employed in the first phase of the study plus pork infusion agar (PIA) and T-Best agar (TBA).

Statistical methods

The first phase of the study was designed to allow separation and quantitation of variance due to analyst, medium and procedure (error variance) by employing a two way analysis of variance on the resulting counts. By obtaining a quantitation of variance contributed by analyst, its magnitude could be evaluated against total variance and used to judge if this factor has a degree of significance which would warrant its consideration in phase two. In addition, the error variance could be used for a standard multiple range test to determine significance.

Results from the heat injured portion of the study were analyzed as a randomized complete block analysis of variance experiment with blocking on each test run and with media type analyzed as subplots. A Duncan's multiple range test was used following analysis of heat activated and heat injured count data to determine which specific media types gave best results.

RESULTS & DISCUSSION

RESULTS of the two way ANOVA of heat activated data (Table 1) indicated that a highly significant difference existed between media types. However, the variance due to analyst performing the pour plate procedure was not statistically significant. Using Duncan's multiple range test it was found that both YEA and TPA gave significantly higher counts ($p<0.01$) using the pour plate procedure than PPIA (Table 2).

The results of the heat activated experiment were reproducible for all three media types between test days and analysts, lending confidence to the consistency of the enumeration procedure. Since variance between analysts was not significant in the heat activated phase, this variable was not controlled in the heat stressed phase. Counts of surviving heat stressed spores are shown in Tables 4 and 6. In certain instances, no colonies were recovered at the lowest dilution used for one medium type. The conservative assumption was then made that 0.99 colonies would have been observed at the next lower dilution and the log of this number was used in the data base. For the heat stressed phase of the experiment, a highly significant difference in media efficiency was found for both heat treatments (Table 3 and 5) and a significant difference noted between test runs as well.

Using Duncan's multiple range test, it was found that PPIA gave significantly ($p<0.05$) higher recovery counts than any of the other media for the one minute treatment (Table 4). For the 2-min heat treatment, PPIA, YEA and TBA were all in the same range of recovery efficiency, significantly ($p<0.05$) above PIA (Table 6). TPA was in the lowest range for both heat and stress experiments.

TPA was found to be an adequate medium for enumeration purposes but totally inadequate for heat stressed spore recovery. Pork pea infusion agar was found less efficient than YEA and TPA as an enumeration medium, but gave the best spore recovery under heat stress conditions. YEA seemed to be the most consistent recovery medium for both heat activated and heat stressed spores when compared to the other media types. The error variance from the heat stressed studies of phase two was much larger than the variance in the first phase of testing and a significant difference was also found between test runs in the second phase. Since the plating, incubation and enumeration procedures were held constant over both phases and it was shown that variance contributed by analyst was insignificant, this loss of precision and lack of consistency from run to run, especially in the two minute heat treatment of phase two, can be attributed solely to the effect of the heat treatment on the spores. A plausible explanation is that an increased period of dormancy or recovery time occurs as heating increases. The limited time of incubation (48 hr) may reduce the consistency of results between tests because of the presence of viable but dormant spores.

The relative efficiency of recovery media remained the same for both heat treatments in phase two although no significant difference was found between PPIA, YEA and TBA in the two minute heat treatment. The variability in results using the pour plate procedure and 48 hour incubation period could indicate that this procedure may not be accurate enough to give a true comparison of recovery media.

Table 1—Analysis of variance of log count[a] results for the enumeration of heat activated[b] P.A. 3679 spores

Source of variation	Sum of squares	d.f.	Mean square	F	$F_{0.95}$	$F_{0.99}$
Total	0.331	29				
Subgroups	0.206	5	0.041			
Between-means of analysts	0.004	1	0.004	0.798	4.26	
Between-means of media types	0.194	2	0.097	18.675	4.26	7.82
Interaction (media type x analyst)	0.007	2	0.004	0.716	3.40	
Error	0.125	24	0.005			

[a] Log count indicates average of duplicate plates per test x dilution factor
[b] 13 min in an 80°C bath

Table 2—Log count[a] results between culture media and analysts for the enumeration of heat activated[b] P.A. 3679 spores.

Media type	Analyst	Test day 1	2	3	4	5	Mean[c]	Mean[d]	Range[e]
TPA	1	7.9684	7.7634	8.0128	8.0569	8.1399	7.9883	7.998	I
	2	7.9868	8.0043	8.0211	8.0293	7.9956	8.0074		
YEA	1	8.0606	8.0170	8.0128	8.0755	8.0969	8.0526	8.036	I
	2	8.0413	8.0374	8.0212	8.0644	7.9294	8.0187		
PPIA	1	7.8633	7.8325	7.9294	7.8633	7.8976	7.8772	7.849	II
	2	7.8388	7.8808	7.8325	7.8633	7.6902	7.8211		

[a] Log count indicates average of duplicate plates per test x dilution factor.
[b] 13 min in an 80°C bath
[c] Means per analyst for each media type
[d] Means per media type
[e] Means with the same Roman numeral designation are in the same range of recovery efficiency according to Duncan's multiple range test at a 99% confidence level using the ANOVA error variance of 0.005

Table 3—Analysis of variance of log count[a] results for the recovery of heat stressed[b] P.A. 3679 spores

Source of variation	Sum of squares	d.f.	Mean square	F	$F_{0.95}$	$F_{0.99}$
Total	15.7656	34				
Between-means of media type	12.4607	4	3.1018	37.5	2.78	4.22
Between-means of tests	1.3195	6	0.2199	2.659	2.51	
Error	1.9855	24	0.0827			

[a] Log count indicates average of duplicate plates per test x dilution factor
[b] 1 min at 121°C

Table 4—Log count[a] results between culture media for the recovery of heat-stressed[b] P.A. 3679 spores

	Test no.								
Media type	1	2	3	4	5	6	7	Mean[c]	Range[d]
PPIA	5.8633	5.9138	5.8513	5.8921	5.9731	6.0792	5.9085	5.9259	I
PIA	5.3424	5.1762	5.3010	5.3010	5.0792	5.4624	5.4624	5.1176	III
YEA	5.3802	5.3802	5.7482	5.6128	5.7076	5.9191	5.9031	5.6636	II
TBA	5.4914	5.301	5.5315	5.0414	5.7076	5.8513	5.8451	5.5385	II
TPA	3.9956	3.9956	4.7782	4.0000	4.7782	3.9956	3.9956	4.2198	IV

[a] Log count indicates average of duplicate plates per test x dilution factor.
[b] 1 min at 121°C
[c] Mean over 7 test runs
[d] Means with the same Roman numeral designation are in the same range of recovery efficiency according to Duncan's multiple range test at a 95% confidence level using the ANOVA error variance of 0.0827

Table 5—Analysis of variance of log count[a] results for the recovery of heat stressed[b] P.A. 3679 spores

Source of variation	Sum of squares	d.f.	Mean square	F	$F_{0.95}$	$F_{0.99}$
Total	19.2671	29				
Between-means of media type	11.6544	4	2.9136	13.3713	2.87	4.43
Between-means of tests	3.2548	5	0.6510	2.9876	2.71	
Error	4.3579	20	0.2179			

[a] Log count indicates average of duplicate plates per test x dilution factor
[b] 2 min at 121°C

Table 6—Log count[a] results between culture media for the recovery of heat-stressed[b] P.A. 3679 spores

	Test no.							
Media type	1	2	3	4	5	6	Mean[c]	Range[d]
PPIA	4.2553	3.2304	4.2788	4.2788	4.0792	3.6335	3.9593	I
PIA	4.0414	3.4472	2.9031	2.4771	2.9031	2.6021	3.1543	II
YEA	4.2553	3.2304	3.6335	3.8541	3.9191	3.5052	3.7314	I
TBA	4.1461	2.2041	3.5441	3.4624	3.8633	3.5315	3.6253	I
TPA	3.3424	2.0000	2.0000	2.0000	2.0000	2.0000	2.2237	III

[a] Log count indicates average of duplicate plates per test x dilution factor
[b] 2 min at 121°C
[c] Mean over 6 test runs
[d] Means with the same roman numeral designation are in the same range of recovery; Efficiency according to Duncan's multiple range test at a 95% confidence level; Using the ANOVA error variance of 0.2179

Other authors have also reported the possible effect of dormancy of heat injured spores on resulting survivor counts. Wheaton and Pratt (1961) found that comparisons of subculture media were not consistent between tests when recovery of two spore crops of heat injured P.A. 3679 was the objective. Using another putrefactive strain they noted that the subculture media did indeed perform consistently in three tests although the amount of recovery was different in each test. Their results were all statistically analyzed with 72 hr counts in Veillon tubes reportedly giving near maximal growth. A more consistent comparison between tests was found by Frank and Campbell (1955) using an endpoint determination in tubes and an incubation time of 60 days.

In summary, yeast extract agar and trypticase peptone agar gave statistically higher counts than pork pea infusion agar for the enumeration of this heat activated P.A. 3679 spore suspension using a pour plate technique. Using the same procedure for the recovery of heat injured spores however, it was found that pork pea infusion agar resulted in the highest mean recovery counts but the reproducibility of the overall procedure diminished with increasing thermal injury. Statistically, the detectable difference between efficiency of all media types for enumerating heat injured spores was difficult to predict due to the increase in overall variance.

REFERENCES

Anderson, A.A. 1951. A rapid plate method of counting spores of Clostridium botulinum. J. Bacteriol. 62: 425.

Frank, H.A. and Campbell, L.L. Jr. 1955. The influence of recovery media on thermal resistance values of spores of a putrefactive anaerobic bacterium. Appl. Microbiol. 3: 300.

National Food Processors Association, 1968. "Laboratory Manual for Food Canners and Processors," Vol. 1, 3rd ed. Avi Publishing Co., Westport, CT.

Odlaug, T.E. and Pflug, I.J. 1977. Recovery of spores of Clostridium botulinum in yeast extract agar and pork infusion agar after heat treatment. Appl. Env. Microbiol. 34: 377.

Pflug, I.J., Scheyer, M., Smith, G.M., and Kopelman, M. 1979. Evaluation of recovery media for heated Clostridium sporogenes Spores. J. Food Prot. 4212: 946.

Stumbo, C.R. 1965. Media. In "Thermobacteriology in Food Processing," p. 29, 1st ed. Academic Press Inc., New York, NY.

Wheaton, E. Pratt, G.B. 1961. Comparative studies on media for counting anaerobic spores. 2. J. Food Sci. 26: 261.

Ms receives 6/22/81; revised 10/26/81; accepted 10/31/81.

Comparison of Selected *Yersinia enterocolitica* Indicator Tests for Potential Virulence

NORMAN J. STERN and JOHN M. DAMARÉ

ABSTRACT

Three recently developed tests used to indicate virulence were performed on 34 strains of *Yersinia enterocolitica*. Agreement in virulence or nonvirulence prediction was found in (a) 29 of 31 strains when tested for calcium growth dependency at 35°C; (b) all 34 strains when tested for the ability to autoagglutinate in tissue culture medium at 35°C; and (c) among all 34 strains when tested for the presence of a 40–48 Mdal plasmid using a modification of the Hansen and Olsen method for plasmid analysis. The autoagglutination assay is recommended for testing potential pathogenicity of foodborne isolates. There are probably several virulence-determining factors for *Y. enterocolitica*, and no one in-vitro test will suffice. The analytical procedure described herein can be used to detect plasmids ranging in size from approximately 2×10^6 to 3×10^8 daltons.

INTRODUCTION

Yersinia enterocolitica is a ubiquitous bacterium indigenous to the gastrointestinal tract of warm blooded animals associated with a variety of human and animal diseases. It has been isolated from a variety of foods (Black et al., 1978; Hanna et al., 1976; Stern, 1981) and is able to multiply to high population levels at normal refrigeration temperatures (Stern et al., 1980a). Differentiation of pathogenic and nonpathogenic strains has been difficult. However, recent detection of a virulence plasmid in *Y. enterocolitica* and also in *Y. pseudotuberculosis* now provides a characteristic of distinguishing potentially virulent strains. The virulence-mediating plasmid ranges in size between 40–48 $\times 10^6$ daltons (Mdal) and was first characterized from strains that are pathogenic to humans (Zink et al., 1980; Gemski et al., 1980a; Portnoy et al., 1981). It is associated with enhanced virulence in mice and the expression of a V and W antigen complex and other virulence determinant properties shared with the plague bacillus, *Y. pestis* (Carter et al., 1980).

In addition to screening for the presence of this plasmid, other methods for testing pathogenicity have been considered and reported (Smith et al., 1981; Mors and Pai, 1980; Stern et al., 1980b). Two recently developed tests appear to hold promise for food microbiologists: a test demonstrating aggulutination of virulent *Y. enterocolitica* strains when grown at 35°C in tissue culture medium (Laird and Cavanaugh, 1980) and an agar medium selecting for calcium dependency of virulent *Y. enterocolitica* at 35°C (Gemski et al., 1980b; Carter et al., 1980). In this study, three tests for determining the potential virulence of *Y. enterocolitica* were compared.

Author Stern is affiliated with the Meat Science Research Laboratory, USDA, Beltsville Agricultural Research Center,, Beltsville, MD 20705. Author Damaré is affiliated with the Food Safety & Inspection Service, Beltsville Agricultural Research Center, Beltsville, MD 20705.

MATERIALS & METHODS

Yersinia enterocolitica strains

Strains and sources are listed in Table 1. Stock cultures were maintained on tryptic soy agar (TSA, Difco) slants, and transferred for both growth and subsequent storage at about 23°C. Strains were streaked onto TSA plates, and isolated colonies were characterized as *Y. enterocolitica* with API-20E strips (Analytab Co., Plainview, NY).

Virulence indicator testing procedures

Tissue culture medium autoagglutination was carried out as described by Laird and Cavanaugh (1980). This test uses the property possessed by potentially virulent isolates of *Y. enterocolitica* to autoagglutinate in tissue culture medium after overnight incubation at 35°C. Avirulent strains are turbid after overnight incubation at 35°C.

Calcium growth dependency was measured according to the method of Gemski and co-workers (1980b). This test depends upon the ability of avirulent strains of *Y. enterocolitica* to grow on calcium chelated medium at 35°C. Potentially virulent strains are calcium dependent at 35°C and therefore show reduced growth under these restrictive conditions.

For detection of the 40 to 48-Mdal plasmids, a modification of methods described by Hansen and Olsen (1978) was employed. Cells were grown in 40 ml of tryptic soy broth (TSB, Difco) + 0.6% yeast extract on a shaker water bath, overnight at 30°C (about 2×10^8 cells/ml). Other growth media may be suitable, but carbon sources such as sugars and polyols were restricted to prevent formation of excessive amounts of polysaccharides. Cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C, then washed once in 10 ml of 0.01M sodium phosphate buffer, pH 7.0, and centrifuged at 10,000 × *g*, for 10 min at 4°C. Washed cells were resuspended in 1.35 ml of 25% sucrose in 0.05M TRIS, pH 8.0 (at the maximum setting on a vortex mixer) to begin spheroplast formation. One-tenth milliliter of freshly prepared lysozyme (10 mg/ml in 0.25M TRIS, pH 8.0) was mixed with four gentle inversions, and the mixture was incubated for 15 min at approximately 23°C. After incubation with lysozyme, 0.5 ml of 0.25M Na_2EDTA, pH 8.0, was added and mixed with five gentle inversions. This mixture was incubated for 15 min at room temperature, to allow spheroplast formation.

To lyse cells, 0.5 ml of 20% sodium dodecyl sulfate (SDS) in TRIS, 0.02M Na_2EDTA, pH 8.0 (TE buffer) was added, and the mixture was inverted five times. To denature the chromosomal DNA, the mixture was placed in a 55°C water bath for 15 sec then inverted gently five times. These heat pulses were repeated for a total of eight to ten times.

To remove the membrane-bound chromosome, the preparation was made 4% SDS by adding 1.7 ml of 10% SDS in TE buffer mixture and mixing gently with five inversions. It was then brought to 1.0M NaCl by adding 1.1 ml of 5M NaCl to the mixed suspension and immediately mixing with 20 slow inversions (failure to mix immediately after the NaCl is added resulted in incomplete removal of linear DNA). The mixture was chilled in an ice bath and refrigerated at 4°C for 6 hr or overnight. Salt-precipitated DNA was separated by centrifugation at 17,000 × *g* for 30 min at 4°C.

To concentrate plasmids, the recovered supernatant was carefully decanted into graduated polystyrene tubes (Corning #25311, NY), and any white floc-like material was carefully removed with a Pasteur pipette. The volume was noted, and the mixture chilled for 5 min in an ice bath. Then, the mixture was gently decanted into chilled 50-ml polycarbonate centrifuge tubes and 0.313 volumes of 42% polyethylene glycol 6000 (PEG) in 0.01M NaH_2PO_4, pH 7.0, was added and gently stirred in with a plastic pipette. The tubes

were then refrigerated for 6 hr or overnight. Precipitated DNA was separated by centrifugation at 800 x *g* for 5 min at 4°C. The pellet was removed and gently resuspended in 0.15 ml of cold 50 mM TRIS + 50 mM NaCl + 5 mM Na_2EDTA, pH 8.0 (TES buffer). The final yield volume was about 0.25 ml of plasmid enriched DNA. The preparation can be stored in small aliquots below 0°C for extended periods. Repeated freezing and thawing induces breakage of the DNA molecules and increases the amount of open circular (nicked) and linear fragments.

Samples of 10–40 μl of the plasmid enriched preparation were analyzed in 1 x 6 x 5 mm wells in a bed of 0.8% medium EEO Agarose (No. A-6877, Sigma, St. Louis, MO) and analyzed on the horizontal gel slab at 100 volts. A 1.0% concentration of agarose was used for the electrophoretic gel legs (jays). The 0.8% agarose gel was prepared by melting at 110°C in 0.9 volume of distilled water and adding 0.1 volume of TRIS-borate buffer (10x-TBB, 890 mM boric acid, 890 mM TRIS, 25 mM Na_2EDTA) just before casting the gel. The gel slab was cast in a level plastic tray (check position with bubble-level). The jays were cast and allowed to completely harden prior to casting the gel slab bed. Approximately 150 ml of agarose were poured to form a 12 x 13.5 x 0.6 cm slab. The well-forming comb was inserted while the agarose bed was still molten and removed carefully after the gel had set and cooled.

The tracking dye consisting of 7% SDS, 0.07% bromphenol blue, and 33% glycerol in distilled water was mixed with each sample at 5 μl/sample before it was added to the wells. Electrophoresis was carried out on a water-cooled Savant HGE 1312 electrophoresis instrument (Hicksville, NY) until the dye reached the end of the gel. The gel was stained in ethidium bromide (10 μg/ml) for 30 min and rinsed in cold flowing water for 15 min. Because ethidium bromide is a mutagen, plastic gloves were used during staining and rinsing to prevent needless exposure.

The DNA plasmid bands were visualized under a hand held, short-wave ultraviolet (UV) light source. The relative positions were recorded, and precise measurements were made from photographs taken through a #24 Wratten (Red) filter with Polaroid 55 P/N film. An *Escherichia coli* reference strain (V517), with eight plasmids of known molecular weights, was analyzed with the *Y. enterocolitica* strains (Macrina et al., 1978). These known plasmid reference weights served as standards used to estimate the molecular weight of plasmids from the *Y. enterocolitica* strains.

RESULTS & DISCUSSION

THE RESULTS OF TESTS for autoagglutination in 34 strains are summarized in Table 1. The positive reactions for strains capable of this agglutination were most often clear-cut. The supernatant fluid was clear and cellular material was agglutinated at the bottom of the test tube. Other times, the agglutinated cellular materials clump along the sides of the tube, leaving a clear supernatant. Avirulent strains generally were tubid after overnight incubation at 35°C. The original report by Laird and Cavanaugh (1980) should be consulted. A photograph of agglutinating and nonagglutinating strains has been published (Stern, 1981). Laird and Cavanaugh (1980) reported that of the 25 agglutinating strains of *Y. enterocolitica*, all proved to be viru-

Table 1—Strains and sources of Yersinia enterocolitica and results of selected virulence indicator tests

Strain identification	Supplier[a]	Isolation source[b]	Virulence indicator test: Autoagglutination test[c]	Virulence indicator test: Horizontal gel electrophoresis[d]	Virulence indicator test: Calcium dependency for growth at 35°C[e]
78-513	Lee	Pork	+	+	+
78-513 (–)[f]			–	–	–
1994	Lee	Oyster	–	–	–
1903	Lee	Oyster	–	–	–
IM 2397	Mehlman		+	+	+
IM 2397 (–)			–	–	–
HSJ	Lee	HCI	–	–	–
IP 383	Lee	HCI	–	–	–
IP 107	Lee	HCI	–	–	–
IP 161	Lee	HCI	–	–	–
IP 102	Lee	Unknown	–	–	–
IP 614	Lee	HCI	–	–	–
WA	Lee	HCI	+	+	–
WA (–)			–	–	–
IP 134	Lee	HCI	–	–	–
75-1261	Lee	Oyster	–	–	–
Q 9896	Lee	Oyster	–	–	–
IP 373	Lee	HCI	+	+	+
IP 533	Lee	HCI	–	–	–
KC 1296	Mehlman	HCI	+	+	–
KC 1296 (–)	Mehlman	Unknown	–	–	–
74-513	Mehlman	Oyster	–	–	–
IP 336	Mehlman	HCI	–	–	–
A 2635	Brenner	Chocolate	+	+	+
A 2635 (–)		Milk	–	–	–
IP 955	Brenner	Water	–	–	–
IP 867	Brenner	HCI	–	–	–
A 2611	Brenner	HCI	–	–	–
MSRL 1	Stern	STS	–	–	–
MSRL 2	Stern	STS	–	–	–
MSRL 4	Stern	STS	–	–	–
MSRL 6	Stern	STS	+	+	ND[g]
MSRL 7	Stern	STS	+	+	ND
MSRL 10	Stern	STS	+	+	ND

[a] Lee = W.H. Lee, Food Safety & Inspection Quality Service, BARC-E, Beltsville, MD; Mehlman = I.J. Mehlman, Food & Drug Administration, Washington, DC; Brenner = D. Brenner, Center for Disease Control, Atlanta, GA; Stern = N.J. Stern, Meat Science Research Laboratory, BARC-E, Beltsville, MD.

[b] HCI = Human Clinical Isolates; STS = Swine throat swab
[c] Autoagglutination test (Laird and Cavanaugh, 1980)
[d] Determination of presence of 40—48 Mdal plasmid
[e] Calcium dependency for growth at 35°C (Gemski et al., 1980b)
[f] (—) = Indicates isogenic strain cured of the 40—48 Mdal plasmid
[g] ND = not done

lent to mice by the oral route. None of the 185 nonagglutinating strains proved virulent.

The presence or absence of the 40–48 Mdal plasmid, as determined by horizontal gel electrophoresis, correlated exactly with the results of the autoagglutination test (Table 1; Fig. 1). This finding corroborates with results reported by Zink et al. (1980) and Damaré et al. (1980). Horizontal gel electrophoresis is best used for confirmation tests, and the autoagglutination test is more easily performed for screening. The electrophoresis system described, although not the simplest indicator of virulence, is extremely versatile: it can be used to detect antibiotic-resistant plasmids, the virulence and toxin determinant plasmids of enterotoxigenic *E. coli*, and other plasmid species ranging in size from approximately 2×10^6 to 3×10^8 daltons.

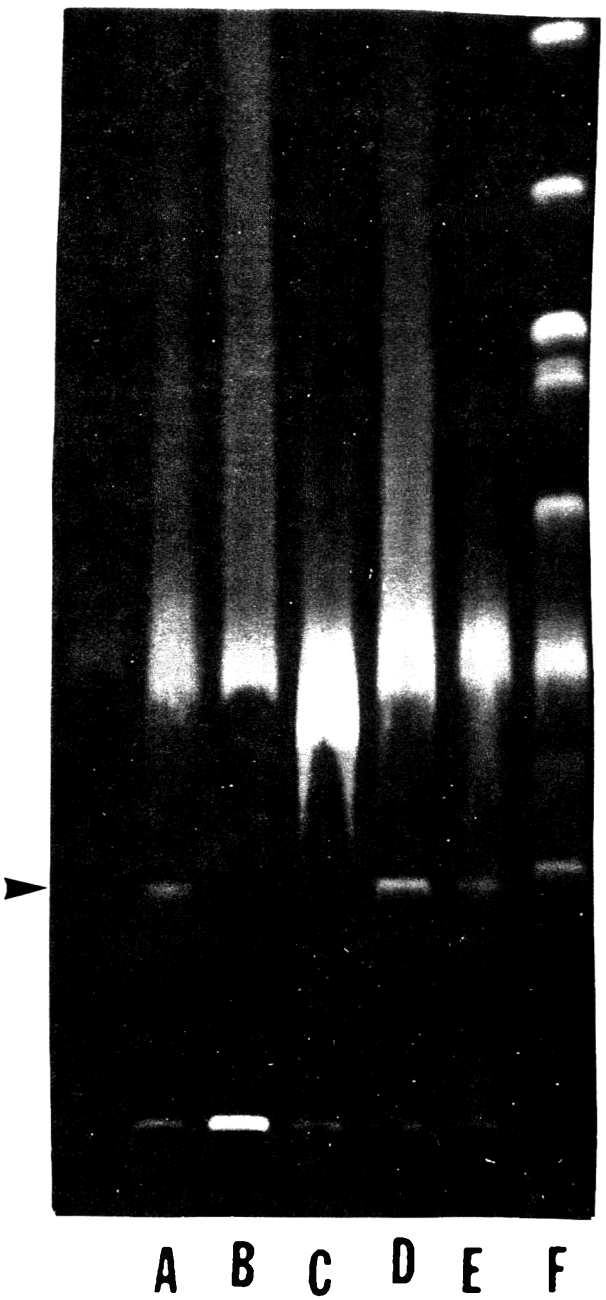


Fig. 1–Horizontal gel electrophoresis of plasmid DNA. Description of procedure found in text. Columns A, D, and E demonstrate strains of Yersinia enterocolitica containing virulence mediating 40–48 Mdal plasmid. Columns B and C demonstrate lack of plasmid content in avirulent Y. enterocolitica. Column F demonstrates plasmids in Escherichia coli V517 reference strain.

The reduced growth of virulent strains 78-513, A 2635, IM 2397, and IP 373 on magnesium oxalate agar compared to blood agar base was expected (Table 2). These four strains were positive for potential virulence by both autoagglutination and horizontal gel electrophoresis tests. Two other strains, WA and KC 1296, did not show calcium-dependent growth as would have been predicted by the other two indicator tests, whereas the remaining 25 strains, as anticipated, were not inhibited on the calcium-chelated magnesium oxalate plates.

Several attempts failed to demonstrate the calcium-dependent growth of agglutination-positive strains WA and KC 1296, although Gemski et al. (1980b) presented data demonstrating this dependency for the WA strain. An explanation for this inconsistency is lacking. Data from five cured (nonagglutinating) strains were comparable to those of the remaining 20 strains (Table 2). Calcium dependency of growth for the MSRL 6, 7, and 10 strains was not recorded because they were spontaneously cured of their ability to agglutinate and no longer contained the 40–48 Mdal plasmid coding for the V and W antigens responsible for agglutination (Carter et al., 1980). Therefore, these cured strains would no longer be inhibited on the magnesium oxalate agar.

Schiemann and Devenish (1980) reported that virulence as predicted by the Sereny and Mongolian gerbil tests was restricted to only a few *Y. enterocolitica* serotypes. Schiemann and Devenish reported that HeLa cell invasiveness was likewise limited to certain serotypes while Aulisio suggested that this test is not necessarily restricted to certain serotypes (personal communications). Heat-stable enterotoxin was almost uniform among all serotypes (Mors and Pai, 1980). It is highly likely that virulence of the organism for humans is controlled by several factors among a given pathogenic strain. Unquestionably, the presence of the 40–48 Mdal plasmid is a key determinant of virulence. In this and other studies, the easiest test for predicting potential virulence appears to be the autoagglutination test developed by Laird and Cavanaugh (1980). However, a screening method that could select virulent *Y. enterocolitica* from among a larger avirulent population still needs to be developed. Such a selective screen would aid in monitoring foods for adulteration with virulent *Y. enterocolitica*.

Our laboratory has attempted to conjugate a plasmid containing *Y. enterocolitica* with a nonplasmid containing

–*Continued on page 588*

Table 2–Growth of selected strains of Yersinia enterocolitica on Magnesium Oxalate Agar and Blood Agar Base at 35°C[a]

Strain	Magnesium oxalate agar	Blood agar base	Calcium dependency indicated
78-513	1[b]	44	yes
78-513 (–)[c]	198	115	no
A 2635	1	48	yes
A 2635 (–)	41	49	no
IM 2397	0	24	yes
IM 2397 (–)	56	51	no
WA	49	56	no
WA (–)	52	47	no
KC 1296	48	56	no
KC 1296 (–)	47	37	no
IP 373	0	60	yes

[a] Gemski et al. (1980b)
[b] Number of colonies on plate
[c] (–) indicates isogenic strain cured of 40–48 Mdal plasmid

Separation and Purification of Hydroxycinnamic Acid Derivatives in Cranberries

A. G. MARWAN and C. W. NAGEL

ABSTRACT

Hydroxycinnamic acid derivatives have been shown to be a complex mixture of closely related compounds. A procedure has been described for their isolation and purification from cranberries. The hydroxycinnamates were extracted from the plant tissue with aqueous alcohol, followed by pre-separation on a polyamide CC-6 column. For easier sample concentration, the eluted compounds were extracted in alcohol. Final separations were made on a High Performance Liquid Chromatograph. Recycling was used to purify the individual compounds. A total of 15 compounds was purified. The method can be used for the separation and purification of individual phenolic compounds from mixtures.

INTRODUCTION

IT IS WELL KNOWN that cranberries have a bitter-sour taste. It has been postulated that the hydroxycinnamic acids and their derivatives are mainly reasponsible for this flavor. The separation and purification of such compounds in cranberries has been the subject of many studies. Goldstein (1976) separated a cranberry extract on a polyethylene glycol dimethacrylate column into five fractions, none of which was further purified. Cansfield and Francis (1970) separated 11 phenolic compounds from a cranberry extract, seven of them were reported to be phenolic acids or phenolic acid derivatives. The most definitive work on cinnamic acids in cranberries was performed by Chu et al. (1973). Using paper chromatography, they isolated 10 fluorescent compounds from a cranberry extract. The 4-glucoside of caffeic acid was found to be the major compound and was the only one defined.

Quantity pre-cleanup and separation of complex mixtures of these compounds usually was achieved by open column chromatography. Blundstone (1970) eluted the hydroxycinnamic acid derivatives of rhubarb extract with 80% methanol on a PVP column. Hanefeld and Herrmann (1976) used a polyamide column to separate caffeic acid esters from the extracts of many fruits. A similar technique was used by Hanson and Zucker (1963) to separate a variety of hydroxycinnamic acid derivatives.

Sondheimer (1958) used a silicic acid column to separate the isomers of chlorogenic acid from a variety of fruits, including grapes. Later, Ribéreau-Gayon (1972) showed that such a column does not differentiate between the esters of quinic acid and the esters of tartaric acid. This is in accordance with the findings of Ong and Nagel (1978) and Baranowski and Nagel (1981) that grapes contain tartaric, but not quinic acid esters of hydroxycinnamic acids.

Krause (1978) described a procedure for separating sugar esters from quinic acid esters of hydroxycinnamic acids on a polyamide column. Also, a procedure was developed by Wulf and Nagel (1976) to separate the substituted benzoic and cinnamic acids on a μ Bondapak/C_{18} column. Ong and Nagel (1978) in a study on the hydroxycinnamates of *Vitis vinifera* grapes, precleaned the grape extract on a polyamide column, and separated the different compounds on a μ Bondapak/C_{18} column by HPLC. A similar approach was used by Baranowski and Nagel (1981) to separate the hydroxycinnamic acid derivatives in White Riesling grapes and wine.

The purpose of this paper is to describe a procedure to separate the hydroxycinnamates in cranberries in large amounts, and with a high degree of purity.

Authors Marwan and Nagel are affiliated with the Dept. of Food Science & Technology, Washington State Univ., Pullman, WA 99164.

MATERIALS & METHODS

Sample preparation

Early Black variety cranberries were obtained from Ocean Spray Cranberries, Inc. (Aberdeen, WA) and stored at −30°C until used. Frozen cranberries (200g) were macerated with 400 ml 95% ethanol in the presence of 1,000 ppm SO_2, added as sodium metabisulfite, in a Waring Blendor at high speed for 5 min. In order to collect enough sample, this procedure was repeated ten times. The mash was removed by filtering under vacuum through Whatman #1 filter paper, and the filtrate was concentrated in a vacuum evaporator at 30°C (Buchler Instruments rotary evaporator, Fort Lee, NJ) to about 300 ml, centrifuged and filtered again. It was acidified to 0.5% formic acid and stored in the refrigerator.

Cleaning and separation on polyamide CC-6 column

A 66 cm x 3 cm glass column was packed with polyamide CC-6 (Brinkmann Instruments, Inc. Westburg, NY), equilibrated with 500 ml of the starting elution solvent which was 0.2% formic acid. The concentrated cranberry extract sample was applied to the column and allowed to percolate in. Elution was started with 0.2% formic acid. Fractions of 20 ml were collected using an automatic fraction collector (Instrumentation Specialities Company, Lincoln, NE). The effluent of the column was monitored by measuring the absorbance of every other fraction at 280 nm and 320 nm on a Beckman DU modified with an optical density convertor, light source stabilizer and cuvette positioner (Gilford Instrument Laboratories, Oberlin, OH). When the absorbance of the fractions leveled off after showing a peak at 320 nm, (formic acid peak), elution was continued with 100% methanol until another peak was resolved (Methanol peak), and then completed with 0.01% ammoniated methanol.

This approach eluted the pigments along with the hydroxycinnamates. Therefore, another polyamide CC-6 column (35 cm x 4.2 cm) was packed and equilibrated with 0.1% sodium acetate buffer, pH 4.0. This solvent allows the hydroxycinnamates to be eluted while the anthocyanins are retained on the column. The fractions of the formic acid peak of the above separation were pooled, concentrated and applied to this column. Elution was with 0.1% sodium acetate buffer, pH 4.0. Fractions of 20 ml were collected and elution was monitored by measuring the absorbance of every other fraction at 280 nm and 320 nm, After the hydroxycinnamates were eluted, the column was flushed with one liter of 95% ethanol and equilibrated again with the 0.1% sodium acetate pH 4.0 buffer, and the same was done for the fractions of the methanol peak of the first separation.

Ethanolic extraction

The collected fractions in the sodium acetate buffer were hard to concentrate, and also most of the sugars came out with the phenolics of the formic acid peak. Therefore, ammonium sulfate-ethanol extraction was used to get rid of the sugars and to get the phenolics in ethanol, which was easier to concentrate. To the combined fractions of formic acid and methanol peaks, separate-

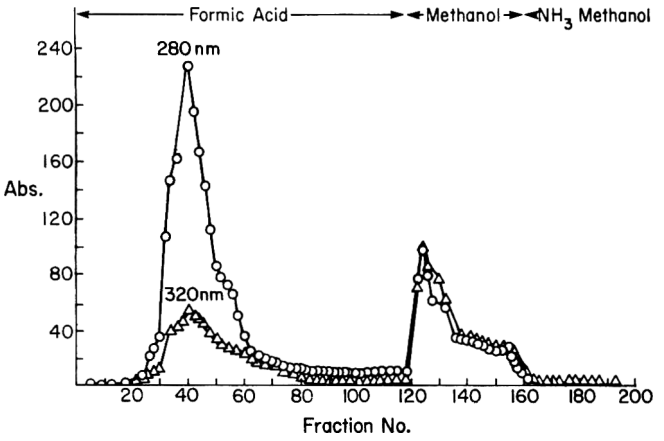


Fig. 1—Polyamide CC-6 separation of the ethanolic extract of cranberries.

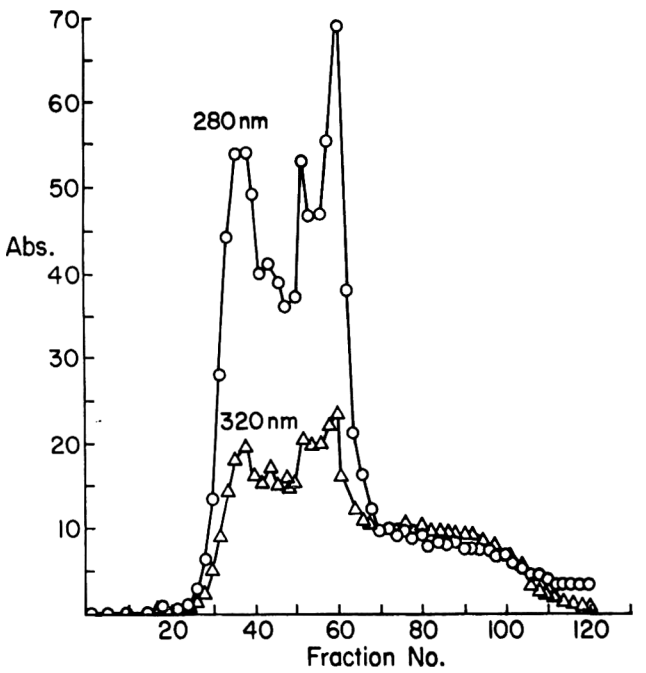


Fig. 2—Sodium acetate buffer elution of the formic acid peak (Fig. 1) on a polyamide CC-6 column.

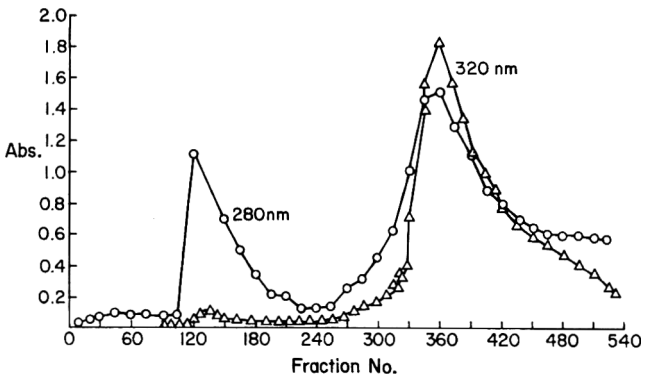


Fig. 3—Sodium acetate buffer elution of the methanol peak (Fig. 1) on a polyamide CC-6 column.

ly, 95% ethanol was added at 25% of their volume. While stirring with a Mag-Mix, ammonium sulfate was added to saturation. The ethanolic layer was separated and kept. This extraction was repeated five times. The ethanolic extracts of each peak were combined and vacuum evaporated at 30°C to about 40 ml for the formic acid peak and to 10 ml for the methanol peak. They then were filtered through a 0.45-μm Millipore filter.

HPLC separation and purification

The solvent delivery system for HPLC consisted of two Waters Associates (Milford, MA) 6000 A pumps, a Model 660 solvent programmer and Model U6K injector. Separations were made on a 25 cm x 9.4 mm (i.d.) Partisil ODS preparative column. The column effluent was monitored by a Micromeritics (Norcross, GA) Model 785 variable wavelength detector set at 320 nm. An Autolab Minigrator (Spectra-Physics, Santa Clara, CA) was used to integrate the peak areas. The detector responses were recorded on a Houston Instruments (Austin, TX) Ominiscribe strip chart recorder.

The following conditions were used for the formic acid peak:

Flow rate = 5 ml/min
Solvent A: 0.5% formic acid
Solvent B: 0.5% formic acid + 8% acetonitrile
Nonlinear gradient setting the solvent programmer on mode #4, run Solvent A for 5 min, then start program from 0–100% B in 20 min.

The column was equilibrated with solvent A for 5 min. Injections of 1.3 ml were made and separate peaks were collected. After this run was completed, the column was flushed with methanol and re-equilibrated with solvent A. This was repeated until enough samples were collected.

The same thing was done for the methanol peak, except isocratic elution was used. The solvent was 7% acetonitrile in 0.5% formic acid. The collected fractions were concentrated to a few milliliters by vacuum evaporation if the volume was small.

For larger volumes the sample was partially concentrated to remove the acetonitrile, then acidified and pumped on the HPLC preparative column until the column became overloaded, which was observed by the recorder response. Then the column was flushed with methanol and the material collected. Methanol is easy to evaporate and thus concentrate the sample without excessive exposure to heat and light.

The concentrated fractions were purified on the HPLC preparative column using the recycle mode and a suitable solvent strength. The purity of the recycled fractions was checked on the HPLC analytical column (Zorbax ODS, 25 cm x 4.6 mm i.d., DuPont, Wilmington, DE). Those fractions which showed 95% or more purity were considered pure. Those which did not show a high degree of purity were concentrated and recycled on the analytical column. Each purified compound was concentrated to dryness either directly by the vacuum evaporator if the volume was small, or after transfer into methanol by the above described technique if the volume was large. Each dried compound was redissolved in 10 ml redistilled 95% ethanol and stored in vials in the refrigerator.

RESULTS & DISCUSSION

FIG. 1 SHOWS that cranberry hydroxycinnamates can be separated primarily into two major groups on a polyamide CC-6 column; A water soluble group (formic acid peak) and an alcohol soluble group (methanol peak). Since the pigments were eluted with the hydroxycinnamates, another solvent system and polyamide column was used. Fig. 2 and 3 show the elution of hydroxycinnamates while the anthocyanins were retained on the column. Material obtained in this manner was then concentrated after transferring it from the aqueous solvent into alcohol. Fig. 4 shows the extraction profile of formic acid peak compounds. This was obtained by multiplying each extraction volume by its absorbance at 320 nm.

Fig. 5 shows the HPLC separation of the formic acid peak compounds. Peaks 3, 4, 6, 7, 8, 9, 10, 11, and 12 were collected, concentrated and individually purified by using the recycling mode of the HPLC system. Fig. 6 illustrates the separation of the methanol peak compounds. Peaks 20, 21, 22, 23, 24, 25, and 26 were collected and also purified by recycling on the HPLC system.

—Text continued on page 588

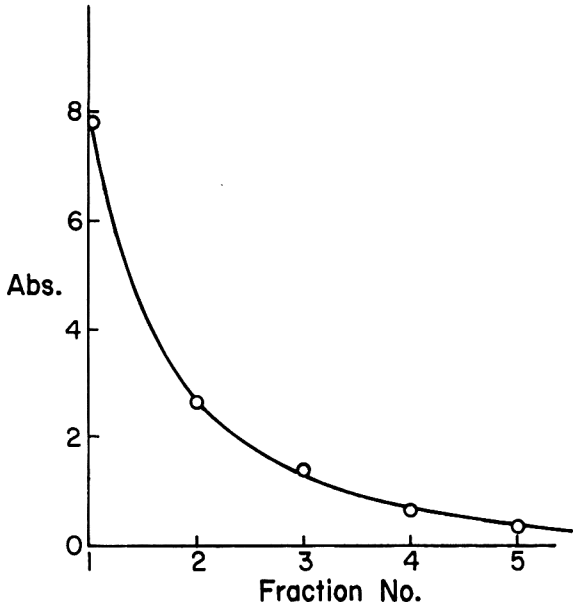


Fig. 4—Ethanol-ammonium sulfate extraction profile of the combined 25—110 fractions (Fig. 2).

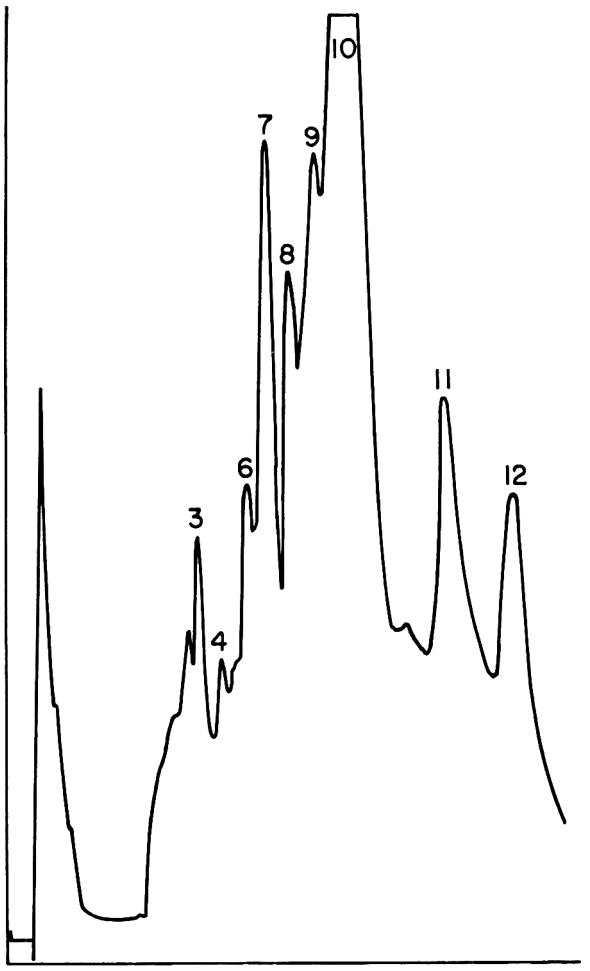


Fig. 5—HPLC preparative column separation of the ethanolic extract of the combined 25—110 fractions (Fig. 2).

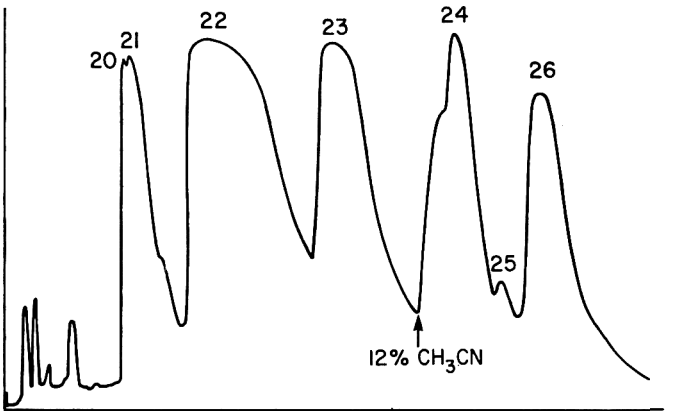


Fig. 6—HPLC preparative column separation of the ethanolic extract of the combined 240—540 fractions (Fig. 3).

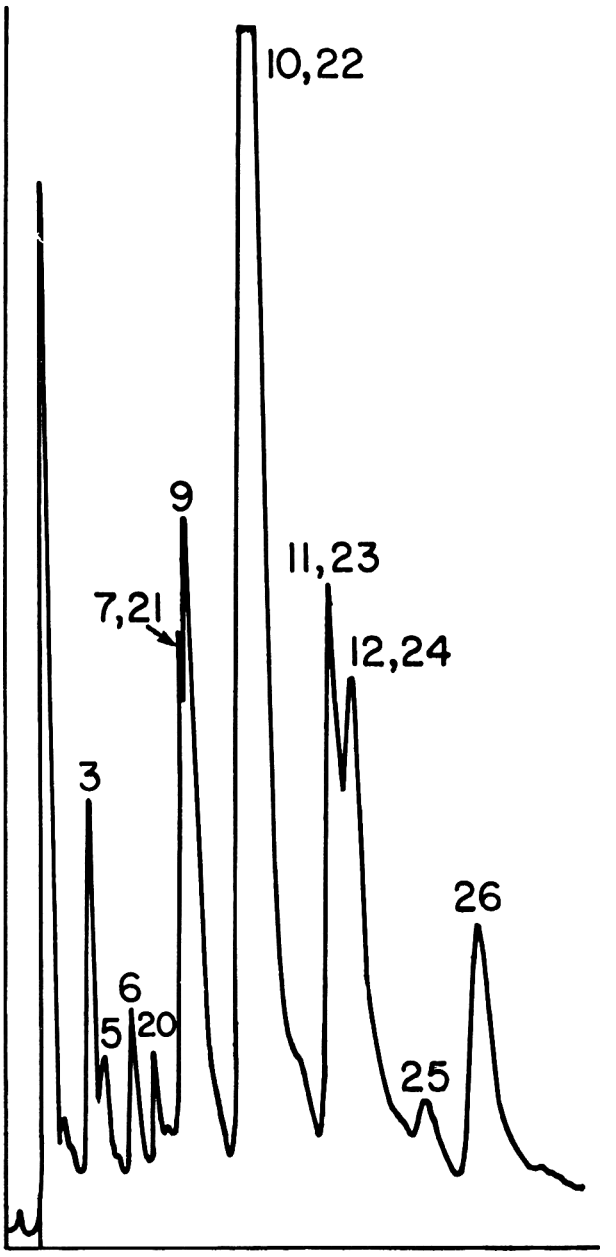


Fig. 7—HPLC separation of the ethanolic extract of cranberry (analytical column).

Fig. 7 shows a typical chromatogram of cranberry juice using 8% acetonitrile in 0.5% formic acid at 1.8 ml/min on the analytical column. The purified compounds were matched with Fig. 7 by their retention times or co-injecting them if it were necessary.

It has been reported that, phenolic compounds with different carbohydrate content are not markedly different in their chromatographic behavior (Wulf and Nagel, 1976). Therefore, the insufficient resolution of compounds 7 and 21, 10 and 22, 11 and 23, and 12 and 24 on the HPLC reversed phase system and their resolution on the polyamide CC-6 column could suggest that each pair of them has the same parent hydroxycinnamic acid but with a different carbohydrate content.

CONCLUSIONS

POLYAMIDE CC-6 column chromatography is a dependable technique for cleanup and pre-separation of complex mixtures of hydroxycinnamates. Individual compounds can then be separated and purified by HPLC. Identifying compounds just by comparing their retention times with standards on HPLC is not dependable, since as shown here a resolved peak on the HPLC analytical column may contain more than one compound. A total of 15 phenolic compounds was isolated and purified from cranberry juice. Their identification will be the subject of a later paper.

REFERENCES

Baranowski, J.D. and Nagel, C.W. 1981. Isolation and identification of hydroxycinnamic acid derivatives in White Riesling wine. Am. J. Enol. Vitic. 32: 5.
Blundstone, H.A.W. 1970. Hydroxycinnamic acid derivatives of Rheum rhaponticum. Phytochem. 9: 1677.
Cansfield, P.E. and Francis, F.J. 1970. Quantitative methods for anthocyanins. 5. Separation of cranberry phenolics by electrophoresis and chromatography. J. Food Sci. 35: 309.
Chu, N.T., Clydesdale, F.M., and Francis, F.J. 1973. Isolation and identificatio of some fluorescent phenolic compounds in cranberries. J. Food Sci. 38: 1038.
Goldstein, G. 1976. Liquid chromatographic separation of plant phenolics using polyethylene glycol dimethacrylate gel. J. Chromatog. 129: 466.
Hanefeld, M. and Herrmann, K. 1976. Quantitative determination of caffeic acid esters and catechins by direct measurement on thin layer chromatograms. J. chromatog. 123: 391.
Hanson, K.R. and Zucker, M. 1963. The biosynthesis of chlorogenic acid and related conjugates of the hydroxycinnamic acids. J. Biol. Chem. 238: 1105.
Krause, J. 1978. Hydroxycinnamic acid derivatives from Spirodela polyrrhiza (L.) Schleiden. Z. Pflanzenphysiol. 88: 465.
Ong, B.Y. and Nagel, C.W. 1978. High-pressure liquid chromatographic analysis of hydroxycinnamic acid-tartaric acid esters and their glucose esters in Vitis vinifera. J. Chromatog. 157: 345.
Ribereau-Gayon, P. 1972. "Plant phenolics." Hanfer Publishing Co., New York.
Sondheimer, E. 1958. On the distribution of caffeic acid and the chlorogenic acid isomers in plants. Arch. Biochem. Biophysics. 74: 131.
Wulf, L.W. and Nagel, C.W. 1976. Analysis of phenolic acids and flavonoids by high-pressure liquid chromatography. J. Chromatog. 116: 271.
Ms received 9/8/81; accepted 10/9/81.

Scientific Paper No. 5995, College of Agriculture Research Center, Washington State Univ., Pullman, WA 99164, Project 0473.

YERSINIA ENTEROCOLITICA VIRULENCE TESTING . . . From page 584

Escherichia coli, but has not been successful. Such transfer of virulence mediating plasmids may provide a plausible explanation for transformation of avirulent varieties of bacteria into virulent forms.

REFERENCES

Black, R.E., Jackson, R.J., Tasai, T., Medevsky, M., Shayegani, M., Feeley, J.C., MacLeod, K.I.E., and Wakelee, A.M. 1978. Epidemic Yersinia enterocolitica infection due to contaminated chocolate milk. N. Engl. J. Med. 298: 76.
Carter, P.B., Zahorchak, R.J., and Brubaker, R.R. 1980. Plague virulence antigens from Yersinia enterocolitica. Infect. Immun. 28: 638.
Damaré, J.M., Smith , R.E., Harris, M.E., Johnston, R.W., and Lee, W.H. 1980. Detection of the virulence associated plasmid in Yersinia enterocolitica isolated from foods. Am. Soc. Microbiol. Abst. Ann. Meet. 80: 189.
Gemski, P., Lazere, J.R., Casey, T., and Wohlhieter, J.A. 1980a. Presence of virulence-associated plasmid in Yersinia pseudotuberculosis. Infect. Immun. 28: 1044.
Gemski, P., Lazere, J.R., and Casey, T. 1980b. Plasmid associated with pathogenicity and calcium dependency of Yersinia enterocolitica. Infect. Immun. 27: 682.
Hanna, M.O., Zink, D.L., Carpenter, Z.L., and Vanderzant, C. 1976. Yersinia enterocolitica-like organisms from vacuum-packaged beef and lamb. J. Food Sci. 41: 1254.
Hansen, J.B. and Olsen, R.H. 1978. Isolation of large bacterial plasmids and characterization of the P2 incompatibility group plasmids pMG1 and pMG5. J. Bacteriol. 135: 227.
Laird, W.J. and Cavanaugh, D.C. 1980. Correlation of autoagglutination and virulence of yersiniae. J. Clin. Microbiol. 11: 430.
Macrina, F.L., Kopecko, D.J., Jones, K.R., Ayers, D.J., and McCowen. 1978. A multiple plasmid-containing Escherichia-coli strain: convenient source of size reference plasmid molecules. Plasmid 1: 417.
Mors, V. and Pai, C.H. 1980. Pathogenic properties of Yersinia enterocolitica. Infect. Immun. 28: 292.
Portnoy, D.A., Moseley, S.L., and Falkow, S. 1981. Characterization of plasmids and plasmid-associated determinants of Yersinia enterocolitica pathogenesis. Infect. Immun. 31: 775.
Schiemann, D.A. and Devenish, J.A. 1980. Virulence of Yersinia enterocolitica determined by lethality in Mongolian gerbils and by the Sereny test. Infect. Immun. 29: 500.
Smith, R.F., Carey, A.M., Damaré, J.M., Hetrick, F.M., Johnston, R.W., and Lee, W.H. 1981. Evaluation of iron dextran and mucin for enhancement of the virulence of Yersinia enterocolitica serotype 0:3. Infec. Immun. (In press).
Stern, N.J. 1981. Isolation of potentially virulent Yersinia enterocolitica from variety meats. J. Food Sci. 46: 41.
Stern, N.J., Pierson, M.D., and Kotula, A.W. 1980a. Growth and competitive nature of Yersinia enterocolitica in whole milk. J. Food Sci. 45: 972.
Stern, N.J., Kotula, A.W., and Pierson, M.D. 1980b. Virulence prediction of Yersinia enterocolitica by pyrolysis gas-liquid chromatography. Appl. Environ. Microbiol. 40: 646.
Zink, D.L., Feeley, J.C., Wells, J.G., Vanderzant, C., Vickery, J.C., Roof, W.D. and O'Donovan, G.A. 1980. Plasmid-mediated tissue invasiveness in Yersinia enterocolitica. Nature 283: 224.
Ms received 8/17/81; revised 10/16/81; accepted 10/20/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 7—10, 1981.

The authors thanks R. Blum for his conscientious technical assistance in this study.

Reference to a brand name does not constitute endorsement by the U.S. Department of Agriculture.

Determination of BHA and BHT in Dehydrated Mashed Potatoes

F. BEAULIEU and D. HADZIYEV

ABSTRACT

The extraction step in BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene) analysis of potato granules, unless precautions are taken, leads to a recovery of only 10–50%. This study showed that BHA (BHT) is retained in granules by retrograded starch and, mostly, its amylose moiety. No satisfactory recovery was obtained using solvents of increasing dielectric constant unless the granules were first hydrated with water. A rapid antioxidant extraction procedure, based on the hydration principle and suitable for quality control labs, is described. Comparative data were acquired for the content of BHA (BHT) in potato granules analyzed by differential pulse voltammetry (using a glassy carbon electrode) and gas-liquid chromatography.

INTRODUCTION

THE ROLE OF phenolic antioxidants in potato granules is to prevent oxidative rancidity of the natural fat of the potato during shipment and storage. The amount allowed in foods is subject to government regulation. Guidelines for dehydrated potato granules permit, for BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene), or their mixture, not more than 50 ppm (Canada), 10 ppm (USA), or 25 ppm for the U.K. and France. The trend in granule production is to decrease the proportion of BHT or omit it completely. In the Add-Back process, antioxidants are usually applied in a formulated emulsifier added to cooked potatoes in the mash-mixing step. Some plants apply BHA (or BHT) separately as an ethanol solution. Steam distillation and volatilization occurring during this step cause initial 40–50% losses of the BHA added, and further losses of about 10% for each recycling of the Add-Back granules. The exact nature of the retention of antioxidants in dry granules is unclear.

Analytical methods for BHA (BHT) determination in granules can be unreliable and most are long and time consuming (Bieth et al., 1978; Halot, 1971). The AOAC method gives results with 40–80% less antioxidant than methods based on continuous extraction of granules. Quality control labs lack a reliable, economical and, above all, a fast method which can be applied in the plant and also be recognized as valid on the export market.

This study elucidates the status of antioxidant retained by model systems consisting of major potato constituents. Also, it describes a rapid and quantitative method for extraction of antioxidants in potato granules, followed by their determination by voltammetry using a glassy carbon electrode and by gas-liquid chromatography (GLC). In addition comparative data are provided for the content of BHA in granules manufactured by an Add-Back granule process.

MATERIALS & METHODS

Potato tubers

Cv. Netted Gem (Russet Burbank) with specific gravity of 1.096 ± 0.002, corresponding to a dry matter content of 25.0%, was grown in Southern Alberta. The tubers were stored at 4°C and were reconditioned at room temperature for 10 days before use. Proximate analysis gave the following percentages on a dry matter basis of peeled tubers: total-N 8.8 (N x 6.25); fat, oil 0.5; crude fiber 2.5; ash 4.0; and starch 75.0. Amylose content of the starch was 21.2%, as determined by potentiometric titration with iodine (Chung and Hadziyev, 1980).

Authors Beaulieu and Hadziyev are with the Dept. of Food Science, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2P5.

Major potato constituents

Cellulose. The tuber cell wall preparation was used as a cellulose matrix. It was isolated from batches of dried tubers mechanically disintegrated in ice-cold water (containing 500 ppm Na-sulfite) using a Virtis homogenizer at full speed. The slurry was then squeezed through Miracloth and washed extensively with deionized water until the residue was free of starch when examined under a polarized-light microscope (Moledina et al., 1978).

Pectin. Citrus pectin, with a 55–60% esterification degree and a mol. weight range of 150–300 x 10^3, was used (ICN-Nutritional Biochemicals, Cleveland, OH).

Protein. Crude proteins were obtained from potato sap extruded from peeled and diced tubers. The dices were wrapped in a cotton cloth and subjected to a pressure of 25 x 10^3 p.s.i. using a hydraulic press (F.S. Carver, Inc., Summit, NJ). The sap was collected, then the cake was dispersed in 10% aqueous NaCl and pressed again. The combined saps were diluted in water and the protein was precipitated at 70°C for 30 min. The fluffy coagulate was collected by low-speed centrifugation and then washed with acetone and ethyl ether. The protein, slightly greyish in color, was air-dried, ground into powder in a mortar with pestle and stored at −20°C.

Starch. Potato starch was isolated from peeled and diced tubers which were homogenized in ice-cold water (containing 500 ppm Na-sulfite) using a Waring Blendor at low speed. The slurry was squeezed through a 100-mesh polyester sieve cloth and the filtrate centrifuged at 700 x *g* for 10 min. The supernatant and the amber-brown protein layered on the starch sediment were removed, and the starch was resuspended in water and recentrifuged. This purification step was repeated until no protein and cell debris were evident under a polarized-light microscope. The final product was treated with ethanol, ethyl ether and acetone, and air-dried.

A batch of the above preparation was used to obtain gelatinized and retrograded starch. Gelatinization was performed at 70°C for 20 min with 100g starch suspended in 1L demineralized water. The gel obtained was cooled to 4°C, the water decanted and the sediment frozen at −25°C for 3 hr. The frozen gel was then freeze-dried and ground to a powder in a Waring Blendor at high speed.

Starch amylose of 273–275°C m.p. (decomposition) with a mol. weight over 150,000, and amylopectin, both from potato, were supplied by Aldrich Chem. Co. Inc. (Milwaukee, WI) and by BDH Lab Chemicals Division (Toronto, Ont).

Antioxidant incorporation into major potato constituents

In order to assay the strength of antioxidant binding to potato constituents, impregnated matrices were prepared by freeze-drying. About 100 ppm of an ethanolic antioxidant solution were added to an aqueous slurry of a potato constituent (protein, starch, etc). Uniform dispersal was achieved with a Kitchen-aid mixer run at low speed for 5 min. Portions of the slurry, 40–60g, were then transferred into plastic containers and freeze-dried at a condenser setting of −50°C and a shelf temperature of 25°C using a Virtis model RePP sublimator. Samples were taken after each 0.5–1 hr and analyzed for antioxidant and moisture contents (Kirleis and Stine, 1978).

—Continued on next page

Antioxidant (BHA)–Amylose inclusion compound preparation

Potato amylose corresponding to 1.5g dry matter was suspended in 15 ml of distilled water, treated with 15 ml of 0.5N NaOH and mixed until the suspension solubilized. The amylose solution was adjusted to pH 6.5–6.8 with 0.5N HCl and then 10 mM K-phosphate buffer pH 7.0 was added (5% v/v). The solution was diluted to 1% with water, heated at 100°C for 1 hr, then cooled to 70°C and deaerated with nitrogen. Under the nitrogen stream, BHA was incorporated (25% on amylose, dry matter basis) and the temperature of the mixture was then gradually lowered to 25°C over 24 hr in a water bath. The precipitated amylose-BHA complex was collected by centrifugation at 6000 x *g* for 15 min, freeze-dried and stored at –20°C (Osman-Ismail, 1972).

X-ray diffraction analysis

Diffraction patterns of amylose-BHA inclusion compounds were recorded on a Philips Model PW-1011-60 diffractometer equipped with a curved crystal AMR monochromator. Copper Kα radiation (1.5418 Å) was used with a time constant of 4 sec, an angular scanning velocity of 1° 2θ and a chart speed of 1 cm/min.

Dehydrated mashed potato granules

The method used by an Alberta processor consisted of steam-peeling, trimming, slicing, washing, precooking, cooling, steam-cooking, mashing by addition of dried A-B granules, conditioning, remixing, air-lift drying, fluidized bed drying, cooling and sifting. Antioxidant (100 ppm on a dry weight basis) was added as an ethanol solution at the mashing step along with other additives.

Antioxidant extraction

Potato constituents. Dehydrated major potato matrices were extracted by a 10 hr continuous Soxhlet procedure using 95% ethanol as solvent (Bieth et al., 1978). As emphasized by the authors, rehydration of the sample (ratio 1:1) had to be performed prior to the extraction.

Potato granules. Pet. ether (b.p. 40–60°C) was the extraction solvent when GLC assay was applied, while benzene was chosen for voltammetry since it was a component of the supporting electrolyte.

The procedure, lasting close to 30 min, involved extraction of the fully rehydrated samples with organic solvent, followed by removal of water and concentration of the organic phase containing the antioxidant.

Potato granule samples of 5g were rehydrated at room temperature with 25 ml water for 10–15 min on a fritted glass funnel (diameter 7.0 cm; pore size 25–50 μm). After removing the excess water by suction, the cake was mixed with 3 x 10 ml solvent and filtered each time. Rehydration (for 2 min) and solvent extraction were repeated. The extract was transferred to a 125 ml separatory funnel, shaken gently and left to stand 5 min. If needed, 10 ml of a 10% sodium chloride solution were added to break the emulsion. The organic layer was then concentrated on a rotary evaporator at 30°C.

Prior to GLC-assay, the initial solvent extraction step was preceded by addition to the cake of a 1 ml solution containing internal standard and a preservative. A stock solution, containing internal standard (62.5 mg of diBHA) and a preservative (1g of paraffin oil) in 25 ml pet. ether, was kept in a freezer. Fresh working solutions were prepared daily by diluting 1 ml of stock solution with 25 ml pet. ether.

Gas chromatographic assay

For GLC assay, the pet. ether extract was evaporated just to dryness. The residue was dissolved in 2 ml of carbon disulfide, and transferred to a sealed reaction vial. Aliquots of 4–5 μl were injected into a Varian model 3700 gas chromatograph equipped with FID detector and a 6 ft x 1/8 in. stainless steel column packed with 3% OV-17 on Gas Chrom Q. The runs were performed isothermally at 165°C with nitrogen (30 ml/min) as a carrier gas. Injector and detector were 200 and 220°C, respectively. Retention times were 4.8 min for BHT, 5.8 for BHA, and 10.0 for diBHA. A peak with retention time of 4 min was observed with BHT when evaporated extracts provided a yellow-brown instead of a colorless residue. The peak was observed when preservative addition was omitted in the granules extraction step.

Differential pulse voltammetric assay

For the voltammetric assay, the benzene antioxidant extract was evaporated at 30°C to below 5 ml. Then the volume was adjusted to 5 ml with benzene, and 10 ml 95% ethanol and 1.5 ml sulfuric acid in 2:1 ethanol:benzene were added to give a final acid concentration of 0.12M (McBride and Evans, 1973). A 10-ml aliquot was then transferred to the voltammetric cell and anodic waves were recorded at room temperature.

A Princeton Applied Research (Princeton, NJ) Model 174A polarographic analyzer was used in differential pulse mode (Brieskorn and Mahlmeister, 1980) in conjunction with a Houston X-Y recorder. Operating conditions were as follows: potential scan rate 2 mV/sec; scan direction +, with a range of 1.5V for BHA and 3.0V for BHT and an initial potential of +0.3V; sensitivity for a full scale recording 50 μA; low pass filter off; and a 50 mV pulse applied for 56.7 msec on the normal voltage ramp.

Peak potentials were 0.74V for BHA and 1.05V for BHT. Quantitation was done by comparison of peak heights with standards.

A glassy carbon electrode vs SCE was used in addition to a Pt-wire counter electrode. In order to obtain reproducible results, the electrode surface was occasionally repolished on a felt cloth tissue wetted with γ-alumina slurry, and retested for its range memory effect and overall performance.

RESULTS & DISCUSSION

Antioxidant recovery from freeze-dried matrices

In a series of freeze-drying assays, it was found by analysis of variance that moisture removal from matrices (at moisture contents of 65% or lower) had a significant influence on the extent of BHA retention. As illustrated in Fig. 1, at a 63.2% moisture level, amylose retained an average of 96.4% BHA, a value which did not change when the moisture content was lowered to 33 or 4.2%. On the other hand, amylopectin at 63.2% moisture lost 20% of BHA, but no further loss was observed with removal of more moisture. Similarly, the 86% BHA retention with native starch, achieved after half of the moisture was removed, did not change during the rest of the drying process. The small 5% BHA loss at a matrix moisture content of 50% and the 1% BHA loss for each additional 10% removal of moisture suggest the strong affinity of BHA towards potato protein.

However, pectin matrix showed a 20–25% variation in BHA retention, because, due to its highly hydrophilic nature, the pectin-BHA slurry was freeze-dried for 10 hr more than the six other matrices. This result agrees with those of Kirleis and Stine (1978) that losses of BHA from a food model system increased substantially when the freeze-drying time was lengthened. The means of the sums of all BHA recoveries found for each matrix at different moisture content levels were then used for further comparisons using statistical tools.

As seen from Table 1, BHA retention depended on the nature of the matrix, and varied from 71.4–96.3%. The explanation for these high retention values (well above the 40–50% retention in the A-B process), probably lies in the fact that the freeze-drying process involves ice crystal sublimation rather than steam distillation and volatilization. In order to find out if there were any significant differences between the matrices, statistical analyses were carried out using the Student-Newman-Keuls Multiple Range Test procedure at the 95% level. This led to the matrices being divided into two subsets.

Selecting a matrix from each subset, the affinity of the antioxidants towards potato amylose (Subset I) and cell wall cellulose (Subset II) was tested by using solvents of increasing dielectric constant. Results are presented in Table 2. As seen for amylose in a solvent polarity range of ϵ = 1.89 (pet. ether) to 24.3 (ethanol), the extent of BHA release was independent of solvent polarity and amounted

to an average of only 3.5–4.3%. The inability of solvents of increasing polarity to extract BHA from amylose suggested that BHA is either entrapped within the realigned molecular network or bound within its helix in the form of an inclusion compound. High recoveries were found with cell wall cellulose as a matrix, being close to 90% for low polarity and 100% for high polarity solvents.

When amylose was hydrated for 10 min in a ratio of 1:1 w/w, antioxidant recovery increased. Low polarity solvents brought about close to a 40% BHA recovery, medium polarity 60% and high polarity up to 96%.

Table 1—Retention of BHA by major potato constituents during freeze-drying

	Matrix	BHA retention (%)[a]
	Starch, gelatinized and retrograded	96.3 (2.6)[b]
	Amylose	95.7 (0.6)
I[c]	Protein	91.4 (2.9)
	Starch, native	86.0 (3.2)
	Amylopectin	78.2 (3.9)
II	Cellulose	73.9 (3.1)
	Pectin	71.4 (14.7)

[a] Mean of the sum of all BHA recoveries found at different moisture levels.
[b] Standard deviation, n = 3.
[c] I and II are subsets calculated from the Student-Newman-Keuls' Multiple Range Test procedure.

X-ray diffraction

When BHA was added to solubilized amylose, rapid precipitation occurred. This strongly suggested the formation of an inclusion complex. The X-ray diffraction patterns of host and guest compounds, their complex and an amylose matrix tenaciously holding BHA (95 ppm) are given in Table 3. As seen from interplanar spacings, pure amylose was characterized by three strong intensity spacings between 3.93–5.83 Å. Similar spacings were found in the unsolubilized freeze-dried amylose matrix with BHA. On the other hand, spacings of the inclusion complex were numerous in the range 2.73–11.63 Å, and were mostly strong in intensity and differed significantly from those of pure crystalline BHA. This was further evidence that amylose and/or starch matrices contain entrapped rather than inclusion clathrate forms of BHA, and indicated the importance of hydration, since it appears necessary to separate the retrograded amylose chains and release the entrapped antioxidants.

Antioxidant extraction from potato granules

The results obtained on matrices were confirmed on samples of dehydrated granules. As seen from Table 4, 8.8–9.5 ppm of the bound antioxidant were recovered from granules (moisture content 7.0%) hydrated to 1:5 w/w (granules:water) and then eluted at room temperature with 30 ml of solvent. The poor performance obtained without rehydration was, again, clearly illustrated. Re-

Table 2—Extent of BHA recovery from some potato constituents by using solvent systems of increasing polarity

Solvent	Polarity (Dielectric constant) ϵ	BHA recovery, % Amylose		Cellulose	
		With water[a]	Without water	With water[a]	Without water
Petroleum ether	1.89	37.6 (5.2)[b]	4.2 (1.8)	87.0 (11.8)[c]	88.1 (11.8)
Benzene	2.60	40.6 (2.9)	4.3 (0.8)	95.0 (12.2)	90.0 (11.9)
Ethyl ether	4.34	59.5 (2.8)	3.5 (1.1)	93.3 (13.9)	102.4 (16.8)
Acetone	20.70	95.6 (1.0)	4.1 (0.7)	102.4 (13.8)	98.4 (11.7)
Ethanol	24.30	96.5 (5.1)	4.3 (1.1)	105.7 (12.5)	96.9 (11.3)

[a] Hydration ratio 1:1 w/w, time 10 min.
[b] Values in parentheses are standard deviations, n = 3.
[c] Values in parentheses are estimated errors calculated from standard curve interval estimators.

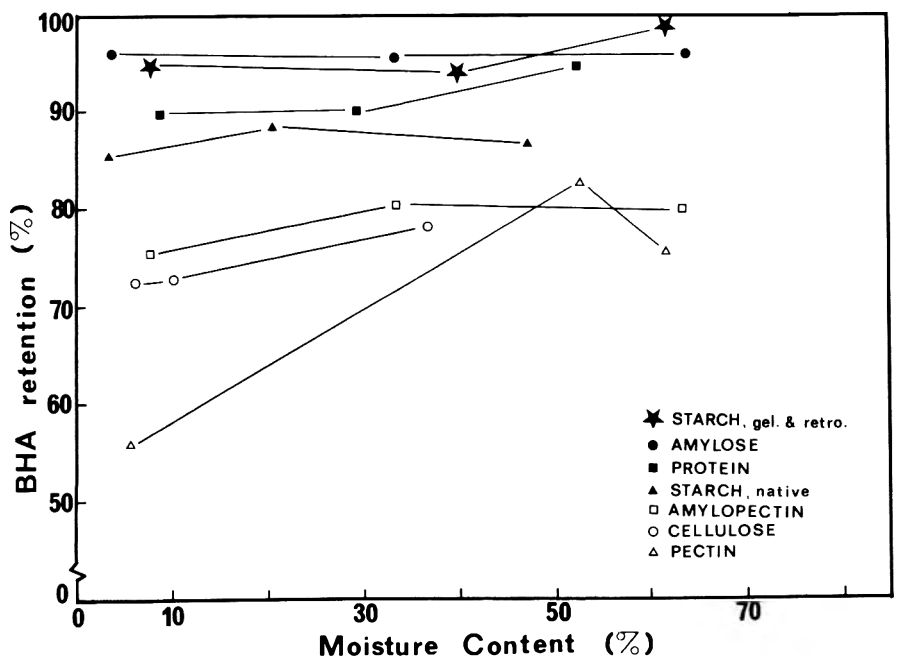


Fig. 1—Effect of moisture loss on BHA retention by major potato constituents.

peated hydration followed by additional extraction with 30 ml solvent released the remaining antioxidant.

The recovery assay using pet. ether or ethyl ether and no rehydration step brought about nearly total extraction of granule lipids. However, BHA was only recovered in trace amounts. This finding might be considered as evidence that the bulk of BHA within granules is not associated with lipids.

Antioxidants retained in a potato granule process were analyzed using the short extraction procedure developed in this study. Results for A-B granules are given in Fig. 2. The data did not differ significantly when benzene was substituted by pet. ether. In addition, the results of the standard GLC method and voltammetry with a glassy carbon electrode agreed closely.

Detection limits in voltammetry were 0.2 and 1.5 ppm for BHA and BHT respectively, while for GLC the limits were 0.2 ppm for both antioxidants. In voltammetry, the calibration curve was a straight line. The best least squares fit coefficients for the equation $x = (y - a)/b$, in which x corresponds to phenolic concentration in ppm and y to peak height in cm, were: BHA (BHT) intercept a, −0.1637 (−0.4714); slope b, 1.9210 (0.4230), with a correlation coefficient better than 0.999.

Moreover, for both methods, no interference from natural potato phenolics was encountered, though in voltammetry some of their wave potentials were close to BHA (BHT). As found in this study, cold water hydration followed by cold organic solvent extraction removes less than half of the chlorogenic acid from granules and much less L-tyrosine and caffeic acid. In the extraction procedure outlined in Materials and Methods, these major phenolics were removed by the water layer. Thus, the water layer can provide an additional source of data on potato phenolics. As proved in our parallel study, complete quantitation of both BHA (BHT) and natural phenolics can readily be achieved with hot instead of cold water hydration, and using hot ethanol as a solvent.

CONCLUSION

STUDIES on the behavior of freeze-dried major constituents of potato showed that retrogradation of the amylose moiety is responsible for the strong antioxidant entrapment within potato granule cells.

The rapid extraction procedure of this study essentially releases the immobilized antioxidant entrapped in the starch amylose matrix upon reconstitution with water. GLC and voltammetry were proved to be reliable for quantitation of

—Continued on page 595

Table 3—Interplanar spacing[a] and intensity[b] values from X-ray diffraction patterns of potato amylose, BHA and their complex

Potato amylose	BHA		Potato amylose: Not solubilized + BHA	Potato amylose: Solubilized + BHA	
5.83 s	14.25 s	17.68 m	5.83 s	6.81 s	11.63 m
5.21 s	12.81 s	9.82 m	5.21 s	5.04 s	3.98 m
3.93 s	6.37 s	7.25 m	3.93 s	4.93 s	3.67 m
	5.98 s	6.66 m		4.75 s	
	5.79 s	4.93 m		4.67 s	
	4.25 s	4.72 m		4.65 s	
		4.02 m		4.48 s	
		3.75 m		3.25 s	
		3.63 m		2.73 s	
		3.56 m			
		3.04 m			
		2.07 m			

[a] Interplanar Spacings are in Å.
[b] Intensities are designated as strong (s) or medium (m).

Table 4—Effect of hydration of potato granules on the extent of BHA recovery by solvent systems of increasing polarity

Solvent	Polarity (Dielectric constant) ϵ	BHA recovery, ppm: With water[a]	BHA recovery, ppm: Without water
Petroleum ether	1.89	8.8 (0.7)[b]	Trace
Benzene	2.60	8.1 (0.7)	Trace
Ethyl ether	4.34	7.8 (0.7)	Trace
Acetone	20.70	8.3 (0.7)	Trace
Ethanol	24.30	9.5 (0.7)	Trace

[a] Hydration ratio 1:5 w/w (granules:water); time 10 min; 30 ml of solvent collected.
[b] Estimated error.

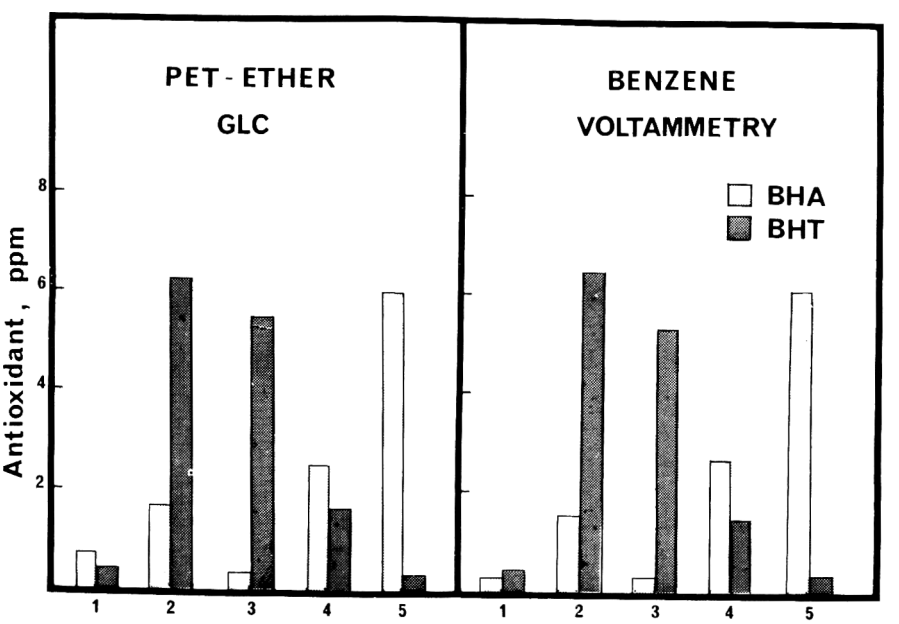


Fig. 2—Antioxidant contents in freshly made Add-Back granules.

Functional Properties of Chitin and Chitosan

DIETRICH KNORR

ABSTRACT

Chitin (poly-β (1⇒4)-N-acetyl-D-glucosamine), chitosan (deacetylated chitin) and microcrystalline chitin (redispersible chitin powder) were compared with microcrystalline cellulose to examine the use of those cellulose-like biopolymers as functional additives for potential application in food formulations. Water binding, fat binding and emulsifying properties were studied. Baking tests were performed with 0.5–2.0% (flour basis) of microcrystalline chitin added to wheat flour bread or to potato protein fortified (8% potato protein concentrate) white bread. Water-binding capacity and fat binding capacity of chitin, chitosan and microcrystalline chitin ranged from 230–440% (w/w) and from 170–315% (w/w). Chitosan and chitin did not produce emulsions but microcrystalline chitin showed good emulsifying properties and was superior to microcrystalline cellulose. Increasing concentration of microcrystalline chitin (0.12–0.8 g/100 ml water) had a positive effect on emulsion stability. Addition of microcrystalline chitin increased specific loaf volume of white bread and protein fortified breads. Water addition of 65% (flour basis) was found to be optimum for "chitin breads."

INTRODUCTION

CELLULOSE-LIKE BIOPOLYMERS such as chitin (poly–β(1⇒4)-N-acetyl-D-glucosamine) which is distributed widely in nature (e.g. in marine invertebrates, insects, fungi and yeasts) and chitosan, (2-deoxy-2-aminoglucose polymer), a deacetylated chitin, are processed in 10^6 kg quantities in Japan and in lesser amounts in the USA (Austin et al., 1981). Chitin is one of the most abundant polysaccharides in the world, comprising 12% of fresh water crayfish meal, 13% of crab meal and 8% of shrimp meal (Patton and Chandler, 1975). The Antarctic Krill, containing 2.3–6.1% of chitin, was recently also suggested for food use (Sidorski et al., 1980) and relative amounts of chitin in the cell wall of fungi at different morphological stages accounted for up to 40% of mycelium and 44% of yeast (Ruiz-Herrara, 1978).

Most of the applications research has been focused on chitosan because the free amino groups in this modified product contribute polycationic, chelating and dispersion forming properties along with ready solubility in dilute acetic acid.

Food applications of chitin, waste of the shellfish industry, and chitosan have been limited (Muzzarelli and Pariser, 1978), although chitin itself is substantially lower in cost and appears amenable to mechanical and chemical modifications (Austin et al. 1981). Studies on the toxicity of chitosan indicated low acute toxicity (Arai et al. 1968). Chitin yields pyrazines on pyrolysis which might open potential food uses as flavor enhancer. Chitosan has been used as an effective aid to recovery of by-products from food processing wastes such as vegetable canning wastes and egg breaking wastes (Bough, 1976; Lotrakul, 1978). The use of chitosan as a hypocholesterolemic agent has recently been reported by Sugano et al. (1980). Bulk and solution properties of chitosan (Filar and Wirick, 1978), film forming capability of chitosan (Averbach, 1978) and thickening ability of microcrystalline chitin (Dunn and Farr, 1974) have been examined. The absorption of mineral and organic acid by chitin (Giles et al., 1958) as well as deacidifying a coffee extract with chitosan have also been discussed (Magnolato, 1979).

There is increasing interest in functional food ingredients. Highly functional cellulose-like biopolymers with low digestibility and solubility could provide new aspects to food functionality. This paper reports on the examination of cellulose-like biopolymers, chitin and chitosan, as highly functional additives for potential applications in food formulations. Compounds are compared with microcrystalline cellulose and potato protein for their water binding, fat binding and emulsifying properties. Baking tests were performed with the addition of chitin to the bread formula.

Author Knorr is with the Dept. of Food Science & Human Nutrition, Univ. of Delaware, Newark, DE 19711.

EXPERIMENTAL

COMMERCIALLY AVAILABLE CHITIN and chitosan (Madera Products Inc., East Albany, OR) were used. Microcrystalline chitin was prepared from chitin on a laboratory scale by hydrolysis with phosphoric acid in 2-propanol, shearing of an aqueous dispersion in a high speed Waring Blendor and freeze drying of the filtered material (Austin et al. 1981). All samples were ground in a Wiley Mill, and passed through a 40 mesh screen to obtain comparable particle size ranges. Commercially available purified microcrystalline cellulose (Avicel, PH 101, FMC Corp., Newark, DE), was used as a reference material, and heat coagulated, hot air dried potato protein concentrate (Agenaprot, Österr. Agrar-Industric Gmbh., Vienna, Austria) was incorporated in the high protein bread formulations.

Fat binding capacity was determined after Lin et al. (1974) using 3 ml of corn oil and 0.5g of sample. Water-binding capacity was determined after Sosulski (1962) with 1.5g sample and 30 ml deionized water. Baking experiments were carried out after Carlson et al. (1981) adding 0.5–2% microcrystalline chitin to the bread formulation. A simple formulation was used to reduce additional effects of other ingredients, consisting of 100% unbleached wheat flour (Ceresota flour, Standard Milling Co., Kansas City, MO), 65% water, 5% dry activated yeast (Standard Brands, Inc., New York, NY) and 2% of sodium chloride and sugar respectively. Loaf volume was determined by a rapeseed displacement method. Water activity of bread center slices was examined by electrodeless conductivity measurement (Type SMT-B, Sena, Zürich, Switzerland.)

Emulsifying properties were studied by emulsifying cottonseed oil (Lot 21781 U.S. Biochemical Corporation, Cleveland, OH) in an ultrasonic unit (Braunsonic 1510, B. Braun Melsungen AG, San Francisco, CA) at a 50 watt setting. Emulsifier levels were between 0.12 and 0.80 g/100 ml water. Deionized water (25 ml) was placed in a 150 ml beaker, together with 50% of the emulsifier used and 25 ml of cottonseed oil. The beaker was placed in an ice-water bath and treated for 3 min. During the first minute the remaining half of the emulsifier was added and additional oil was added continuously with a peristaltic pump (Masterflex Model 7015, Cole Parmer Instruments, Chicago, IL) at a flowrate of approx 30 ml per min (total 60 ml). Immediately after sonification, excess oil was pipetted off and measured to obtain the actual amount of oil used and the emulsifying capacity determined. Emulsions were transferred to 25 ml graduated cylinders, allowed to stand for 48 hr, heated in a water bath at 80°C for 15 min and cooled to room temperature (Swift et al., 1951).

–Continued on next page

RESULTS & DISCUSSION

Water-binding capacity of chitin, chitosan and microcrystalline chitin ranged from 230–440% (w/w) with chitosan having the greatest water-binding capacity (Fig. 1). There are several possible explanations for the differences in water-binding properties between chitin and chitosan. These include differences in the crystalinity of the products and differences in the amount of salt forming groups. Since Austin et al. (1981) showed that approx. 3–28% of total dry crab shells account for covalently bound protein (total protein 12–73%) and since protein residues remain with the chitin even after the most drastic alkali treatment (Austin et al., 1981), differences in the protein content of the material, especially between microcrystalline chitin and chitin or chitosan might also affect water-binding properties. Fat binding capacity ranged from 170–215%, with chitosan having the lowest and chitin having the highest fat binding capacity. (Fig. 1).

The effect of increasing emulsifying agent concentration on emulsifying capacity is shown in Table 1. A progressive decrease in the emulsion capacity with increasing agent concentration is shown. This effect has been observed with potato protein concentrates (Holm and Eriksen 1980) and other systems (Acton and Saffle, 1970; Crenweldge et al., 1975). Microcrystalline chitin showed good emulsion capacity. Chitin and chitosan, however, did not produce emulsions under the test conditions employed and changes in the concentration of chitin or chitosan did not affect the emulsion capacity. The effect of increasing agent concentration on the stability of lauryl sulfate, microcrystalline chitin and microcrystalline cellulose is given in Fig. 2. As expected, the emulsion stability was highest for lauryl sulfate at all concentrations with the varying concentration causing unusual effects on the stability. The emulsion stability for microcrystalline chitin and cellulose increased as the agent concentration increased. Stability differences were most pronounced at lower agent concentrations.

The effect of microcrystalline chitin addition on specific loaf volume of wheat flour breads and potato protein fortified wheat flour breads (replacement level of the flour by potato protein concentrate was 8%) is given in Fig. 3. Loaf volume depressions have been reported with addition of various fiber sources (Pomeranz et al., 1977) as well as with protein concentrates (Knorr and Betschart, 1978). The increase of loaf volume with increasing microcrystalline chitin addition indicates that the surfactant properties of chitin may be of special interest. It should also be noted that water activity of bread crumb samples decreased from a_w = 0.43 to a_w = 0.38 with increasing microcrystalline chitin addition. Knorr and Betschart (1981) recently discussed the effects of water addition on loaf volume of protein fortified breads. Consequently, the combined effects of variable water and microcrystalline chitin addition on the specific loaf volume of potato protein fortified wheat breads was examined.

As shown in Fig. 4, an optimum loaf volume of 6.0 $cm^3 \cdot g^{-1}$ was reached with a 65% water and 2% microcrys-

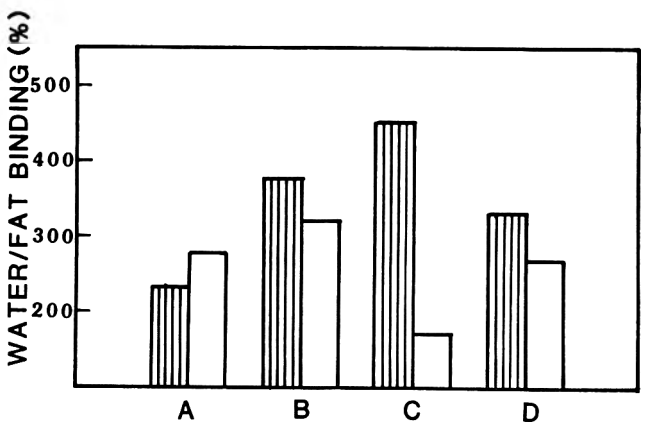


Fig. 1—Fat and water-binding capacity of microcrystalline cellulose (A), chitin (B), chitosan (C) and microcrystalline chitin (D), ▥ water binding capacity; □ fat binding capacity.

Table 1—Effect of agent concentration on emulsifying capacity of microcrystalline cellulose, microcrystalline, chitin and lauryl sulfate

Agent Conc ($g \cdot 100\ ml^{-1}$)	Emulsifying capacity (ml oil/g agent) Microcrystalline cellulose	Microcrystalline chitin	Lauryl sulfate
	Mean ± Standard Deviation		
0.12	200 ± 47	900 ± 47	1416 ± 71
0.26	167 ± 24	558 ± 35	858 ± 35
0.52	65 ± 5	327 ± 38	588 ± 16
0.80	15 ± 14	185 ± 7	495 ± 7

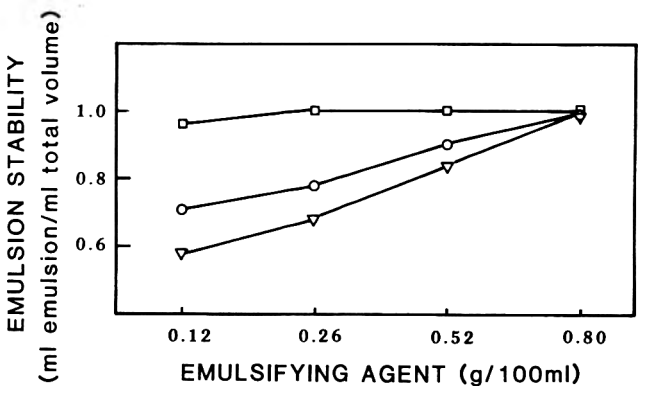


Fig. 2—Effect of emulsifying agent concentration on emulsion stability of lauryl sulfate (□), microcrystalline cellulose (▽), and microcrystalline chitin (○).

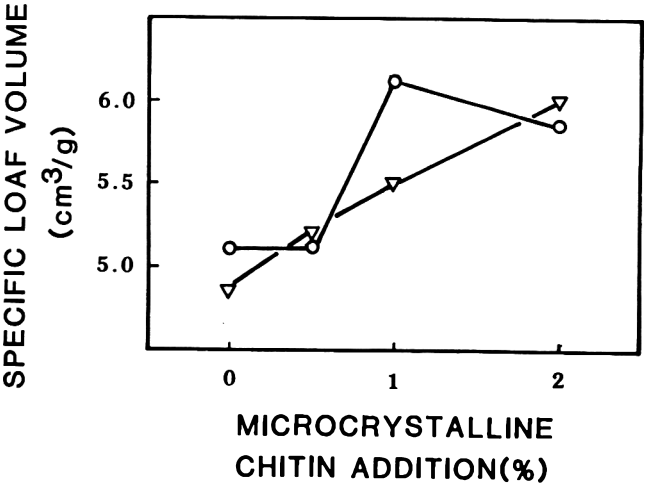


Fig. 3—Effect of increasing addition of microcrystalline chitin on specific loaf volume of wheat bread (○) and potato protein fortified (8% replacement level of wheat flour with potato protein concentrate) wheat bread (▽).

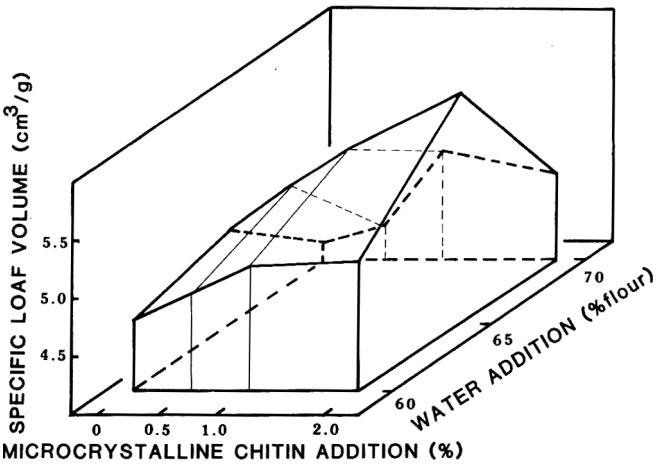


Fig. 4—Effect of water and microcrystalline chitin addition on specific loaf volume of potato protein fortified (8% replacement level of wheat flour with potato protein concentrate) wheat bread.

talline chitin addition. While the loaf volume decreased with water addition beyond 65%, it generally increased with increasing chitin addition at all levels of water addition. The data from Fig. 3 and 4 indicate a positive effect of microcrystalline chitin on loaf volume of wheat breads with chitin providing surfactant properties which can overcome loaf volume depression through protein fortification of wheat breads.

In summary, the data indicate that cellulose-like biopolymers, chitin in particular, could provide a number of unique functional properties for food processing. Thus, chitin, a natural polymer which is insoluble in ordinary solvents and has low digestibility, could be an "inert" but highly functional additive with numerous applications in foods.

REFERENCES

Acton, J.C. and Saffle, R.L. 1970. Stability of oil-in-water emulsions. J. Food Sci. 35: 852.

Arai, K., Kinumaki, T., and Fugita, T. 1968. On the toxicity of chitosan. Bull Toka Regional Fisheries Res. Lab. No. 56: 89.

Austin, P.R., Brine, C.J., Castle, J.E., and Zikakis, J.P. 1981. Chitin: New facets of research. Science 212: 749.

Averbach, B.L. 1978. Film-forming capability of chitosan. In "Proceedings of the First International Conference on Chitin/Chitosan," Ed. Muzzarelli, R.A.A. and Pariser, E.R. Massachusetts Institute of Technology, Cambridge, MA.

Bough, W.A. 1976. Chitosan: a polymer from seafood waste, for use in treatment of food processing wastes and activated sludges. Process Biochem. 11(1): 13.

Carlson, B.L., Knorr, D., and Watkins, T.R. 1981. Influence of tomato seed addition on the quality of wheat flour bread. J. Food Sci. 46: 1029.

Crenweldge, D.D., Dill, C.W., Tybor, P.T., and Landmann, A. 1974. A comparison of the emulsification capabilities of some protein concentrates. J. Food Sci. 39: 175.

Dunn, H.J. and Farr, M.P. 1974. Microcrystalline chitin. U.S. Patent No. 3,847,897.

Filar, L.J. and Wirick, M.G. 1978. Bulk and solution properties of chitosan. In "Proceedings of the First International Conference on Chitin/Chitosan," Ed. Muzzarelli, R.A.A. and Pariser, E.R. Massachusetts Institute of Technology, Cambridge, MA.

Giles, Ch., Hassan, A.A.A., Laidlaw, M., and Subramanian, R.V.R., 1958. Absorption at organic surfaces. Some observations on the constitution of chitin and on its absorption of inorganic and organic acids from aqueous solutions. J. Soc. Dyers & Colorists 74: 647.

Holm, F. and Eriksen, S. 1980. Emulsifying properties of undenatured potato protein concentrate. J. Food Technol. 15: 71.

Knorr, D. and Betschart, A.A. 1978. The relative effect of an inert substance and protein concentrates upon loaf volume of breads. Food Sci. & Technol. 11: 198.

Knorr, D. and Betschart, A.A. 1981. Water absorption and loaf volume of protein-fortified breads. J. Food Sci., R. Technol. 14:

Lin, M.J.Y., Humbert, E.S., and Sosulski, F.W. 1974. Certain functional properties of sunflower meal products. J. Food Sci. 39: 317.

Lotrakul, V. 1978. Selection of chemicals for recoveries of grease, fats and proteins in food processing waste water. Ph.D. thesis, The Unvi. of Oklahoma, Norman, OK.

Magnolato, D. 1979. A process for deacidifying a coffee extract and the deacidified extract obtained. UK patent application GB 2029688A.

Muzzarelli, R.A.A. and Pariser, E.R. 1978. "Proceedings of the First International Conference on Chitin/Chitosan." Massachusetts Institute of Technology, Cambridge, MA.

Patton, R.S. and Chandler, P.T. 1975. In vivo digestibility evaluation of chitinous materials. J. Dairy Sci. 58: 397.

Pomeranz, Y., Shogren, M.D., Finney, K.F., and Bechtel, D.B. 1977. Fiber in breadmaking: Effects on functional properties. Cereal Chem. 54: 25.

Ruiz-Herrara, J. 1978. The distribution and quantitative importance of chitin in fungi. In "Proceedings of the First International Conference on Chitin/Chitosan," Ed. Muzzarelli, R.A.A. and Pariser, E.R. Massachusetts Institute of Technology, Cambridge, MA.

Schlotzbauer, W.S., Chortyk, O.T., and Austin, P.R. 1976. Pyrolysis of chitin, a potential tobacco extender. J. Agric. Food Chem. 24: 177.

Sikorski, Z.E., Bykowski, P., and Knyszewski, J. 1980. The utilization of krill for food. In "Food Processing Engineering, Vol. I," Ed. Linko, P., Mälkki, Y., and Olkku, J. Applied Science Publisher Ltd., London.

Sosulski, F.W. 1962. The centrifugation method for determining flour absorption in hard red spring wheats. Cereal Chem. 39: 344.

Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., Fukuda, N., and Hasegawa, Y. 1980. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33: 787.

Swift, C.E., Lockett, C., and Fryar, A.J., 1951. Comminuted meat emulsions — the capacity of meats for emulsifying fats. Food Technol. 15: 468.

Ms received 7/18/81; revised 10/2/81; accepted 10/6/81.

The author is thankful to P. Austin, B.L. Carlson, J. Deschamps and S. Latlief for valuable suggestions and assistance.

BHA AND BHT IN POTATO GRANULES . . . From page 592

the extracted antioxidants. Data for BHA content in the granules obtained with and without a rehydration step suggested that the current methods of incorporation of antioxidant in a granule process are not efficient since antioxidant was shown to be held within the starch-amylose matrix rather than being associated with potato lipids (a major source of rancidity in granules).

REFERENCES

Bieth, H., Laugel, P., and Hasselmann, M. 1978. Problèmes posés par l'analyse du BHA et du BHT dans certains aliments deshydrates. Ann. Fals. Exp. Chim. 71: 69.

Brieskorn, C.H. and Mahlmeister, K. 1980. Verbesserte Erfassungsgrenzen phenolischer Antioxidantien mittels differentieller Puls-Voltammetrie. Z. Lebensm. Unters. Forsch. 171: 348.

Chung, I. and Hadziyev, D. 1980. Tuber and starch characteristics of Alberta grown potatoes. Can. Inst. Food Sci. Technol. J. 13: 143.

Halot, D. 1971. Détermination des antioxydants dans les matières alimentaires. Chim. Analytique 53: 776.

Kirleis, A.W. and Stine, C.M. 1978. Retention of synthetic phenolic antioxidants in model freeze-dried food systems. J. Food Sci. 43: 1457.

McBride, H.D. and Evans, D.H. 1973. Rapid voltammetric method for the estimation of tocopherols and antioxidants in oils and fats. Anal. Chem. 45: 446.

Moledina, K.H., Fedec, P., Hadziyev, D., and Ooraikul, B. 1978. Effect of pre-cooking in potato granule production by a freeze-thaw process. Potato Res. 21: 301.

Osman-Ismail, F. 1972. The formation of inclusion compounds of starches and starch fractions. Ph.D. dissertation No. 4829, ETH Zurich, Juris Druck + Verlag Zurich.

Ms received 6/16/81; revised 9/21/81; accepted 9/24/81.

The authors thank Dr. G.J. Packer for helpful discussions and gas chromatographic runs, and the National Science and Engineering Research Council of Canada for providing grants in aid of the project.

Heat Transfer and Moisture Loss of Spherical Fresh Produce

KAN-ICHI HAYAKAWA and JORGE SUCCAR

ABSTRACT

A computerized procedure was developed for simulating the post harvest cooling and moisture loss of produce by using a reliable mathematical model. This model was derived by assuming temperature and time variable respiration heat generation and temperature variable thermophysical property values. The developed procedure was employed to examine influence of five key dimensionless parameters on the cooling rate and moisture loss. These parameters are related to the rates of changes in density and thermal conductivity with changes in produce temperature, surface heat transfer conductance, transpiration rate, and environmental relative humidity. Theoretical results obtained from our examination were verified through heat transfer and moisture loss experiments by using fresh potatoes and tomatoes.

INTRODUCTION

MOST FRESH PRODUCE should be cooled to proper temperatures shortly after the harvest to reduce the rate of quality degradation and to extend its shelf life. The determination of a proper method for this cooling process could be greatly assisted through the use of reliable mathematical procedures for estimating the heat and moisture transfer characteristics of the produce undergoing cooling processes. Therefore, several researchers developed their procedures for this estimation. Some representative works in this or related areas were reported by Baird and Gaffney (1976), Bakker-Arkema et al. (1967), Eshleman et al. (1976), Brugger and Buelow (1980), Hayakawa (1978), Misra and Young (1979), and Lentz and van den Berg (1973). The close study of the above and other published works shows that negligible temperature gradient, constant, and/or no heat generation in the produce was assumed in most of the published work. The assumption on the negligible temperature gradient is likely valid for a slow cooling process and another on constant or no heat generation for cooling produce of low respiratory activity or for cooling most produce at a high or moderate rate. Since these assumptions may not be applicable in some cases, the present author published his mathematical model for simulating the thermal response and moisture loss without using these assumptions (Hayakawa, 1978). To develop this model, the overall configuration of the produce was idealized to be an infinitely wide slab and no time effect was taken into consideration on the rate of respiratory heat generation. The present paper is to report a new mathematical model applicable to spherical fresh produce by assuming this time effect. In addition, temperature variable density and thermal conductivity are included in the same model.

MATHEMATICAL MODEL

ACCORDING TO close examination of published data on the thermophysical properties of fresh produce and of water (Anonymous, 1977; Dickerson, 1968; Gaffney et al., 1980; Polley et al., 1980; and van den Berg and Lentz, 1975), there are less than 1.5(%), 5, and 20 variations in the values of specific heat, density and thermal conductivity of fresh produce respectively when produce temperatures are changed within a range applicable to most post harvest handling operations. We also observe that the variations in the density and thermal conductivity may be approximated with linear functions of produce temperature. Therefore we obtain the following equation for simulating heat transfer in an individual, homogeneous spherical produce by using a general relationship given by Carslaw and Jaeger (1959). (All symbols used are defined in the nomenclature.)

$$c_p(\rho_o + \rho_1 T)\frac{\partial T}{\partial T} = (k_o + k_1 T)\left(\frac{\partial^2 T}{\partial r^2} + \frac{2}{r}\frac{\partial T}{\partial r}\right) + k_1\left(\frac{\partial T}{\partial r}\right)^2 + Q(T,T) \quad (1)$$

The initial and boundary conditions used for solving Eq (1) are:

$$T = T_o \text{ for } 0 \leq r \leq R \text{ and } t = 0 \quad (2)$$

$$\frac{\partial T}{\partial r} = 0 \text{ at } r = 0 \text{ for } t > 0 \quad (3)$$

$$(k_o + k_1 T)\frac{\partial T}{\partial r} = h_{qo}(|T_a - T|)^{n_q}(T_a - T) - (L_o + L_1 T)\cdot h_{mo}\left(\left|P_s\frac{T_a + 273.15}{T + 273.15} - P_a\right|\right)^{n_m}\cdot\left(P_s\frac{T_a + 273.15}{T + 273.15} - P_a\right)$$
$$= (-1)^i h_{qo}(|T_a - T|)^{n_q+1} - (-1)^j h_{mo}(L_o + L_1 T)\cdot\left(\left|P_s\frac{T_a + 273.15}{T + 273.15} - P_a\right|\right)^{n_m+1}$$
$$\text{at } r = R \text{ and } t > 0 \quad (4)$$

$$i = \begin{cases} 1 & \text{when } T_a < T \text{ at } r = R \\ 2 & \text{when } T_a > T \text{ at } r = R \end{cases}$$

$$j = \begin{cases} 1 & \text{when } P_s\frac{T_a + 273.15}{T + 273.15} < P_a \text{ at } r = R \\ 2 & \text{when } P_s\frac{T_a + 273.15}{T + 273.15} > P_a \text{ at } r = R \end{cases} \quad (5)$$

$$h_{qo} = h_{qow},\ n_q = n_{qw},\ h_{mo} = h_{mow},\ n_m = n_{mw} \text{ when } j = 1$$
$$h_{qo} = h_{qod},\ n_q = n_{qd},\ h_{mo} = h_{mod},\ n_m = n_{md} \text{ when } j = 2 \quad (6)$$

In Eq (4), the temperature ratio multiplied to P_s is obtained by using an ideal gas law by assuming that water vapor transspired from the produce reaches the surrounding medium temperature in a zone beyond a boundary layer of the produce and that in some cases there is the partial condensation of the vapor in the surrounding medium. In the same equation, the latent heat of vaporization of moisture is assumed to change linearly with temperature and equal to that of water because of high moisture content in the produce. According to Weiss (1977), we have:

$$L_o + L_1 T = 2500.80 - 2.3668T \quad (7)$$

Since Teten's equation is simple to use and is accurate for estimating saturation water vapor pressure, P_s, we have:

Authors Hayakawa and Succar are with the Food Science Dept., Cook College, Rutgers Univ., New Brunswick, NJ 08903.

$$P_s = 0.61078 \exp\left[\frac{17.2693882T}{T + 237.30}\right] \quad (8)$$

The powers, n_q and n_m, applied to the temperature difference and vapor pressure difference in Eq (4) are for estimating surface heat transfer and transpiration rate coefficients. They become equal to zero for forced convection and to nonzero values for natural convection.

When there is vapor condensation on the produce surface, it is likely that surface heat transfer and transpiration coefficients are different from those applicable to dry produce surface. Therefore, Eq (6) is assumed.

We observe that the rate of respiration heat generation in fresh produce is a function of produce temperature and exposure time to tempratures specific to the cultural variety of the produce according to data presented in published articles (Anonymous, 1977; Buescher, 1979; Fukushima et al., 1980; Gaffney and Baird, 1975 and 1977; Kusunose and Sawamura, 1980; Lutz and Hardenburg, 1968; Willis and McGlasson, 1971; Wu and Salunke, 1975). The heat is produced at variable rates within a range of temperatures. When the produce is exposed to temperatures outside of this range, the production of heat is temporarily or permanently ceased and a local or entire produce tissue is inactivated when the exposure time exceeds a limiting value specific to the cultural variety because of metabolic damage on the tissue. The published articles also indicates that the heat is generated at abnormal rates when the produce suffers from cold injury. It is also observed from published data that the rate of the heat production changes as a function of time after harvesting until it reaches an equilibrium rate.

Based on the above stated information available from the literature, we assume changes in the equilibrium rate of heat generation as a function of produce temperatures as shown in Fig. 1. The symbol T_l and T_e respectively represent the lower and upper limits for the heat production. Cold inactivation temperatures applicable to a local and average produce temperatures are represented respectively with T_{lld} and T_{lmd}. It is assumed that the local or whole tissue of the produce is inactivated when its local temperature is maintained for t_{lld} or longer or when its average temperature is kept for t_{lmd} or longer. The cold injury temperatures and exposure times are represented similarly with symbols T_{cjl}, T_{cjm}, t_{cjl}, and t_{cjm} and also high inactivation temperatures and times by T_{hld}, T_{hmd}, t_{hld}, and t_{hmd}.

Changes in the rate of heat generation are approximated with the following functions of produce temperatures:

$$Q = \begin{cases} a_1(T - T_l) & T_l \leq T < T_b \\ b_1(T - T_b) + b_o & T_b \leq T < T_c \\ c_1\, 10^{(T - T_c)/c_2} & T_c \leq T < T_d \\ d_1(T - T_d) + d_o & T_d \leq T < T_e \end{cases} \quad (9)$$

It is assumed that different volumetric elements in one individual produce generate heat at different rates as represented by Eq (9) when a temperature distribution in the produce is not uniform. When the produce suffers from cold injury, Eq. 9 are slightly modified by replacing constants in these equations, with respective constants applicable to the cold injured produce, e.g. a_{1j}, b_{1j}, . . . d_{2j}.

According to a published article (Anonymous, 1977), there are four different types of changes in the heat generation as functions of time after harvesting, before it reaches an equilibrium level. Therefore the following four equations are obtained:

(a) Most vegetables other than root crops

$$Q/Q_e = 1 + [(Q_o - Q_e)/Q_e] \exp(-E_1 t) \quad (10)$$

(b) Fruit ripened after harvest (climacteric)

$$Q/Q_e = \begin{cases} Q_a + Q_2 t - (Q_2/t_d)t^2 \text{ for } o < t < t_d & (11) \\ 1 + Q_c \exp[-E_2(t - t_d)] \text{ for } t_d < t & (12) \end{cases}$$

(c) Fruit which does not ripen after harvest

$$Q/Q_e = 1 \quad (13)$$

(d) Vegetables which lose dormancy during storage

$$Q/Q_e = 1 - Q_4 \exp[-E_4 t] \quad (14)$$

Finally the amount of moisture lost from the produce may be estimated by:

$$w_l = -4\pi R^2 (-1)^j h_{mo} \int_o^t \left(\left| P_s \frac{T_a + 273.15}{T + 273.15} - P_a \right| \right)^{n_m + 1} dt \quad (15)$$

In the above equation, a moisture loss is represented with a negative value and a gain through vapor condensation on the product surface by a positive value.

The equations given above are transformed to dimensionless expressions to simplify further analyses as shown below:

$$g_p \frac{\partial u}{\partial Fo_r} = g_k \left[\frac{\partial^2 u}{\partial \eta^2} + \frac{2}{\eta}\frac{\partial u}{\partial \eta}\right] + q(u, Fo_r) + \epsilon_{ki} \left(\frac{\partial u}{\partial \eta}\right)^2 \quad (16)$$

$$u = u_o(\eta) \quad \text{for } Fo_r = o \quad o \leq \eta \leq 1 \quad (17)$$

$$\frac{\partial u}{\partial \eta} = o \quad \text{for } Fo_r = o \quad \eta = o \quad (18)$$

$$g_k \frac{\partial u}{\partial \eta} = (-1)^i H_{qo} (|u_a - u|)^{n_q + 1} - (-1)^j g_L H_{mo} \cdot \left(\left| w \frac{u_a + u_k + 2u_{20}}{u + u_k + 2u_{20}} - w_a \right|\right)^{n_m + 1} \quad (19)$$

$$Q = \begin{cases} \beta_{a1}(u - u_l) & u_l \leq u < u_b \\ \beta_{b1}(u - u_b) + \beta_{bo} & u_b \leq u < u_c \\ \beta_{c1}\, 10^{(u - u_c)/\beta_{c2}} & u_c \leq u < u_d \\ \beta_{d1}(u - u_d) + \beta_{do} & u_d \leq u \leq u_e \end{cases} \quad (20)$$

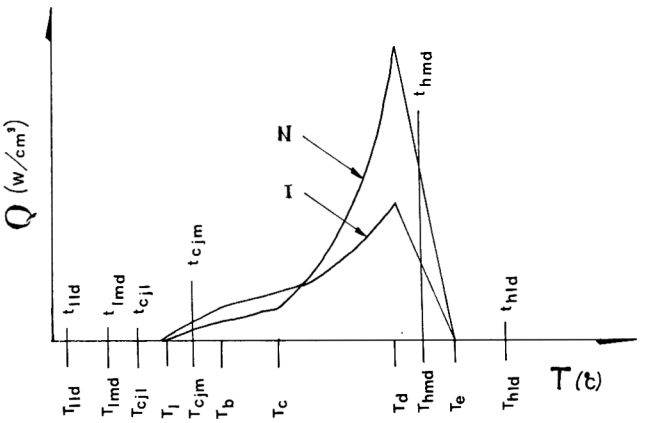


Fig. 1—Assumed rate of heat generation by fresh produce. Curve N for normal produce and Curve I for cold injured produce.

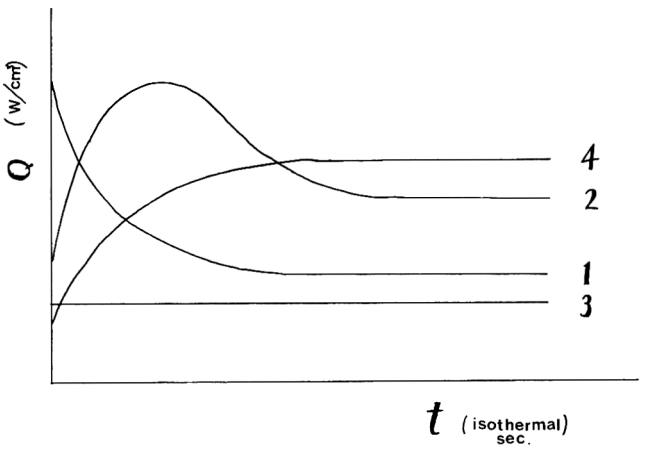


Fig. 2—Four different types of changes in the rate of heat generation by fresh produce as functions of time measured from the harvest.

Dimensionless expressions for Eq (10) through (14) are easily obtained by replacing t with $Fo_r R^2/\alpha_r$. Dimensionless moisture loss, Γ, is obtained from the second term in the right side of Eq (19).

$$\Gamma = -(-1)^j H_{mo} \int_0^{Fo_r} \left(w \frac{u_a + u_k + 2u_{20}}{u + u_k + 2u_{20}} - w_a \right)^{n_m+1} d\,Fo_r$$

$$= L_r w_l / [c_p \rho_r (T_o - T_r) R^3] \qquad (21)$$

ANALYSIS OF MATHEMATICAL MODEL

SINCE DIMENSIONAL Eq (1) and its boundary condition as well as dimensionless Eq (16) and its boundary condition are all nonlinear, the equations cannot be solved analytically. Therefore, a finite element method was used to solve these equations (Huebner, 1975). A finite element formula was derived by applying Galerkin's method to Eq (1) or (16) together with the respective boundary conditions. The derived element formula is shown in Appendix A.

When all element equations were assembled, we have simultaneous, first order ordinary differential equations. Since these equations are nonlinear and could be stiff because of exponentially produced heat, Gear's stiff method was used to solve these equations (Gear, 1971). A Fortran program package was prepared to solve Eq (1) and another package to solve Eq (16).

A proper number for subdividing the domain of r or ρ should be determined to produce accurate solutions while keeping computer-time costs to the minimum possible level. For this determination, we derive an analytical solution for estimating transient heat conduction in a spherical solid in which heat is generated at constant rate and whose surface is subjected to convective heat exchange with surrounding medium. This analytical solution is given to Appendix A.

Although no evaporative loss of the moisture is assumed for the derivation, the analytical solution provides means for selecting proper number of subdivision through the comparison of numerical results produced by the computer program packages against those computed from the analytical solution.

It is most likely that the steeper temperature gradient in a sample body we have, the larger number of finite elements is required and also that a steep temperature gradient may result from a high rate of the internal heat generation and from a large Biot number value. Therefore, use rates for the heat generation, which are 10 to 100 times higher than the maximum rate given in an article (Lutz and Hardenburg, 1968). According to our literature survey, the upper limit of frequently observed Biot number values is less than 5, which is used for our comparative analysis. The analysis revealed that a proper number of subdivisions was 28. The values of η's for those subdivisions are presented in Appendix A.

To perform a parametric analysis for the heat transfer and moisture loss of the produce we obtain ranges of all dimensional physical quantities included in Eq (1) through (9) from published articles (Anonymous, 1977; Arce and Sweat, 1980; Buescher, 1979; Dickerson, 1968; Fukushima et al., 1980; Gaffney et al., 1980; Hayakawa et al., 1979; Kusunose and Sawamura, 1980; Lutz and Hardenburg, 1968; Miller, 1978; Misener and Shove, 1976; Mowry and Heldman, 1972; Polley et al., 1980; Singh et al., 1975; Sastry and Buffington, 1980; Sastry et al., 1978) as shown in Table 1. The parametric values associated with the heat production, especially those on the upper and lower limits for the production and on the upper and lower inactivation temperatures, are not available in the literature. Therefore, tabulated values on these parameter are chosen through consultation with a plant physiologist and a food enzymologist at Cook College, Rutgers University together with information provided in a reference book on food enzymology (Whitaker, 1972). From the values given in Table 1, we estimate a range of each dimensionless parameter included in Eq (16) through (19). The results are presented in Table 2.

Most post harvest cooling processes last for only a few hours while it takes from 1–50 days after harvest for the produce to reach equilibrium respiration. Therefore time-variations in the heat generation are not examined in our parametric analysis.

Since there are over 40 dimensionless parameters, it is almost impossible to examine the influence of all these parameters on the heat transfer and moisture loss of the produce. Therefore, the influence of five parameters associated with key, thermophysical property values are analyzed by simulating post harvest cooling processes of fresh produce with the least, moderate, or most significant contributions of heat generation to the cooling rates. The

*Table 1—Ranges of dimensional, physical and biological property values of spherical fresh produce**

Symbol	Range	Symbol	Range
C_p	3.3 – 4.1 g/(JC°)	T_d	21 – 32°C
k_o	0.005 – 0.0059 w/(cmC°)	T_e	43°C**
k_1	8.0(–6) –9.0(–6) w/(cm $(C°)^2$)	a_1	3.6(–6) – 2.4(–4) w/($cm^3C°$)**
ρ_o	0.905 – 1.01 g/cm^3	b_1	6.1(–7) – 1.1(–4) w/($cm^3C°$)**
ρ_1	–3.7(–4) – –4.6(–4) g/($cm^3C°$)	b_o	6.1(–6) – 1.2(–4) w/(cm^3)
h_{qod}	0.005 – 0.0080 w/($cm^2C°$)	c_2	15 – 40°C
n_{qd}	0 – 0.33	d_1	–1.7(–6) – –1.45(–3) w/($cm^3C°$)
h_{qow}	0.0005 – 0.0080 w/($cm^2C°$)	d_o	3.5(–5) – 1.6(–2) w/(cm^3)**
n_{qw}	0 – 0.33	$T_{//d}$	–0.6 – –0.1°C**
h_{mod}	5(–8) – 7(–7)g/(cm^2sec kpa)	$T_{/md}$	–0.6 – –0.1°C**
n_{md}	0 – 0.33	T_{cjm}	–0.6 – 10°C
h_{mow}	8(–5) – 2 (–4)g/(cm^2 sec kpa)	$T_{cj/}$	–0.6 – 10°C**
n_{mw}	0 – 0.33	T_{hmd}	43 – 47°C
R	0.5 – 5 cm	$T_{h/d}$	43 – 50°C**
T_o	25 – 40°C	$t_{//d}$	1.2(+5) – 8.6(+5) sec**
T_a	–7 – 5°C	$t_{/md}$	1.2(+5) – 8.6(+5) sec**
rh	0.80 – 0.95	t_{cjm}	8.6(+4) – 6.1(+5) sec
T_l	–1.7 – –0.5°C**	$t_{cj/}$	8.6(+4) – 6.1(+5) sec**
T_b	0°C**	t_{hmd}	600 sec
T_c	1.7 – 10°C	$t_{h/d}$	600 sec**

* A signed integer placed in a pair of parenthesis is a power of 10 which should be multiplied to obtain a proper value. For example 5(–8) = 5×10^{-8}

**Values assumed through consultation with a plant physiologist and a plant enzymologist.

selected parameters are: $\epsilon_{\rho l}$, ϵ_{kl}, H_{qod}, H_{mod}, and W_a. The first two are respectively related to rates for changes in dimensionless density and dimensionless thermal conductivity with respect to increase in produce temperature. The third and fourth parameters are dimensionless surface heat conductance and dimensionless transpiration coefficient respectively. The last one is dimensionless water vapor pressure. Table 3 shows the ranges of the five parameters within which our analysis is performed. The same table also shows a frequently observed range of values for each parameter.

To examine the influence of the selected parameters, the full replicate of a central composite design of experiments was used (Davies, 1960). For this use, the limits of each parameter should be transformed to ±2.37844, which is estimated by $2^{5/4}$ because the number of the parameters is five and because the design is the full replicate. The computerized simulation of cooling processes is performed at the following values of each transformed variable: −2.37844, −1, 0, +1, and +2.37844. Since most of these values should be placed within the frequently observed range of each parameter, we used the following equations for transforming parametric variables to corresponding design variables.

$$\epsilon_{\rho l} = 0.0035738X_1 - 0.0085 \quad (22)$$

$$\epsilon_{kl} = 0.0269249X_2 + 0.0640385 \quad (23)$$

$$H_{qod} = 10^{0.2860342X_3 + 0.211787} \quad (24)$$

$$H_{mod} = 10^{2 - 1/(0.0284754X_4 + 0.2301986)} \quad (25)$$

$$W_a = 0.0315338X_5 + 0.875 \quad (26)$$

*Table 2—Ranges of dimensionless property values of spherical produce**

Symbol	Range	Symbol	Range
ϵ_{kl}	0 – 0.12	F_{ohmd}	0.025 – 3.6
ϵ_{pl}	−0.017 – 0	F_{ohld}	0.025 – 3.6
ϵ_{Ll}	−0.038 – −0.024	β_{al}	1.5(−4) – 1.1
H_{qod}	0.04 – 7.5	β_{bo}	2.0(−6) – 7.4(−2)
H_{qow}	0.04 – 7.5	β_{bl}	2.6(−5) – 0.52
H_{mod}	7(−5) – 4.4(−2)	β_{co}	1.3(−5) – 0.10
H_{mow}	0.018 – 19	β_{cl}	0.38 – 1.6
U_1	−0.107 – −0.038	β_{do}	3.1(−5) – 0.61
U_b	−0.04 – −0.025	β_{dl}	−3.7 – −5.6(−5)
U_c	0.017 – 0.36	β_{llj}	7.5(−4) – 5.5
U_d	0.5 – 1.2	β_{boj}	9.8(−6) – 0.37
U_e	1.1 – 1.7	β_{blj}	−5.9 – −2.3(−5)
U_{cjl}	−0.04 – 0.36	β_{coj}	9.8(−7) – 3.7(−2)
U_{cjm}	−0.04 – 0.36	β_{clj}	2.0
U_{hmd}	1.1 – 1.9	β_{doj}	1.4(−6) – 0.15
U_{hld}	1.1 – 1.9	β_{dlj}	−0.35 – −2.4(−6)
Fo_{cjl}	3.5 – 3.6(+3)	U_k	6.3 – 10.9
Fo_{cjm}	3.5 – 3.6(+3)	U_{20}	0.025 – 0.04

* A signed integer placed in a pair of parenthesis is a power of 10 which should be multiplied to obtain a proper value. The values of n_{qd}, n_{qw}, n_{md}, and n_{mw} are assumed to be zeros.

Table 3—Selected dimensionless parameters for evaluating their influence on heat transfer and moisture loss of spherical produce[a]

No.	Parameter	Range examined	Range observed frequently
1	$\epsilon_{\rho l}$	−0.017 ~ 0	−0.017 ~ −0.006
2	ϵ_{kl}	0 ~ 0.12	0.03 ~ 0.12
3	H_{qod}	0.04 ~ 7.5	0.70 ~ 2.8
4	H_{mod}	7.01(−5) ~ 4.4(−2)	5.5(−4) ~ 2.0(−2)
5	W_a	0.80 ~ 0.95	0.80 ~ 0.95

[a] A signed integer placed within each pair of parentheses is a power of 10 which should be multiplied to obtain a proper value.

Table 4 shows the values of the remaining dimensionless parameters, which are used for our analysis. These values are obtained through the careful examination of published information collected for the present investigation. It should be noted that some parametric values applicable to the moderate heat generation are skewed toward limiting values because their frequently observed values are not located at the median points of the ranges. When there is over 10 times difference between the upper and lower limits of a parameter, a logarithmic mid point or skewed logarithmic mean is assigned to the cases for the moderately significant heat generation. The reason for this assignment is that the distribution of such parametric values is likely logarithmic. For all simulations, we assumed that the initial and environmental u values are equal to 1.0 and 0.0 respectively. We simulate 43 cooling processes for each of the three cases related with the rate of heat generation by using our computer programs developed for the present study.

Criteria, which are chosen to evaluate the efficiency of simulated cooling processes, are: the values of modified Fourier number, Fo_r, and dimensionless moisture loss, Γ, when the dimensionless average temperature, $\bar{u}$, of produce becomes equal to 0.5, 0.1 or 0.02. The first $\bar{u}$ value was selected since a half cooling time has been used to evaluate cooling processes (Stewart and Couey, 1963). The latter two values are chosen since the average produce temperature is likely located within these values at the end of a cooling process and since an equilibrium, average temperature might not reach lower than 0.02 for some produce, which generates heat at high rates.

Abridged results of the estimated Fo_r and Γ values are shown in Table 5 together with the number of cooling processes with which the average produce temperature does not reach a specified level. As expected, the number of these processes is largest for the cases of most significant heat generation. Since the Fo_r and Γ values for such processes are fairly large or infinitely large, these values are not included in the ranges of values shown in Table 5. From this table, we generally observe that the higher rate of heat generation yields greater moisture loss and also requires longer cooling time for the average temperature to reach a specified level.

All estimated values of Fo_r and Γ were subjected to nonlinear regression analyses separately for each class of heat generation by using a statistical computer program package available at Rutgers University (Barr et al., 1976). Since some of the estimated values are infinitely large, several different reciprocal functions were used to transform Fo_r and Γ values. Among them, $(Fo_r)^{-1}$ and $(-\Gamma)^{-0.25}$ give the most satisfactory results. Tables 6 and 7 show the values of significant coefficients for regression polynomial terms. We generally observe from these two tables as follows. The values of the coefficients for the least significant heat generation are similar to those of corresponding coefficients for the moderately significant heat generation while those of the most significant heat generation are somewhat different from corresponding values for the other two groups. Surface heat and moisture transfer conductances greatly influenced the Fo_r and Γ values. The environmental relative humidity has significant influence on the moisture loss although it has almost no influence on the Fo_r values. The rates of changes in the produce density, $\epsilon_{\rho l}$, and thermal conductivity, ϵ_{kl}, have slight influence on the Fo_r values. The Fo_r tends to increase with increase in $\epsilon_{\rho l}$ and to decrease with increase in ϵ_{kl}. However there are virtually no influence of these two parameters on the Γ values.

SAMPLE APPLICATION

FRESH POTATOES of Superior variety and tomatoes of

Ace variety are subjected to cooling processes in laboratory refrigerators in order to collect experimental data on their thermal response and moisture loss. These collected data are then compared with results obtained by using the computer programs developed for the present investigation. The sample potatoes used for the cooling were purchased from a local farm and the sample tomatoes were harvested at an experimental farm in mid September, 1980, about 1 wk before initiating the experiments. The tomatoes were treated with ethylene gas to synchronize their maturity shortly after the harvest. When they were used for the experiment, red color was fully developed in them.

Two copper constantan thermocouple junctions, which were made from 36 gauge wires, were carefully placed either at the center or surface of each produce used for the experiment. Thermocouple wire with thick insulation, Duo-Wrapped Wire (Thermo-Electric Co.), is used to make the junction placed at the center in order to have enough physical strength in placing it at the center. The junctions placed on the surface are made from teflon coated wire. Personal information provided by Gaffney (1980) was used for exactly placing them on the surface. The samples with thermocouples installed are placed in an incubator for overnight to obtain uniform temperature distribution and then subjected to cooling processes either in a laboratory refrigerator or in a forced air constant temperature chamber. During these cooling processes, sample temperatures are recorded with a digital recorder and sample weights are monitored by a balance placed in the refrigerator or in the chamber. Typical results obtained with the potato, which is cooled in the forced air chamber, are shown in Fig. 3. Fig. 4 shows other typical results for the tomato cooled in the refrigerator.

To utilize the computerized procedure developed in the present investigation, the thermophysical property values and thermal response property values of the samples are required. By carefully examining published articles, we obtain these property values as shown in Table 8. Since there are no data on the respiration heat of fully ripened tomato, tabulated constants for heat generation by the sample tomatoes are estimated from those of ripening produce. Among the values listed in this table, the surface heat conductance and transpiration coefficient are estimated by using the polynomial equations developed through the parametric analysis since they are dependent on cooling processes.

To use the polynomial equations, we need to estimate the average temperature of the produce undergoing a cooling process. This temperature is estimated from the surface and central temperatures of a sample as follows. According to our preliminary experiments, we observed that temperature distributions in both tomato and potato were virtually quadratic. Through a simple analysis, we have:

$$\bar{T} = (2 \cdot \text{central temp} + 3 \cdot \text{surface temp})/5 \qquad (27)$$

Table 4—Values of dimensionless parameters used for computerized simulation of cooling processes[a]

Symbol	Parametric values L	M	H	Symbol	Parametric value L	M	H
U_l	−0.038	−0.073	−0.11	c1	1.3(−5)	7.6(−4)	0.10
U_b	−0.025	−0.033	−0.04	c2	0.38	0.70	1.60
U_c	0.36	0.10	0.017	do	3.1(−5)	7.9(−3)	0.61
U_d	0.50	0.88	1.25	dl	−5.6(−5)	−0.014	−3.70
U_e	1.06	1.38	1.69	aij	7.5(−4)	0.064	5.50
U_{cjl}	0.36	0.06	−0.039	boj	9.8(−6)	2.6(−3)	0.37
U_{cjm}	0.36	0.06	−0.039	blj	−2.3(−5)	−0.012	−5.90
U_{lld}	−0.10	−0.15	−0.20	clj	9.8(−7)	1.8(−3)	0.037
U_{lmd}	−0.05	−0.08	−0.10	c2j	2.0	2.0	2.0
U_{hmd}	1.10	1.50	1.90	doj	1.4(−6)	4.4(−3)	0.15
U_{hld}	1.10	1.50	1.90	dlj	−2.4(−6)	−9.2(−4)	−0.35
Fo_{cjl}	3.50	110.	3.6(+3)	U_k	10.9	8.85	6.80
Fo_{cjm}	3.50	110.	3.6(+3)	U_{20}	0.04	0.03	0.025
Fo_{lld}	5.00	160.	5.0(+3)	V_{ul}	26.4	34.3	42.2
Fo_{lmd}	5.00	160.	5.0(+3)	H_{mow}	19.0	4.35	0.18
Fo_{hmd}	0.025	0.30	3.60	H_{qow}	7.0(−5)	1.75	0.044
Fo_{hld}	0.025	0.30	3.60	n_{qd}	0.0	0.0	0.0
al	1.5(−4)	0.013	1.10	n_{qw}	0.0	0.0	0.0
bo	2.0(−6)	5.2(−4)	0.074	n_{md}	0.0	0.0	0.0
bl	2.6(−6)	3.6(−3)	0.52	n_{mw}	0.0	0.0	0.0

[a] Symbols L, M, and H represent respectively the least, moderately, and most significant contributions of heat generation to heat transfer. A signed integer placed within each pair of parentheses is a power of 10 which should be multiplied to obtain a proper value.

Table 5—Summary of computer output on simulated cooling processes[a]

$\bar{U}$	Fo_r or Γ	Least significant heat generation N_f	range		Moderately significant heat generation N_f	range		Most significant heat generation N_f	range	
0.5	Fo_r	1	0.0583	0.674	1	0.0585	0.725	9	0.152	2.10
	Γ		−3.71(−4)	−0.280		−5.02(−4)	−0.309		−0.00108	−1.36
0.1	Fo_r	1	0.222	2.259	1	0.225	2.38	17	0.592	1.93
	Γ		−6.52(−4)	−0.488		−8.46(−4)	−0.510		−0.00197	−0.143
0.02	Fo_r	1	0.260	3.00	1	0.298	3.15	21	0.995	1.09
	Γ		−7.04(−4)	−0.530		−9.05(−4)	−0.548		−0.0118	−0.166

[a] The symbol N_f signifies the number of cooling processes with which the average produce temperature did not reach a specified level because of the combination of physical property values chosen for the simulation. The Fo_r and Γ values associated these processes are not included in the ranges shown in the table.

The above equation estimates very accurately the average temperature according to a series of tests performed by using temperature distributions estimated by our computer programs if surrounding medium temperatures remain nearly constant during cooling processes.

We obtain the following values from data collected from a separate test. $T_r = T_a = 6.28°C$, $t_2 = 6{,}520$ sec, $W_{l2} = 0.113$ gr, R = 3.64 cm, and $W_a = 0.22$.

By using the definitions of x_1, x_2, and x_5, we have: $x_1 = 0.189$, $x_2 = -1.150$, $x_5 = -20.8$. Because of extremely low relative humidity used for the experiment, the value of x_5 is out of the range used for the parametric analysis. By applying the definitions of Fo_r and Γ, we have: $Fo_{r2} = 0.729$ and $\Gamma_2 = 0.079$. However we proceed our calculations since the influence of x_5 on heat transfer is almost negligible and that on moisture loss is relatively small for the range of x_5 values examined in our study. By selecting the coefficients of Fo_{r2} and Γ_2 for the low heat generation, we have:

$$1/0.729 = 1.16432 - 0.001680 \cdot 0.189 + 0.002295 \cdot (-1.150) + 0.863355 \cdot x_3 - 0.021153 \cdot x_4 \cdots + 0.114622 \cdot x^3{}_4 - 0.002569 \cdot (-20.8)^3 \quad (28)$$

$$1/0.079^{0.25} = 2.221977 - 0.001082 \cdot 0.189 + 0.000218 \cdot (-1.150) + 0.0462064 \cdot x_3 - 0.679195 \cdot x_4 \cdots + 0.041952 \cdot x^3{}_4 - 0.068070 \cdot (-20.8)^3 \quad (29)$$

The above equations are solved by using Newton-Coates' method with the following initial approximations for initiating iterative calculations: $h_{qod} = 0.0015$ w/($cm^2C°$) and $h_{mod} = 5.0 \times 10^{-8}$g/($cm^2$Kpa sec). The solution obtained are: $x_3 = 0.17985$ and $x_4 = -0.03843$. When these values are converted to the respective dimensional quantities, we have: $h_{qod} = 0.0023$ and $h_{mod} = 5.5 \times 10^{-8}$. By starting with these values, we refined the parametric values through iterative computations to minimize the sums of squares of differences between the estimated and observed values by using the computer programs. Finally refined parametric values are: $h_{qod} = 0.0028$ and $h_{mod} = 1.8 \times 10^{-7}$. These values are listed in Table 8. The parametric constants shown in this table are then used to estimate the temperature and moisture loss of tomatoes in separate experiments. Typical results are shown in Fig. 3. There is close agreement between predicted and experimentally observed data.

We tried to estimate the values of h_{qod} and h_{mod} for cooling the tomatoes through similar computations. However initially estimated values are all outside of the ranges used for the parametric analysis. This strongly indicates to us that the rate of respiration heat of tomatoes with fully developed red color could be very small especially when they are kept overnight at a temperature higher than 30°C. Therefore we assumed that $T_{hmd} = T_{hld} = 10°C$ and $t_{hmd} = t_{hld} = 0$. This assumption signifies no heat generated by

*Table 6—Regression coefficients for estimating Fo_{r1}, Fo_{r2}, Fo_{r3}**

Coefficient	Low heat generation			Medium heat generation			High heat generation		
	Fo_{r_1}	Fo_{r_2}	Fo_{r_3}	Fo_{r_1}	Fo_{r_2}	Fo_{r_3}	Fo_{r_1}	Fo_{r_2}	Fo_{r_3}
c_o	3.910300	1.164321	0.892934	3.856530	1.152934	0.883182	2.542207	0.598941	0.193073
x_1	−0.010816	−0.001680	−0.001056	−0.010693	−0.001647	−0.001030	−0.007412	−0.000858	−0.000277
x_2	0.012324	0.002295	0.001342	0.012243	0.002551	0.001732	0.012934	0.002209	0.000891
x_3	3.005669	0.863355	0.660460	3.013170	0.864865	0.661574	3.076038	0.832733	0.467152
x_4	−0.073738	−0.021153	−0.018412	−0.099295	−0.026537	−0.022435	−0.174339	0.005494	−0.022427
x_5	0.001808	0.001427	0.001805	0.001819	0.001427	0.001808	0.001092	0.001235	0.001729
$x_1 \cdot x_1$	0.000171	−0.000266	−0.000248	0.000224	−0.000244	−0.000227	0.001857	−0.001284	−0.003230
$x_1 \cdot x_3$	−0.000172	−0.001061	−0.000672	−0.007206	−0.001076	−0.000683	−0.007187	−0.000973	−0.000375
$x_2 \cdot x_2$	0.000178	−0.000152	−0.000234	−0.000034	−0.000378	−0.000360	0.001588	−0.001240	−0.003230
$x_2 \cdot x_3$	0.013027	0.002195	0.001098	0.012945	0.002613	0.001579	0.013718	0.002557	0.001206
$x_3 \cdot x_3$	0.843684	0.194620	0.144123	0.848679	0.195579	0.144964	0.944174	0.255150	0.242850
$x_3 \cdot x_4$	0.010770	0.003397	0.002627	0.015954	0.004103	0.002861	0.049657	−0.016218	−0.018462
$x_4 \cdot x_4$	−0.042444	−0.012219	−0.010509	−0.580041	−0.015547	−0.012766	−0.088726	−0.044183	−0.003230
$x_3 \cdot x_3 \cdot x_3$	0.114622	0.014674	0.009214	0.111560	0.014047	0.008731	0.049564	−0.000614	0.020882
$x_4 \cdot x_4 \cdot x_4$	−0.009358	−0.002569	−0.002149	−0.012864	−0.003218	−0.002543	−0.016259	−0.021712	0.003965
r^2	0.999997	0.999910	0.999862	0.999996	0.999923	0.999884	0.999682	0.997741	0.968367

* Tabulated coefficients are for computing $1/Fo_{ri}$ (i = 1, 2, or 3). Subscripts 1, 2, 3 signify Fo or Γ values applicable to average produce temperatures of 0.5, 0.1, and 0.02 respectively.

*Table 7—Regression Coefficient for estimating Γ_1, Γ_2, and Γ_3**

Coefficient	Low heat generation			Medium heat generation			High heat generation		
	Γ_1	Γ_2	Γ_3	Γ_1	Γ_2	Γ_3	Γ_1	Γ_2	Γ_3
c_o	2.557997	2.221977	2.180138	2.364127	2.076072	2.041941	1.678860	1.213386	0.721926
x_1	−0.001657	−0.001082	−0.000994	−0.001537	−0.001032	−0.000960	−0.001081	−0.000570	−0.000326
x_2	0.000045	0.000218	0.000208	−0.000084	0.000120	0.000161	0.000327	0.000284	0.000192
x_3	0.545825	0.462064	0.450992	0.520889	0.444864	0.435559	0.969279	1.314122	1.270405
x_4	−0.783595	−0.679195	−0.666405	−0.731571	−0.640259	−0.629668	−0.831717	−0.231766	−0.433364
x_5	0.013335	0.022575	0.026851	0.009256	0.016452	0.019838	0.004596	0.008329	0.007845
$x_1 \cdot x_1$	−0.000539	−0.000677	−0.000800	−0.000416	−0.000481	−0.000553	0.004055	0.007246	−0.012077
$x_2 \cdot x_2$	−0.000521	−0.000643	−0.000780	−0.000436	−0.000511	−0.000588	0.004033	0.007251	−0.012077
$x_3 \cdot x_3$	−0.107304	−0.095169	−0.094632	−0.098015	−0.087165	−0.086707	−0.054482	−0.36014	0.231431
$x_3 \cdot x_4$	−0.168659	−0.143802	−0.140054	−0.155274	−0.134479	−0.131557	0.017049	−0.367302	−0.356754
$x_4 \cdot x_4$	0.310673	0.269771	0.264371	0.286395	0.251660	0.247358	0.234111	0.135045	−0.012077
$x_3 \cdot x_3 \cdot x_3$	0.046724	0.041952	0.041046	0.040939	0.037757	0.037078	−0.053099	−0.127723	−0.122198
$x_4 \cdot x_4 \cdot x_4$	−0.078249	−0.068070	−0.066795	−0.072520	−0.063725	−0.062688	−0.023758	−0.135536	0.076610
r^2	0.999848	0.999813	0.999787	0.999884	0.999863	0.999846	0.989135	0.965852	0.895127

* Tabulated coefficients are for computing $(\Gamma_i)^{-0.25}$ (i = 1, 2, or 3)

the sample tomato. The values of h_{qod} and h_{mod} are iteratively estimated by using the computer programs. The estimated values are: $h_{qod} = 0.0017$ and $h_{mod} = 3.0 \times 10^{-7}$. These estimated values are shown in Table 8. Finally all tabulated values are used to estimate the cooling performance of another tomato. Fig. 4 shows typical results obtained. There is excellent agreement in the values predicted and observed. The determined surface heat conductances are within the range of values applicable to the cooling processes of similar produce (Arce and Sweat, 1980). According to Sastry et al. (1978), the literature values of transpiration coefficient applicable to cured potatoes are between 4×10^{-8} (g/(cm^{2}sec Kpa)) and 7×10^{-8}. Our value is considerably greater than the upper limit of this literature value. Extremely low relative humidity used for our experiment could be a cause for this difference. On the other hand, our value of the coefficient for cooling tomatoes is close to the literature values which range from 4.0×10^{-7} to 7.8×10^{-7}.

Estimated and observed central as well as surface temperatures are not presented in Fig. 1 and 2 to avoid the complexity. We observe that there are less than 1C° differences between experimentally determined and theoretically computed temperatures applicable to either one of these two locations.

DISCUSSION & CONCLUSION

TO DEVELOP the mathematical model, realistic rates for the respiration heat generation are assumed by carefully examining the published information on the physiological response of fresh produce to temperature. In addition, temperature variable density and thermal conductivity are assumed. In our knowledge, there is no comparable assumptions utilized for the analysis of heat transfer in fresh produce in the published articles except one published by the senior author and another by Gaffney et al. (1980). The latter authors assumed that a term corresponding to

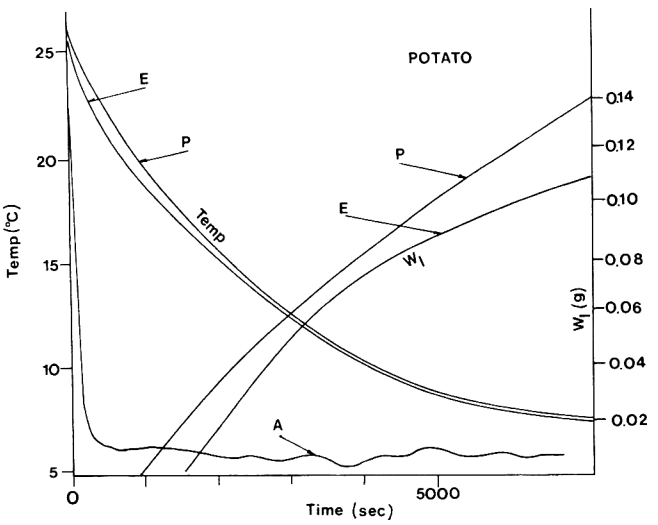


Fig. 3—Average temperature and moisture loss of Superior potato exposed to a 22% relative humidity forced air. A = air temperature, E = observed curve, and p = predicted curve.

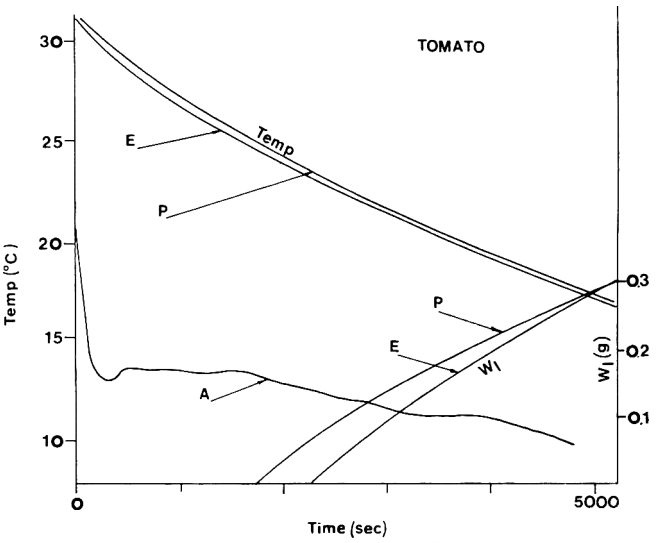


Fig. 4—Average temperature and moisture loss of Ace tomato exposed to a 85% relative humidity still air. A = air temperature, E = observed curve, and P = predicted curve.

Table 8—Parametric values used for computerized simulation of cooling potatoes and tomatoes

Symbol	Unit	Potato	Tomato	Symbol	Unit	Potato	Tomato
C_p	g/(gC°)	3.46	4.00	C_2	C°	43.3	24.4
k_o	W/(cmC°)	0.0054	0.0051	d_1	W/(cm^{3}C°)	−1.50(−5)	−3.3(−5)
k_1	W/(cmC°^{2})	9(−6)	9(−6)	d_o	W/cm^3	8.35(−5)	3.67(−4)
ρ_o	g/cm^3	1.0043	1.0643	$T_{//d}$	°C	−0.6	−0.5
ρ_1	g/(cm^{3}C°)	−0.00041	−0.00041	$T_{/md}$	°C	−0.6	−0.5
h_{qod}	W/(cm^{2}C°)	0.0028	0.0017	T_{cjm}	°C	1.7	4.4
n_{qd}	—	0	0	$T_{cj/}$	°C	0	0
h_{qow}	W/(cm^{2}C°)	0.0028	0.0017	T_{hmd}	°C	46.1	10*
n_{qw}	—	0	0	$T_{h/d}$	°C	48.9	10*
h_{mod}	g/(cm^{3}skp)	1.8(−7)	3.0(−7)	$t_{//d}$	sec	604,800	604,800
n_{md}	—	0	0	$t_{/md}$	sec	604,800	604,800
h_{mow}	g/(cm^{2}skp)	1(−4)	3(−4)	t_{cjm}	sec	259,200	259,200
n_{mw}	—	0	0	$t_{cj/}$	sec	259,200	259,200
T_1	°C	0	0	t_{hmd}	sec	600	0*
T_b	°C	1.1	2.2	t_{hld}	sec	600	0*
T_c	°C	4.4	4.4	a_{1j}	W/(cm^{3}C°)	6.39(−6)	1.21(−5)
T_d	°C	37.8	32.2	b_{1j}	W/(cm^{3}C°)	−5.33(−7)	−6.03(−6)
T_e	°C	43.3	43.3	b_{oj}	W/cm^3	3.55(−6)	2.68(−5)
a_1	W/(cm^{3}C°)	3.20(−6)	6.03(−6)	c_{ij}	W/cm^3	1.77(−6)	1.34(−5)
b_1	W/(cm^{3}C°)	3.20(−6)	6.03(−6)	c_{2j}	C°	88.9	55.6
b_o	W/cm^3	3.55(−6)	1.34(−5)	d_{ij}	W/(cm^{3}C°)	−7.58(−7)	−3.81(−6)
c_1	W/cm^3	1.42(−5)	2.68(−5)	d_{oj}	W/cm^3	4.21(−6)	4.24(−5)

* See the text for a reason of using these values.

$k_1(\partial T/\partial r)^2$ in Eq (1) was negligible. This assumption could be valid for a low to moderate rate of cooling since we have a small temperature gradient in this case. Because of the realistic nature of the model, the computer programs, which are obtained from it, could be used for the preliminary examination of interrelationship between the thermal response and physiological activity of spherical fresh produce provided that it is reasonably homogeneous.

The model, which is represented with a heat conduction equation, may be solved by using a proper finite difference method. However, the finite element method is used for the present investigation since this method may easily accomodate different intervals for subdividing one radial length. According to Misra and Young (1979), they obtained accurate solutions when the number of subdivided elements is as small as 10 to solve their heat conduction equation applicable to a spherical body. However, 28 elements are required in our case for a very high rate of heat generation and for a moderately large Bi value. This difference could be due to the fact that no internal heat generation was assumed in their analysis.

The polynomial equations obtained through the parametric analysis could provide useful means for estimating the thermal response and moisture loss of spherical produce when one has reliable physical property values and parametric constants for the respiration heat generation especially when there are slight or moderate contributions of heat generation to heat conduction in the produce. When this contribution is high, the polynomial equations are not reliable since the average temperature of the produce does not reach the specified levels in this case.

In conclusion, the computerized procedures are developed for predicting the thermal response and moisture loss. For this development, the time variable and temperature variable rates of heat generation are assumed. The computerized procedures are used for the parametric analysis to examine the influence of five selected dimensionless groups which are associated with the rates of changes in thermal conductivity and in density, surface heat conductance, transpiration coefficient, and environmental relative humidity. Among those five, the surface heat conductance and transpiration coefficient strongly influence the thermal response and moisture loss of produce. Sample applications of the computerized procedure and of the polynomial regression equations are presented to predict the temperature and moisture loss of fresh potatoes and tomatoes, which are undergoing cooling processes.

APPENDIX A

Finite element equations

Since the finite element equations of the dimensional heat conduction equation, Eq (1), is similar to those of the dimensionless equation, Eq (16), only the former ones are given below.

Galerkin's method is applied to a finite element, whose nodal values are represented with subscripts 1 and 2.

$$4\pi\int_{r_1}^{r_2} I_i < k\left(\frac{\partial^2 T}{\partial r^2}+\frac{2}{r}\frac{\partial T}{\partial r}\right)+Q+\left(\frac{\partial k}{\partial T}\right)\left(\frac{\partial T}{\partial r}\right)^2 - c_p\rho\frac{\partial T}{\partial t} > r^2\,dr = 0 \qquad (a1)$$

where I_i is the following linear interpolation function.

$$I_i = (r_2 - r)/(r_2 - r_1) \text{ and } I_2 = (r - r_1)/(r_2 - r_1) \qquad (a2)$$

The integration of the first term becomes:

$$\int_{r_1}^{r_2} L_i r^2 k\frac{\partial^2 T}{\partial r^2}\,dr = L_i r^2 k\frac{\partial T}{\partial r}\Big|_{r_1}^{r_2} - \int_{r_1}^{r_2}\frac{\partial}{\partial r}(L_i r^2 k)\frac{\partial T}{\partial r}\,dr \qquad (a3)$$

The first term of the right side of Eq (a3) becomes equal to nil for all elements except one having a boundary nodal point, at where the boundary condition, Eq (3) or (4), is introduced. By performing integration as described in most reference books on finite element analysis, we obtain the following equations.

$$\begin{Bmatrix}\frac{\partial T_1}{\partial t}\\ \frac{\partial T_2}{\partial t}\end{Bmatrix} = \frac{60}{c_p(r_2-r_1)}\cdot\frac{1}{m_1m_3-m_2}\cdot\begin{Bmatrix}-m_2S\\ m_1S\end{Bmatrix} + \frac{1}{c_p(m_1m_3-m_2^2)}\cdot\begin{Bmatrix}V_1\\ V_2\end{Bmatrix} + \frac{60}{c_p(m_1m_3-m_2^2)} < \begin{bmatrix}\frac{F}{(r_2-r_1)^2}\end{bmatrix} \begin{bmatrix}-m_2-m_3 & m_2+m_3\\ m_1+m_2 & -m_1-m_3\end{bmatrix} + \frac{1}{12(r_2-r_1)^2}\cdot\begin{bmatrix}P_1 & -P_1\\ P_2 & -P_2\end{bmatrix} > \begin{Bmatrix}T_1\\ T_2\end{Bmatrix} + \frac{5k_1}{c_p(m_1m_3-m_2^2)}\cdot(T_1-T_2)^2 \cdot\begin{Bmatrix}m_3A-m_2B\\ -m_2A+m_1B\end{Bmatrix} \qquad (a4)$$

where

$$A = 3r_1^2 + 2r_1r_2 + r_2^2 \qquad B = r_1^2 + 2r_1r_2 + 3r_2^2 \qquad (a5)$$

$$m_1 = 10r_1^2\rho(T_1) + 2(2r_1r_2\rho(T_1) + r_1^2\rho(T_2)) + r_2^2\rho(T_1) + 2r_1r_2\rho(T_2) + r_2^2\rho(T_2) \qquad (a6)$$

$$m_2 = 2r_1^2\rho(T_1) + 2r_1r_2\rho(T_1) + r_1^2\rho(T_2) + r_2^2\rho(T_1) + 2r_1r_2\rho(T_2) + 2r_2^2\rho(T_2) \qquad (a7)$$

$$m_3 = r_1^2\rho(T_1) + 2r_1r_2\rho(T_1) + r_1^2\rho(T_2) + 2\left(r_2^2\rho(T_1) + 2r_1r_2(T_2) + 10r_2^2\rho(T_2)\right) \qquad (a8)$$

$$S = R^2 < h_{qo}(-1)^j(|T_a - T_2|)^{n_g+1} - L\,h_{mo}(-1)^j(|P_s\cdot\frac{T_a+273.15}{T_2+273.15} - P_a|)^{n_m+1} > \qquad (a9)$$

$$V_1 = m_3[J_1Q(T_1) + J_2Q(T_2)] - m_2[J_2Q(T_1) + J_3Q(T_2)] \qquad (a10)$$

$$V_2 = -m_2[J_1Q(T_1) + J_2Q(T_2)] + m_1[J_2Q(T_1) + J_3Q(T_2)] \qquad (a11)$$

$$J_1 = 12r_1^2 + 6r_1r_2 + 2r_2^2 \qquad J_2 = 3r_1^2 + 4r_1r_2 + 3r_2^2 \qquad (a12)$$

$$J_3 = 2r_1^2 + 6r_1r_2 + 12r_2^2$$

$$\left.\begin{aligned}P_1 &= -m_3A[k(T_1) - k(T_2)] + m_2B[k(T_1) - k(T_2)]\\ P_2 &= m_2A[k(T_1) - k(T_2)] - m_1B[k(T_1) - k(T_2)]\end{aligned}\right\} \qquad (a13)$$

$$F = \frac{1}{12}\left(2r_1^2k(T_1) + 2r_2^2k(T_2) + (r_1+r_2)^2[k(T_1) + k(T_2)]\right) \qquad (a14)$$

It should be noted that the first term in the right side of Eq (a3) appears only in the outmost boundary element.

–Continued on next page

Analytical solution for heat conduction in a spherical body

When heat is generated at a constant rate, Q, and when there are the following boundary and initial conditions, one may easily derive an analytical solution for estimating a transient state temperature distribution in a sphere.

$$v = 1.0 \text{ when } F_o = 0 \text{ and } 0 \leqslant \eta \leqslant 1 \quad (b1)$$

$$\frac{\partial v}{\partial \eta} = 0 \text{ at } \eta = 0 \text{ for } F_o > 0 \quad (b2)$$

$$\frac{\partial v}{\partial \eta} = \text{Biv at } \eta = 1 \text{ for } F_o > 0 \quad (b3)$$

Eq (b3) assumes that there is convective heat exchange between the spherical surface and surrounding medium whose temperature is kept at zero. The following solution is derived by using a superposition theorem (Carslaw and Jaeger, 1959).

$$v = \frac{Q}{6\,Bi}\{Bi(1-\eta^2)+2\} + \frac{2}{\eta}\sum_{n=1}^{\infty} \exp(-\beta_n^2 Fo) \cdot \frac{\beta_n^2 + (Bi-1)^2}{\beta_n^2 + Bi(Bi-1)} \cdot \frac{1}{\beta_n^2} \cdot \sin(\beta_n \eta) \cdot \sin \beta_n \left[\frac{Q}{6}\left\{2 - Bi\left(\frac{6}{\beta_n^2} + \frac{2}{Bi}\right)\right\} + Bi\right] \quad (b4)$$

Optimum nodal values of eta

The nodal values of a normalized location variable, η, for the optimum finite element subdivision are obtained after a series of computational experiments. These values are: 0.0, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.30, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.975, and 1.0.

APPENDIX B – NOMENCLATURE

- A Expression defined by Eq (a5).
- a_1 Rate of changes in heat generation with respect to produce temperatures when they are within T_l and T_b [W/(cm^{3}C°)].
- B Expression defined by Eq (a5)
- Bi Biot number = $h_q R/k$, where h_q and k are assumed to be invariable.
- Bi_r Modified Biot Number = $h_{qr} R/k_r$.
- b_1 Similar to a_1. This is applicable to produce temperatures between T_b and T_c [W/(cm^{3}C°)]
- b_o Rate of heat generation at T_b(W/cm^3)
- c_p Specific heat of produce [Joule/(gC°)]
- c_1 Rate of heat generation at T_c(w/cm^3)
- c_2 Constant used in one of Eq (9) (C°)
- d_1 Similar to a_1. This is applicable to produce temperatures between T_d and T_e [W/(cm^{3}C°)]
- d_o Rate of heat generation at T_d(W/cm^3).
- E_1, E_2, E_4 Constants used in Eq 10, 12, and 14 respectively (1/sec).
- F Expression defined by Eq (a13).
- Fo Fourier number = $\alpha t/R^2$, where α is assumed to be invariable
- Fo_r Modified Fourier number = $\alpha_r t/R^2$
- Fo_{lld}, Fo_{lmd}, Fo_{cjl}, Fo_{cjm}, Fo_{hmd}, Fo_{hld} values of modified Fourier number, which correspond to t's with same subscripts.
- g_k = k/k_r
- g_ρ = ρ/ρ_r
- h_{qo} Constant for estimating surface heat conductance. For a forced convective heat exchange process, this is equal to surface heat conductance since $n_q = 0$[W/(cm^{2}C°)]
- h_{mo} Constant for estimating the transpiration coefficient. For a forced convective mass exchange process, this is equal to the transpiration coefficient since $n_m = 0$ [g/(cm^{2}sec kpa)].
- H_{qo} = $h_{qr} R \cdot |U_{ar}|^{-n_q}/kr$
- H_{mo} = $L_r h_{mo} R P_r^{n_m+1}/[k_r(T_o - T_r)]$
- h_{qr} = $h_{mo}(|T_{ar} - T_r|)^{n_q}$ Surface heat conductance when surrounding medium temperature is T_{ar} and produce surface temperature is equal to T_r.
- I_i Linear interpolation function. See eqs. a2.
- J_1, J_2, J_3 Expressions defined by eqs. a12.
- k Thermal conductivity of produce which is linearly related to produce temperature. $k = k_o + k_1 T$ [W/(cmC°)].
- k_o, k_1 See k for their definitions.
- $k(T_1)$, $k(T_2)$ Values of k at T_1 and T_2 respectively [W/(cmC°)].
- L Latent heat of moisture vaporization, which is linearly related to produce temperature. $L = L_o + L_1 T$ (Joule/g).
- L_o, L_1 See L for their definition.
- m_1, m_2, m_3 Expressions defined by Eq (a6), (a7) and (a8) respectively.
- n_m, n_q Constants used for estimating surface heat conductance or transpiration coefficient.
- N_f See Table 5.
- p Water vapor pressure (Kpa)
- p_1, p_2 Expressions defined by (a13).
- $Q(T,t)$ Rate of heat generation per unit volume of produce. This rate is generally a function of produce temperature and time measured from the harvest (W/cm^3).
- $Q(T_1)$, $Q(T_2)$ The values of Q at T_1 and T_2 respectively (W/cm^3).
- Q_e Equilibrium rate of heat generation (W/cm^3)
- Q_a, Q_c, Q_o, Q_2, Q_4 Constants used in Eq (10), (11), (12) and (14).
- $q(u, Fo_r) = R^2 Q(T,t)/(k_r(T_o - T_r)$
- R Radius of spherical produce (cm).
- r Radial variable (cm).
- r_1, r_1 Nodal values of finite element (cm).
- rh Relative humidity (fraction).
- S Expression defined by Eq (a8).
- T Temperature (°C).
- T_b, T_c, T_d, T_e, T_l Produce temperatures which define applicable ranges of temperaures to estimate the rate of heat generation. See Eq (9) (°C).
- T_{lld}, T_{lmd} Cold inactivation temperatures which are applicable to the local and average produce temperatures respectively. The local tissue is inactivated when local produce temperature is held at or lower than T_{lld} for t_{lld} sec. or longer. Similarly, t_{lmd} is related to T_{lmd} (°C).
- T_{cjl}, T_{cjm} Cold injury temperatures which are respectively applicable to local and average produce temperatures. Cold injury times t_{cjl} and t_{cjm} are respectively associated with T_{cjl} and T_{cjm} (°C).
- T_{hld}, T_{hmd} Thermal inactivation temperatures which are respectively applicable to local and average produce temperatures, and which are respectively associated with thermal inactivation times t_{hld} and t_{hmd} (°C)
- T_r Reference temperature (°C).
- t_{lld}, t_{lmd} See T_{lld} and T_{lmd} (sec)
- t_{cjl}, t_{cjm} See T_{cjl} and T_{cjm} (sec)
- t_{hld}, t_{hmd} See T_{hld} and T_{hmd} (sec).
- u = $(T - T_r)/(T_o - T_r)$

u_b, u_c, u_d, u_e, u_l Dimensionless temperatures of T's with same subscripts.

u_k $= (273.15 - T_r)/(T_o - T_r)$

u_{2o} $= T_r/(T_o - T_r)$

u_{lld}, u_{lmd}, u_{cjl}, u_{cjm}, u_{hmd}, u_{hld} Dimensionless temperatures of T's with same subscript.

v Dimensionless temperature estimated by Eq (b4).

v_{ul} $= (1{,}056.62\ T_r)/(T_o - T_r)$

v_1, v_2 Expressions defined by Eq (a10) and 'all' respectively.

w $= p/p_r$

W_l moisture loss (g)

x_1, x_2, x_3, x_4, x_5 Statistical design variables defined by Eq (22), (23), (24), (25) and (26) respectively.

α Nonvariable thermal diffusivity (cm^2/sec)

α_r $= k_r/(c_p p_r)$. Thermal diffusivity of produce at T_r(cm^2/sec).

β_{bo}, β_{co}, β_{do} Dimensionless parameters obtained from constants identified with their subscripts by multiplying them with $R^2/[(T_o - T_r) \cdot k_r]$. For example, $\beta_{bo} = b_o R^2/[(T_o - T_r) \cdot k_r]$.

β_{bl}, β_{d_1} Dimensionless parameters obtained from constants identified with their subscripts by multiplying them with R^2/k_r. For example, $\beta_{bl} = R^2 b_1/k_r$.

β_{cl} $= C_2/(T_o - T_r)$.

β_n The nth positive root of $\beta \cot \beta = 1 - Bi$.

Γ $= L_r W_1/[(T_o - T_r) C_p P_r R^2]$

ρ density of produce, which is linearly related to its temperature. $\rho = \rho_o + \rho_1 T$ (g/cm^3)

ρ_o, ρ_1 See ρ for their definition.

$\rho(T_1)$, $\rho(T_2)$ Produce densities at T_1 and T_2 respectively.

ϵ_{kl} $= (T_o - T_r) k_1/k_r$

$\epsilon_{\rho l}$ $= (T_o - T_r) \rho_1/\rho_r$

η $= r/R$

subscript

a Quantities related to surrounding heat exchange medium.

d Quantities associated with dry produce surface.

o Initial value when it is used with T.

r Quantities at reference temperature.

s Saturation

w Quantities related to wet produce surface.

REFERENCES

Anonymous. 1977. Thermal properties of foods. In "ASHRAE Handbook & Product Directory," p. 29.1. American Society of Heating, Refrigerating, and Air Conditioning Engineers, New York, NY.

Arce, J.A. and Sweat, V.E. 1980. Survey of published heat transfer coefficients encountered in food refrigeration process. ASHRAE Trans. 86, Part 2.

Baird, C.D. and Gaffney, J.J. 1976. A numerical procedure for calculating heat transfer in bulk loads of fruits or vegetables. Presented at the annual Meeting of American Society of Heating, Refrigerating, and Air Conditioning Engineers. Seattle, WA.

Bakker-Arkema, R.W., Bickert, W.G., and Patterson, R.J. 1967. Simultaneous heat and mass transfer during cooling of a deep bed of biological products under varying inlet air conditions. J. Agr. Eng. Res. 12: 297.

Barr, A.J., Goodnight, J.H., Sall, J.P., and Helwig, J.Y. 1976. "A User's Guide to SAS," SAS Institute, Inc. Raleigh, NC.

Brugger, M.F. and Buelow, F.H. 1980. Two-dimensional finite difference model of the transient environment within a potato pile. Presented at the 1980 Winter Meeting of American Society of Agricultural Engineers, Chicago, IL Dec. 2—5.

Buescher, R.W. 1979. Influence of high temperature on physiological and compositional characteristics of tomato fruits. Lebensm.-Wiss, U.-Technol. 12: 162.

Carslaw, H.S. and Jaeger, J.C. 1959. "Conduction of Heat in Solids," 2nd ed., Oxford Univ. Press, London, England.

Davies, O.L. 1960. "The Design and Analysis of Industrial Experiments," 2nd ed. Imperial Chemical Industries Ltd. London, England.

Dickerson, R.W. Jr. 1968. Thermal properties of foods. In "The Freezing Preservation of Foods," Vol. 2, p. 26. AVI Publishing Co., Westport, CT.

Eshleman, W.D., Baird, C.D., and Gaffney, J.J. 1976. A numerical simulation of transient heat flow in irregular shaped foods. ASAE paper No. 76-6504. Presented at the 1976 Winter Meeting of American Society of Agricultural Engineers, Chicago, IL.

Fukushima, T., Yarimizu, K., Kitamura, T., and Iwata, T. 1980. The relation between water stress and the climacteric in respiration of some fruits. Scientia Horticulture, 12: 259.

Gaffney, J.J. and Baird, C.D. 1975. Susceptibility of West Indian avocados to chilling injury as related to rapid cooling with low temperature air or water. Florida State Horticultural Society 88: 490.

Gaffney, J.J. and Baird, C.D. 1977. Forced-air cooling of bell peppers in bulk. Trans. ASAE. 20: 1174.

Gaffney, J.J. 1980. Method for accurately positioning thermocouples in fruits and vegetables. Personal communications.

Gaffney, J.J., Baird, C.D. and Eshleman, W.D. 1980. Review and analysis of the transient method for determining thermal diffusivity of fruits and vegetables. ASHRAE Trans. 86, Part 2: 261.

Gear, C.W. 1971. "Numerical Initial Value Problems in Ordinary Differential Equations," Prentice-Hall, Englewood Cliffs, NJ.

Hayakawa, K. 1978. Computerized simulation for heat transfer and moisture loss from an idealized fresh produce. Trans. ASAE, 21: 1015.

Hayakawa, K., Brian, D., Vaccaro, E., and Gilbert, S.G. 1979. Development of a new procedure for direct determination of respiration heat generation by fresh produce. Lebensm.-Wiss. u-Technol. 12: 189.

Huebner, K.H. 1975. "The Finite Element Method for Engineers," John Wiley & Sons, New York, NY.

Kusunose, H. and Sawamura, M. 1980. Ethylene production and respiration of post harvest acid citrus fruits and Wase Satsuma Mandarin fruit. Agric. Biol. Chem. 44: 1917.

Lentz, C.P. and van den Berg, L. 1973. Factors affecing temperature, relative humidity and moisture loss in fresh fruit. ASHRAE J. 15(8): 55.

Lutz, J.M. and Hardenburg, R.E. 1968. The commercial storage of fruits, vegetables, and florist and nursery stocks. Agricultural Handbook No. 66. Superintendent of Documents, U.S. Government Printing Office, Washington, DC.

Miller, W.M. 1978. Surface moisture drying characteristics of citrus fruit. Presented at the 1978 Summer Meeting of American Society of Agricultural Engineers, Logan, UT, June 27—30.

Misener, G.C. and Shove, G.C. 1976. Moisture loss from Kennebec potato tubers during initial storage period. Trans. ASAE 19: 967.

Misra, R.N. and Young, J.H. 1979. The finite element approach for solution of transient heat transfer in a sphere. Trans. ASAE 22: 944.

Mowry, J.K. and Heldman, D.R. 1972. Analysis of moisture removal from the surface of blueberry fruit using air flow. Presented at the 1972 Winter Meeting of American Society of Agricultural Engineers, Chicago, IL, Dec. 11—15.

Polley, S.L., Snyder, O.P., and Kotnour. 1980. A compilation of thermal properties of foods. Food Technol. 34(11): 76.

Sastry, S.K. and Buffington, D.E. 1980. Transpiration rates of stored tomatoes under various environmental conditions. Presented at the 1980 Winter Meeting of American Society of Agricultural Engineers, Chicago, IL Dec. 2—5.

Sastry, S.K., Baird, C.D., and Buffington, D.E. 1978. Transpiration rates of certain fruits and vegetables. ASHRAE Trans. 84, Part 1.

Stewart, J.K. and Coney, H.M. 1963. Hydrocooling vegetables. A practical guide to predicting final temperatures and cooling times. Marketing Research Report No. 637. USDA Ag. Marketing Service, Market Quality Res. Unit, Fresno, CA.

Singh, R.P., Heldman, D.R., Cargill, B.F., and Bedford, C.L. 1975. Trans. ASAE 18: 1975.

Van den Berg, L. and Lentz, C.P. 1975. Effect of composition on thermal conductivity of fresh and frozen foods. J. Inst. Can. Sci. Technol. 8: 79.

Weiss, A. 1977. Algorithms for the calculation of moist air properties on a hand calculator. Trans. ASAE 20: 1133.

Whitaker, J.R. 1972. Effect of temperature on enzyme-catalyzed reactions. In "Principles of Enzymology for Food Sciences," Marcel Dekker, Inc., New York, NY.

Wills, R.B.H. and McGlasson, W.B. 1971. Effect of storage temperatures on apple volatiles associated with low temperature breakdown. J. Hort. Sci. 46: 115.

Wu, M.T. and Salunkhe, D.K. 1975. Effect of alternating storage temperatures on ripening of tomato fruits. Lebensm.-Wiss. u.-Technol. 8: 119.

Ms received 6/22/81; revised 10/16/81; accepted 10/18/81.

A paper of the Journal Series, New Jersey Agricultural Experimental Station, Cook College, Rutgers, The State Univ. of New Jersey, Dept. of Food Science, New Brunswick, NJ 08903.

This work was performed as a part of NJAES Project No. 1024, supported by the New Jersey Agricultural Experiment Station, by Hatch Act Fund and by Computer Time Fund provided by the Center for Computer and Information Services, Rutgers, The State Univ. of New Jersey.

Volatile Flavor Compounds from Shallots

J. L. WU, C. C. CHOU, M. H. CHEN, and C. M. WU

ABSTRACT

Approximately 0.030%, 0.012% and 0.005% (v/w), in wet weight, of volatile oils were obtained from raw, baked and deep-fried shallots, respectively. These oils have been studied by a gas chromatograph coupled to a mass spectrometer. The flavor components of these oils can be classified in the following categories: thiols, monosulfides, disulfides, trisulfides, thiophenes and oxygen compounds. After heating (baking or deep-frying) of the shallot, a sharp increase in the amount of dimethylthiophenes was observed and the alkyl propenyl disulfides decreased.

INTRODUCTION

SHALLOT (*Alium cepa* L., Aggregatum g.) is an important flavoring vegetable in a wide range of Chinese dishes. It is commonly used as seasoning only after deep oil frying. However, the deep oil fried shallot could not be used as a seasoning in commercialized food products because of its containing the rancid oils. In our study, both baked and deep oil fried shallots were used as the experimental samples. The volatile oils obtained from raw, baked and deep-fried shallots were analyzed by GC-MS analyses, leading to an understanding of the flavor of formation mechanism. Dembele and Dubois (1973) reported the composition of raw shallots. However, this study emphasizes the comparison of raw, baked and deep-fried shallots.

All authors are affiliated with the Dept. of Food Science, Food Ind. Res. & Dev. Inst., P.O. Box 246, Hsinchu 300, Taiwan, Republic of China.

EXPERIMENTAL

Isolation of volatile shallot oils

The AOAC (1975) method was used to isolate the volatile shallot oils. For the deep-fried shallot volatile oils, shallots deep-fried at 150–160°C for 10 min were used; for the baked shallot volatile oils, shallots were baked at 70°C for 60 min and then at 140°C for 30 min. These heating conditions were judged to produce the best flavor shallots.

GC-MS analyses of shallot volatile oils

A Hewlett Packard 5985B GC-MS was used. The GC column was a SCOT Carbowax 20M, 0.02 in. i.d. x 50 ft, stainless steel column. One microliter of shallot oil sample was injected. It was in the splitter mode with a split ratio of 80:1. The carrier gas was helium. GC operating conditions: initial temperature, 80°C, held for 10 min, then programmed first at 2°C/min to 140°C, and then at 1°C/min to 180°C. Operating conditions of the mass spectrometer were: ionization voltage, 70 eV; source temperature, 200°C; accelerating voltage, 1800 V.

RESULTS & DISCUSSION

APPROXIMATELY 0.030%, 0.012% and 0.005% (v/w), in wet weight, of volatile shallot oils were obtained from raw, baked and deep-fried shallots, respectively. The GC chromatograms of raw, baked and deep-fried volatile shallot oils obtained on a SCOT Carbowax 20M column are shown in Fig. 1, 2 and 3, respectively. Peak identification was in

Fig. 1—Gas chromatogram of shallot oil.

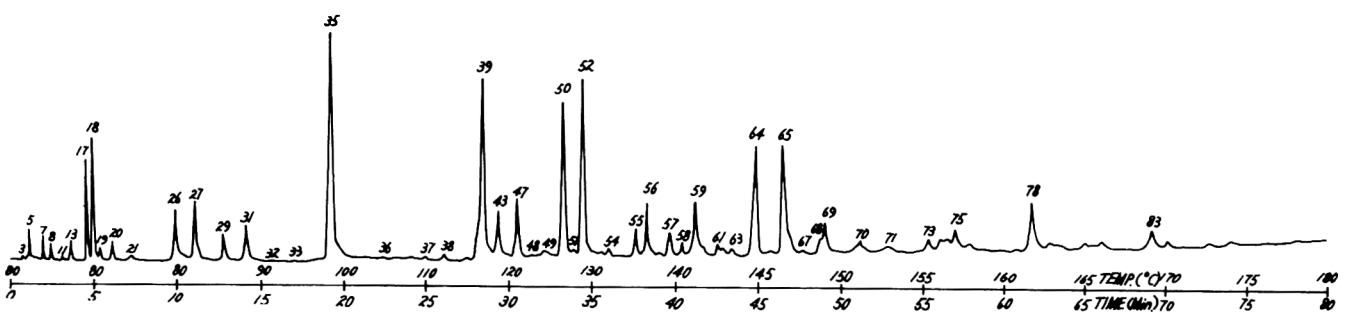


Fig. 2—Gas chromatogram of baked shallot oil.

accordance with the retention times and mass spectra cited in the previous literatures.

The compounds identified as presented in Table 1 with their mass spectral data and their peak numbers correspond to the numbers in Fig. 1, 2 and 3. Propanethiol, 2,5-dimethylthiophene, 3,4-dimethylthiophene, dipropyl disulfide, propyl cis-propenyl disulfide, propyl trans-propenyl disulfide, methyl propyl trisulfide and dipropyl trisulfide were identified in these shallot oils (peaks 3, 7, 12, 13, 18, 27, 29, 31, 35 and 43) by comparing their relative retention times which were reported by Dembele and Dubois (1973) and mass spectral patterns with those by MSDC (1974). 3,4-Dimethylthiophene and dimethyl trisulfide (peaks 18 and 26) were identified by comparing their relative retention times (Dembele and Dubois, 1973) and mass spectral patterns (TNO, 1980). Methyl cis-propenyl disulfide and

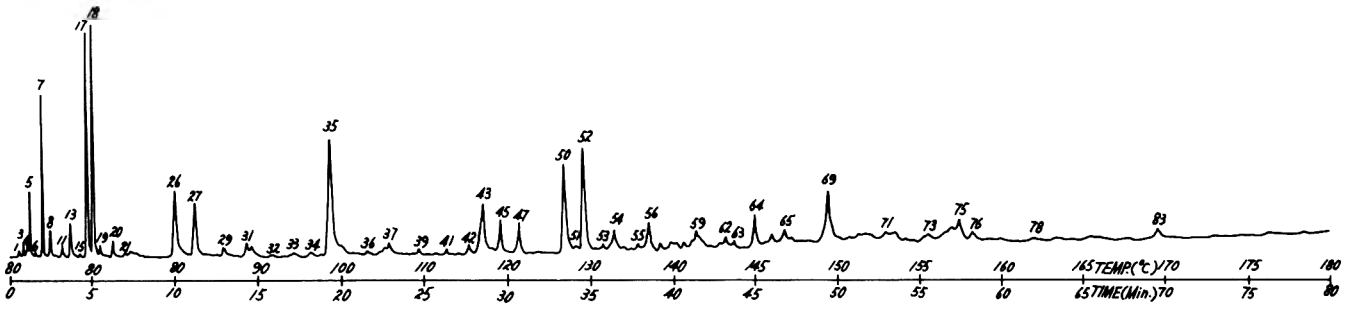


Fig. 3—Gas chromatogram of deep-fried shallot oil.

Table 1—Identity of volatile compounds of shallot

Peak No.[a]	compound	M.W.	Characteristic MS data[b] m/e (relative intensity)			
1	methanethiol		47.2(100.0)	48.2(100.0)	45.2(64.7)	18.3(64.7)
		48	18.3(64.70	45.2(64.7)	47.2(100.0)	48.2(100.0)
3	propanethiol		76.3(100.0)	47.2(7.6)	78.3(6.8)	61.3(5.0)
		76	69.3(3.1)	76.3(100.0)	77.3(4.8)	78.3(6.8)
4	propylenethiol		74.2(100.0)	73.3(15.9)	18.3(13.1)	76.2(12.1)
		74	71.3(11.2)	73.3(15.9)	74.2(100.0)	76.2(12.1)
7	dimethyl disulfide		94.2(100.0)	79.2(28.8)	96.2(9.9)	95.8(3.9)
		94	95.3(3.9)	96.2(9.9)	97.3(0.5)	98.3(0.3)
11	propyl propenyl sulfide		98.3(100.0)	111.3(63.9)	112.3(52.8)	97.3(45.8)
		116	98.3(100.0)	111.3(63.9)	112.3(52.8)	116.3(41.7)
13	2,5-dimethylthiophene		111.3(100.0)	112.3(78.0)	97.3(34.5)	113.3(10.1)
		112	111.3(100.0)	112.3(78.0)	113.3(10.1)	114.3(4.4)
17	methyl propyl disulfide		122.2(100.0)	80.2(52.8)	124.2(9.5)	123.2(6.4)
		122	124.2(9.5)	125.2(0.6)	126.2(0.3)	138.4(0.5)
18	3,4-dimethyl thiophene		111.3(100.0)	112.3(70.7)	97.3(33.4)	113.3(9.5)
		112	112.3(70.7)	113.3(9.5)	114.3(3.4)	122.2(1.5)
19	methyl cis-propenyl disulfide		120.2(100.0)	72.3(13.9)	80.2(10.2)	75.3(10.1)
		120	120.2(100.0)	121.2(6.1)	122.2(9.2)	123.2(0.5)
20	methyl trans-propenyl disulfide		120.2(100.0)	72.3(12.8)	75.3(11.8)	80.2(9.9)
		120	121.2(5.9)	122.2(9.5)	123.2(0.5)	124.2(0.3)
26	dimethyl trisulfide		126.2(100.0)	79.2(22.2)	111.1(17.9)	128.2(13.8)
		126	127.2(4.9)	128.2(13.8)	129.2(0.5)	130.2(0.7)
27	dipropyl disulfide		150.3(100.0)	108.3(64.2)	152.3(9.2)	126.2(8.7)
		150	151.3(7.9)	152.3(9.2)	153.3(0.6)	154.3(0.4)
29	propyl cis-propenyl disulfide		148.3(100.0)	106.2(65.5)	150.4(10.1)	72.2(9.6)
		148	148.3(100.0)	149.4(8.7)	150.4(10.1)	151.2(0.9)
31	propyl trans-propenyl disulfide		148.3(100.0)	106.3(66.2)	72.2(8.9)	150.3(8.8)
		148	148.3(100.0)	149.3(8.2)	150.3(8.8)	151.3(0.7)
35	methyl propyl trisulfide		154.3(100.0)	112.1(63.5)	156.3(13.3)	79.2(11.7)
		154	156.3(13.3)	157.3(0.8)	158.3(0.6)	182.4(0.3)
39	methyl propenyl trisulfide		152.3(100.0)	88.3(62.1)	154.3(21.7)	73.3(20.5)
		152	154.3(21.7)	155.4(1.5)	156.3(1.9)	158.3(1.4)
43	dipropyl trisulfide		182.4(100.0)	75.3(45.4)	98.1(13.6)	184.4(13.5)
		182	184.4(13.5)	185.4(1.0)	186.4(0.6)	207.5(0.6)
47	1-methylthiopropyl ethyl disulfide		89.3(100.0)	79.2(8.2)	73.2(5.9)	61.2(5.9)
		182	160.3(1.2)	166.3(0.6)	168.3(1.9)	182.4(2.4)
50	propyl cis-propenyl trisulfide		180.4(100.0)	106.2(53.3)	115.3(43.4)	116.3(33.1)
		180	182.4(15.5)	183.4(1.4)	184.4(1.2)	207.6(1.3)
52	propyl trans-propenyl trisulfide		180.4(100.0)	106.3(59.5)	115.3(46.3)	116.3(35.3)
		180	182.4(16.0)	183.3(1.4)	184.4(1.0)	207.6(1.8)
65	2-n-hexyl-5-methyl-2,3-dihydrofuran-3-one		98.3(100.0)	111.3(64.1)	182.5(8.2)	99.2(6.0)
		182	183.6(1.1)	186.4(0.3)	205.6(0.2)	207.6(0.5)
78	2-n-octyl-5-methyl-2,3-dihydrofuran-3-one		98.2(100.0)	111.3(74.6)	89.3(15.7)	99.3(8.7)
		210	207.6(3.0)	208.7(0.7)	210.7(6.4)	211.6(0.8)

[a] Refers to peaks in Fig. 1, 2 and 3.
[b] Determined with a Hewlett Packard 5985B gas chromatograph-mass spectrometer.

methyl trans-propenyl disulfide (peaks 19 and 20) were identified by comparing their relative retention times and mass spectral patterns (Brodnitz et al., 1969). 2-n-Hexyl-5-methyl-2,3-dihydrofuran-3-one (peak 65) was identified by comparing its relative retention time (Dembele and Dubois, 1973) and mass spectral pattern (Boelens et al., 1971). 1-Methylthiopropyl ethyl disulfide (peak 47) was identified by comparing its mass spectral pattern (Boelens et al., 1974). Methyl propyl disulfide, propyl cis-propenyl trisulfide, propyl transpropenyl trisulfide and 2-n-octyl-5-methyl-2,3-dihydrofuran-3-one (peaks 17, 50, 52 and 78) were identified by comparing their relative retention time (Dembele and Dubois, 1973) and mass spectral patterns (Schreyen et al., 1976). Methanethiol and propylenethiol (peaks 1 and 4) were identified by comparing their mass spectral patterns (MSDC, 1974). Propyl propenyl sulfide (peak 11) was identified by comparing its relative retention time (Dembele and Dubois, 1973) and mass spectral data which was shown in Table 1. Methyl propenyl trisulfide (peak 39) was identified by comparing its relative retention time (Brodnitz et al., 1969) and mass spectral data which was also shown in Table 1. The methanethiol, propylenethiol, methyl cis-propenyl disulfide, methyl propenyl trisulfide and 1-methylthiopropyl ethyl disulfide were not previously reported to occur in the shallot essential oil (Dembele and Dobois, 1973).

Table 2—Percentages of constituents of raw, baked and deep-fried shallot oils[a]

Peak no.	Compound	Shallot oil (%) Raw	Baked	Deep-fried
	Thiols			
1	methanethiol	b	b	0.12
3	propanethiol	0.06	0.07	0.45
	Unsat. monosulfide			
11	propyl propenyl sulfide	0.28	0.09	0.40
	Sat. disulfides			
7	dimethyl disulfide	1.99	0.38	3.75
17	methyl propyl disulfide	3.59	2.95	10.93
27	dipropyl disulfide	4.16	3.42	4.50
	Unsat. disulfides			
19	methyl cis-propenyl disulfide	2.91	0.39	0.48
20	methyl trans-propenyl disulfide	5.09	0.63	0.83
29	propyl cis-propenyl disulfide	2.79	1.38	0.71
31	propyl trans-propenyl disulfide	4.43	1.99	0.98
	Sat. trisulfides			
26	dimethyl trisulfide	18.81	2.76	5.61
35	methyl propyl trisulfide	19.93	15.32	12.93
43	dipropyl trisulfide	5.55	2.63	4.13
47	1-methylthiopropyl ethyl disulfide	6.41	3.12	3.14
	Unsat. trisulfide			
39	methyl propenyl trisulfide	4.85	14.38	b
50	propyl cis-propenyl trisulfide	4.50	9.18	9.21
52	propyl trans-propenyl trisulfide	5.47	10.58	9.59
	Thiophenes			
12	2,4-dimethylthiophene	b	b	b
13	2,5-dimethylthiophene	0.17	0.51	1.70
18	3,4-dimethylthiophene	1.36	3.93	11.65
	Oxygen compounds			
65	2-n-hexyl-5-methyl-2,3-dihydrofuran-3-one	3.13	7.77	1.42
78	2-n-octyl-5-methyl-2,3-dihydrofuran-3-one	1.11	4.21	1.46
	Unknown compounds			
5	(M.W. 282)	0.13	0.50	1.57
8	(M.W. 268)	b	0.46	1.25
54	?	b	0.39	1.72
59	(M.W. 191)	1.43	3.32	2.29
64	(M.W. 205)	1.02	7.41	3.57
69	?	0.84	2.24	5.63

[a] Percentages were calculated according to the peak area of each peak to the total peak area in GC-MS chromatogram.
[b] Amounts of these constituents were too little to estimate.

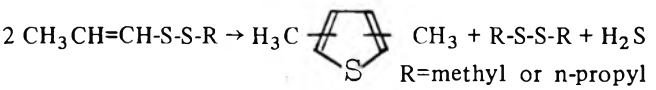
All of the identified components of shallot volatile oils contain at least one sulfur atom per molecule except compounds 65 and 78, and all are aliphatic sulfides except compounds 12, 13 and 18. The percentages of main constituents of raw, baked and deep-fried shallot oils are shown in Table 2.

The main constituents of shallot oils are: (1) methyl propyl trisulfide, dimethyl trisulfide, propyl propenyl disulfide and 1-methylthiopropyl ethyl disulfide (raw shallot oil); (2) propyl propenyl trisulfide, methyl propyl trisulfide, methyl propenyl trisulfide, 2-n-hexyl-5-methyl-2,3-dihydrofuran-3-one and 2-n-octyl-5-methyl-2,3-dihydrofuran-3-one (baked shallot oil).; and (3) propyl propenyl trisulfide, methyl propyl trisulfide, 3,4-dimethylthiophene, methyl propyl disulfide, and dimethyl trisulfide (deep-fried shallot oil).

According to the percentages of important components in the whole shallot volatile oils, the following results were obtained:

1. In the heating processing either by baking or deep-frying, the amount of dimethylthiophenes, unsaturated alkyl trisulfides and several small high molecular compounds increased while saturated alkyl trisulfides and unsaturated alkyl disulfides decreased.

2. Methyl propyl trisulfide had the highest percentage of content in all three shallot volatile oils. Methyl propenyl disulfide and propyl propenyl disulfide had lesser amounts, but their changes in heating were conspicuous. The following equation is proposed as the main chemical reaction occurring in the baking or frying. This reaction was also proposed by Boelens et al. (1971) in the onion.



3. Compared with baked shallots, deep-fried shallots treated at a higher temperature and shorter time had higher contents of dimethylthiophenes, saturated alkyl disulfides, propanethiol and methanethiol in the volatiles. However, the baked shallots had higher oxygen compounds.

REFERENCES

AOAC. 1975. Determination of essential oil in emulsion. In "Official Methods of Analysis," 12th ed, p. 344. Association of Official Analytical Chemists, Washington, DC.
Boelens, M., de Valois, P.J., Wobben, H.J., and van der Gen, A. 1971. Volatile flavor compounds from onion. J. Agric. Food Chem. 19: 984.
Boelens, M., van der Linde, L.M., de Valois, P.J., van Dort, H.M., and Takken, H.J. 1974. Organic sulfur compounds from fatty aldehyde, hydrogen sulfide, thiols and ammonia as flavor constituents. J. Agric. Food Chem. 22: 1071.
Brodnitz, M.H., Pollock, C.L., and Vallon, P.P. 1969. Flavor Components of Onion Oil. J. Agric. Food Chem. 17: 760.
Dembele, S. and Dubois, P. 1973. Composition of essences shallots (Allium cepa L. Var. aggregatum) Ann. Technol. agric. 22: 121.
MSDC. 1974. "Eight Peak Index of Mass Spectra," 2nd ed. Mass Spectrometry Data Centre, AWRE, Aldermaston, UK.
Schreyen, L., Dirinck, P., Van Wassenhove, F., and Schamp, N. 1976. Volatile flavor components of leek. J. Agric. Food Chem. 24: 336.
TNO. 1980. "Compilation of Mass Spectra of Volatile Compounds in Food," Central Institute for Nutrition and Food Research, TNO, Zeist, The Netherlands.
Ms received 5/15/81; revised 9/14/81; accepted 9/17/81.

Bioavailability of Iron Produced by the Corrosion of Steel in Apples

A. ROSANOFF and B. M. KENNEDY

ABSTRACT

The bioavailability of iron formed by the corrosion of low-carbon steel (99% Fe) in contact with Red Delicious apples was measured in a rat model using a depletion-repletion experiment. The percentage of ingested iron converted to hemoglobin iron (Conversion Efficiency) was 74, 57, and 56%, respectively, for daily doses of 110, 190, and 285 μg of this iron. (Conversion Efficiency for $FeSO_4$ was 46–50%). When compared with $FeSO_4$ = 100, the relative biological value of this iron was found to range from 93–153. Ingestion of one Red Delicious apple that has been exposed to eight large iron nails for 24 hr could provide 10–15 mg iron having good bioavailability.

INTRODUCTION

ADDITION OF IRON TO FOODS due to preparation in iron cookware has often been cited as being significant (Burroughs and Chan, 1972; Walker and Arvidsson, 1953; Moore, 1965). Although the possible role of iron cookware in iron nutrition has been debated (Sharon, 1972; Monsen et al., 1967; MacKay et al., 1945), some studies have shown that intake of food exposed to iron cookware can produce a significant rise in hemoglobin values in humans (Devadas et al., 1973). However, no bioavailability studies for such nutritional iron have been found in the literature. Since the contribution of iron to foods by contact with iron alloys appears to be significant in some cases, the study of the nutritional properties of such iron, especially bioavailability, seems warranted.

Apples show a particularly high rate of iron uptake when in contact with reduced iron. Moore (1965) showed a 100-fold increase in iron content for apple butter cooked 2 hr in an iron Dutch oven. Rosanoff and Kennedy (1979) found that the iron content of raw apples in contact with steel (99% Fe) can increase by several milligrams over a day's exposure time.

The purpose of this study was to measure the bioavailability of iron formed by the corrosion of iron into apple (iron/apple) in a rat model.

MATERIALS & METHODS

Bioavailability test

Sixty-eight male weanling rats of the Sprague-Dawley albino strain, weighing 39–51g (Simonson Labs, Inc.) were placed on an iron-deficient diet (1.8 ppm Fe) for 23 days (Table 1). Hemoglobin values determined after this depletion period were all less than 6.0 g/dl. One animal died during the depletion period. The remaining 67 anemic rats were randomly assigned into eight groups (eight or nine per group) which had mean weights ranging from 95–110g and mean hemoglobin values from 3.5–3.6 g/dl. Each group was placed on one of the eight repletion regimes shown in Table 2. Lyophilized apple was found to be extremely hygroscopic and therefore inappropriate for direct incorporation into the basal diet. Therefore, each animal repleted on the test iron (iron/apple) was fed, in addition to the basal diet, 0, 0.22, 0.38, or 0.57 mg iron every other day as frozed iron/apple supplements that had been allowed to thaw before presentation. In every case, these iron/apple supplements were consumed by the animals within an hour of presentation, which was always during morning, daylight hours. There was no loss of supplement due to dropping. Four groups were fed the basal diet having 0, 6, 12, or 24 ppm iron as ferrous sulfate which was added dry to the basal diet rather than incorporated into frozen apple supplements or another form of liquid supplement in order to insure that the chemical form of the iron presented to these animals was, in fact, ferrous sulfate. These four groups were fed ad-libitum. After 12 days, hemoglobin values were again determined for each animal.

Authors Rosanoff and Kennedy are with the Dept. of Nutritional Sciences, 119 Morgan Hall, Univ. of California, Berkeley, CA 94720.

Preparation of iron/apple supplements

Iron/apple was prepared using Red Delicious apples grown in the Pacific Northwest Region and newly purchased, round, common nails of low-carbon steel (SAE 1008, 99% iron). Consistent doses of the iron/apple in three levels (200, 400, and 600 μg iron/dose) were prepared from apples with 10–15 nails inserted for 20–50 hr. The apples were peeled, cored, and crushed with an acid-washed procelain mortar and pestle. The resulting liquid-pulp was divided into three aliquots. These were diluted with similarly prepared pulp made from apples that had not been in contact with iron to give the desired iron concentrations. Mixed blends were frozen into 1-g cubes. Analysis of iron for four to six cubes chosen at random from each preparation showed less than 1% internal variation for all three iron/apple supplements (Table 2). Each cube contained a 2-day supply of iron and was thus fed every other day to minimize the intake of apple with the iron/apple. A preparation of frozen untreated apple cubes contained less than 10 μg Fe/cube. One group of iron-depleted animals and a group of positive control animals that were never depleted of iron received untreated frozen apple cubes every other day.

Iron analyses

Iron determinations were made using 2,2'-bypyridine (AACC, 1969). Specimens to be analyzed were weighed, dried 4–8 hr in acid-washed quartz crucibles under an infra-red lamp, and ashed in a muffle furnace at 550°C for 24 hr. Ash residues were dissolved in HCl while heating; the solutions were filtered and brought to volume using distilled water. Iron was reduced to the ferrous state with hydroquinone and the pH of the solutions adjusted to 4.5

Table 1—Composition of basal diet

Ingredient	g/100g Diet
Casein	21.0
Sucrose (confectioners)	67.5
Corn oil	5.0
Choline pre-mix[a]	1.0
B-vitamin pre-mix[b]	1.0
Vitamins A, D, E, pre-mix[c]	1.0
Mineral pre-mix[d]	3.5

[a] Choline pre-mix in sucrose, g/kg: choline bitartrate, 180.
[b] B-vitamin pre-mix in sucrose, mg/kg: thiamin HCl, 500; riboflavin, 1,000; niacinamide, 6,000; calcium-d-pantothenate, 3,000; pyridoxine HCl, 960; folic acid, 200; biotin, 100; B_{12} triturate in mannitol, 2,000; menadione, 40.
[c] Vitamins A, D, E, pre-mix in corn oil, IU/g: vitamin A, 1,000; vitamin D, 100; vitamin E, 22.
[d] Mineral pre-mix after Williams & Briggs, less iron citrate, g/kg: $CaCO_3$, 207; $CaHPO_4$, 323; Na_2HPO_4, 186; KCl, 208.6; $MgSO_4$, 65.7; $MnSO_4 \cdot H_2O$, 4.4; $CuSO_4$, 0.37; $ZnCO_3$, 0.48; KIO_3, 0.03 (Cohen et al., 1967).

with acetate buffer. The solutions were allowed to stand for 30 min for complete color development and then read on a spectrophotometer at 520 nm. Standard solutions were prepared using analytical grade $FeSO_4 \cdot 7H_2O$.

Hemoglobin tests

Percent hemoglobin values were determined using the cyanmethemoglobin method (Crosby et al., 1954). Blood was taken from the tail vein after cutting the tip with a razor blade, drawn directly into heparinized microcaps (Drummond, 20 microliters, 1¼% volumetric tolerance), and shaken immediately with 5.0 ml Drabkins reagent (Sigma Chemicals). Within 10 min, each solution was poured into a spectrophotometer tube and the color read in a spectrophotometer at 540 nm. Determinations were made in duplicate. Reference standards used were by Hycel.

General animal care

Animals were caged individually in stainless steel, mesh-bottomed cages. Each animal was given the diet (Table 1) in a glass jar; all animals had free access to distilled water. Iron/apple supplements were administered in glass castor cups that had been rinsed with distilled water. The temperature of the animal room ranged from 21–29°C throughout the experiment except for 1 day when the temperature reached 32°C. Weights of animals and food intakes were determined three times per week. Food intakes took into account spills collected from beneath the cages. About 30% of the animals developed diarrhea at some point in the experiment. All but one of these diarrhetic animals were on low-iron diet regimes. Cages were kept clean and changed as necessary.

Calculations

Gain or loss in hemoglobin-iron (Hb-iron) over the repletion period was calculated for each animal on the assumption that blood volume = 5.56% of body weight for Sprague-Dawley rats (Everett et al., 1956) and that hemoglobin is 0.34% iron by weight.

Regression of net gain in Hb-iron (mg) on iron intake (mg) was calculated for the four standard groups and the four test groups. In the case of the four standard groups, each group's mean iron intake in mg was used as the fixed x-value for every animal in that group. Both lines were tested for assumption of linearity using the analysis of variance technique described by Chiang and Selvin (1974) with an alpha level = 0.05.

Iron conversion efficiency, or the percentage of iron intake converted to Hb-iron, was calculated by dividing the net change in Hb-iron in mg by iron intake in mg and multiplying by 100.

Relative biological value (RBV) was calculated using the graphic technique (Pla and Fritz, 1970). The least squares estimate for a straight line was calculated using the 34 $FeSO_4$ standard data pairs where y = iron intake in mg and x = change in Hb-iron in mg. The 95% confidence band for this least squares line was also calculated (Fig. 4) using techniques described by Chiang and Selvin (1974). The intake of iron as ferrous sulfate that produced the same gain in hemoglobin-iron as each of the three test doses of iron/apple was determined using this regression line with its 95% confidence limits, and the following formula was used to calculate RBV for each test dose:

$$RBV = 100 \times \frac{\text{mg Fe intake from test dose}}{\text{mg Fe intake from standard that gave the same change in Hb-iron}}$$

RESULTS

Iron/apple production

When a nail was inserted into an apple and removed some hours later, a brown-black area of about 0.25 cm was found around the wound. When dry-ashed, this same area appeared reddish brown in color. No such area appeared when an apple was pierced with a wooden stick sharpened to a point. Iron analyses of apples with nails inserted at room temperature showed the increase in iron content to vary directly with both metallic surface area and time of exposure (Fig. 1 and 2).

Bioavailability of iron/apple

Table 3 shows the results of the hemoglobin repletion test. Groups on zero added iron (basal groups, Table 2) both showed a net loss in Hb-iron whereas all other groups displayed a gain that was positively correlated with iron intake. Graphical representation (Fig. 3) showed a positive correlation between net gain in Hb-iron and total iron intake for both the standard ($r^2 = 0.97$) and test ($r^2 = 0.95$) groups. Statistical test showed assumption of linearity to be valid for the regression of gain in Hb-iron on iron intake as $FeSO_4$ (Table 4) but invalid in the case of the 32 iron/apple data pairs (Table 5). The effect of small amounts of untreated apple intake on weight gain and Hb gain were insignificant (Student's t-test, $\alpha = 0.05$) for iron-depleted animals and positive control animals that were never depleted of iron (Table 6).

The range for the conversion efficiency of iron sulfate was 46–50% (Table 3) and compares well with other studies (Miller, 1977; Mahoney et al., 1974). The conversion efficiency of iron/apple was greater than that of $FeSO_4$ in each case showing 74% in the low level supplement group and 57% and 56% for the medium and high supplement groups, respectively. Mahoney et al. (1974) have shown the efficiency of converting dietary iron to Hb-iron remains constant over the range of iron intakes from 0.12–0.5 mg/day when fed ad libitum. However, Smith and Otis (1937) showed a linear relationship between hemoglobin response and iron intake only for doses below

Table 2—Iron intake of $FeSO_4$ (standard) and iron/apple (test) groups

	Group	Number of animals	Iron: Addition basal diet[a] (mg/kg)	Iron: Apple supplement[b,c] (mg/cube)	Total iron intake for 12-day repletion[c] (mg)
Standard ($FeSO_4$)	Basal	9	0	0	0
	Low	8	6	0	0.7 ± 0.13[d]
	Medium	9	12	0	1.6 ± 0.21[d]
	High	8	24	0	3.4 ± 0.29[d]
Test (iron/apple)	Basal	7[e]	0	<0.01	<0.08
	Low	8	0	0.22 ± 0.0080[f]	1.3 ± 0.020
	Medium	9	0	0.38 ± 0.011[f]	2.3 ± 0.027
	High	8	0	0.57 ± 0.043[f]	3.4 ± 0.11

[a] Iron as $FeSO_4 \cdot 7H_2O$, diets fed ad libitum.
[b] Iron as apple/iron in frozen cubes, one cube fed every other day.
[c] Mean and standard deviation.
[d] Calculated directly with diet intake data.
[e] One animal died during repletion.
[f] Number of frozen cubes analyzed for basal, low, medium, and high were 6, 6, 4, and 8, respectively.

0.25 mg iron/day when rats consumed the whole day's supply of iron within a 1-hr period. The medium and high iron/apple doses in this experiment were both above 0.25 mg iron and were very likely not on the linear portion of the dose response curve established by Smith and Otis (1937) for these feeding conditions. The 95% probability of nonlinearity for these data (Table 5) support this hypothesis. The relative biological values calculated by the graphic technique (Fig. 4) are shown in Table 7 as the 95% confidence limits. The RBV as measured in the low level supplement group was in the range 132–152 (RBV standard: $FeSO_4$ = 100). RBV values for the medium and high doses of iron/apple were less, ranging from 93–112 or about equal to that of ferrous sulfate.

It is clear that both standard and test groups of animals responded similarly in both hemoglobin and weight gain, placing the bioavailability of iron/apple in the same range as that of ferrous sulfate. However, the differing methods of iron presentation due to the physical and chemical limitations mentioned above makes the direct mathematical comparison of the two sets of data inappropriate. A 56–74% conversion efficiency ratio denotes this iron/apple to be of relatively high bioavailability (Mahoney et al., 1974), and although a precise value for RBV is not available from these data, by the criteria of Pla and Fritz (1970) this form of iron falls into the category of good sources of iron, i.e., RBV ⩾ 70.

DISCUSSION

MOST ORGANIC SALTS of iron show an availability similar to that of ferrous sulfate, i.e., RBV = 100, and inorganic compounds of iron generally show a similar or lower RBV than ferrous sulfate (Pla and Fritz, 1970). The RBV of elemental iron powders is reported in the range 10–60 and appears to be at least partly dependent on particle size and method of iron reduction (Pennell et al., 1975; Motzok et al., 1978). The absorptive properties of iron chelates have been studied in various experimental animals and in man. Several, but not all, of the Fe (III) chelates studied so far have shown absorptive rates similar to or greater than that

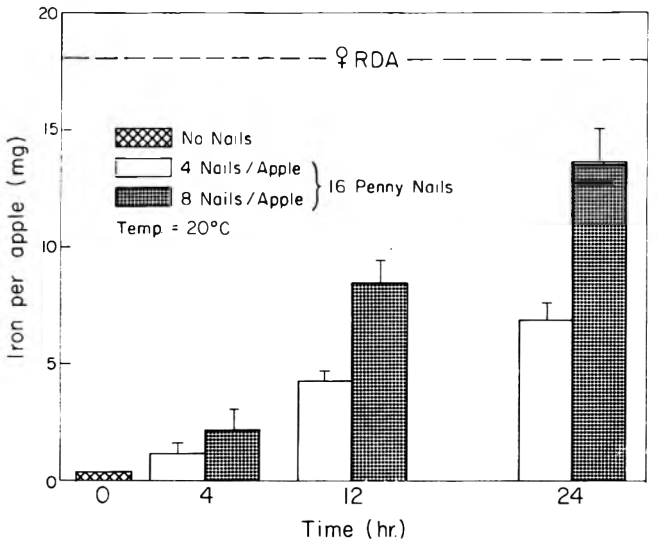


Fig. 1—Effect of insertion time and number of nails on total iron per apple. T *denotes ± one std deviation.*

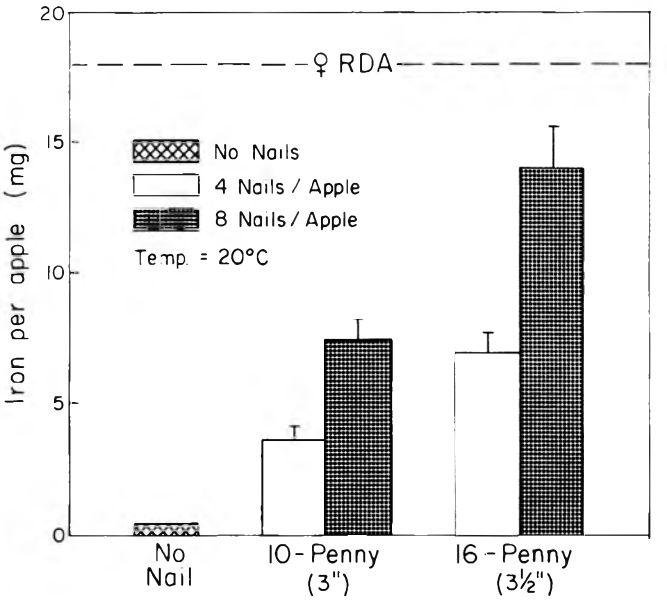


Fig. 2—Effect of nail size (surface area) on total iron per apple (24-hr treatment). T *denotes ± one std deviation.*

Table 3—Initial and final weights and hemoglobin values for repletion period, calculated change in hemoglobin-iron, and change in hemoglobin-iron as percent of iron intake (conversion efficiency) for $FeSO_4$ (standard) and iron/apple (test) groups

Group code	No. of animals	Iron intake[a] (mg)	Weight[a] Initial (g)	Weight[a] Final (g)	Hemoglobin[a] Initial (g/dl)	Hemoglobin[a] Final (g/dl)	Gain or loss in Hb-Iron[a] (mg)	% of iron intake converted to Hb-Iron[b,a]
			Ferrous sulfate standard					
Basal	9	0	111 ± 21	129 ± 28	3.6 ± 0.49	2.8 ± 0.24	−0.057[c] ± 0.079	–
Low	8	0.70 ± 0.13	106 ± 18	147 ± 28	3.6 ± 0.48	3.8 ± 0.46	0.32 ± 0.15	46[c] ± 13
Medium	9	1.6 ± 0.21	108 ± 16	164 ± 26	3.5 ± 0.41	4.6 ± 0.49	0.73 ± 0.16	47 ± 6.9
High	8	3.4 ± 0.29	98.4 ± 17	173 ± 17	3.6 ± 0.43	8.0 ± 1.1	1.9 ± 0.41	56 ± 9.9
			Iron/apple tests					
Basal	7	0.05 ± 0.0054	98.6 ± 21	110 ± 29	3.6 ± 0.6	2.96 ± 0.36	−0.051 ± 0.088	–
Low	8	1.3 ± 0.02	102 ± 12	168 ± 13	3.6 ± 0.5	5.2 ± 0.56	0.96 ± 0.14	74 ± 10.6
Medium	9	2.3 ± 0.027	109 ± 10	189 ± 13	3.5 ± 0.47	5.7 ± 0.53	1.3 ± 0.16	57 ± 6.9
High	8	3.4 ± 0.11	106 ± 15	187 ± 22	3.6 ± 0.52	7.4 ± 1.0	1.9 ± 0.35	56 ± 10.6

[a] Mean ± standard deviation.
[b] Conversion efficiency.
[c] Calculated from individual values.

of ferrous sulfate (Christopher et al., 1974; Pollycove et al., 1972). In absorptive properties, the iron/apple of this study would best be categorized with the iron chelates or ferrous salts. The production of an iron chelate or chelates or the conversion of reduced iron to the highly available ferrous ion form via this corrosion process are possibilities worth further investigation.

Toxicity of iron/apple must be considered. The possibility of iron overload from the ingestion of apples too long exposed to steel is now being investigated and will be discussed in a forthcoming paper. The amount of non-iron metals in various steels is very small (NBS 260) and their rate of corrosion into apple from steel has not been measured. The possibility of toxic elements present in iron/apple is small but should be investigated before conclusions on the safety of this product can be made.

CONCLUSION

APPLES having contact with reduced iron show both an iron nutrient density and content that are one to two orders of magnitude greater than those of other common foods (Table 8). This study has shown that this corrosion of iron into apples can not only generate a dramatic increase in food iron but also produces a form of iron showing good bioavailability (Table 9). It can be assumed that

Table 4—Analysis of variance testing assumption of linearity for regression of gain in Hb-iron (mg) on iron intake (mg) as ferrous sulfate[a]

Source	SS	d.f.	MS
Regression	18.16682675	1	18.1668275
About	0.1320273	2	0.0660136
Within	1.578917	30	0.05263056
Residual	1.710944	32	0.053467
Total	19.87777	33	

[a] The mean iron intake of each group was used as the fixed x value for every animal in that group. H_0: $E(y/x) = a + bx$; H_1: $E(y/x) \neq a + bx$; $\alpha = 0.05$.
Critical point: $F_{(2,30).95} = 3.32$.
$F = \frac{\text{about regression}}{\text{within regression}} = \frac{0.0660136}{0.0526305} 1.25$, accept H_0.

Table 5—Analysis of variance testing assumption of linearity for regression of gain in Hb-iron (mg) on iron intake (mg) as iron/apple[a]

Source	SS	d.f.	MS
Regression	14.314	1	14.314
About	0.446138	2	0.223069
Within	1.247862	28	0.0445665
Residual	1.694	30	0.0564666
Total	16.008	31	

[a] H_0: $E(y/x) = a + bx$; H_1: $E(y/x) \neq a + bx$; $\alpha = 0.05$.
Critical point: $F_{(2,28).95} = 3.34$.
$F = \frac{0.223069}{0.0445665} = 5.0053$, reject H_0.

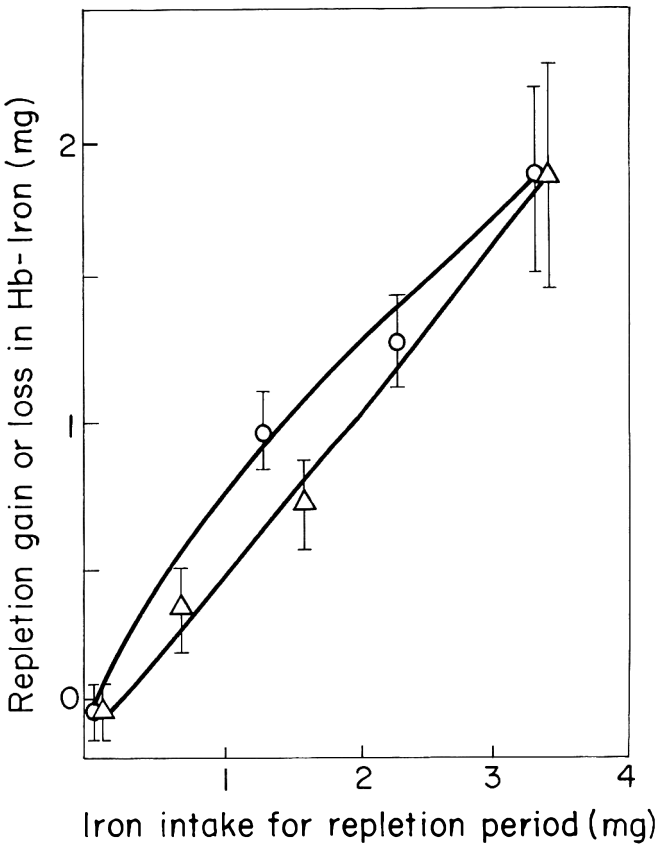


Fig. 3—Effect of iron intake on change in Hb-iron for iron supplied as $FeSO_4$ (Δ) and as iron/apple (○). Vertical lines denote std deviation.

Table 7—Biological values for three iron/apple test doses relative to the standard ($FeSO_4$)

Test group	Iron intake of test group (mg)	Intake of standard giving same response as test dose[a] (mg of Fe) Low	High	Relative biological value of iron/apple (%)
Low	1.3	1.71	1.98	132 – 152
Medium	2.3	2.24	2.57	97 – 112
High	3.4	3.16	3.64	93 – 107

[a] Upper and lower values of 95% confidence interval.

Table 6—Test of positive and negative control groups fed or not fed untreated apple for difference in gain in hemoglobin concentration and weight

	Adequate diet		Iron-deficient diet	
	+ Untreated apple fed every other day	No supplement	+ Untreated apple fed every other day	No supplement
n	8	8	7	9
Repletion weight gain (g)	78.2 ± 6.31	75.9 ± 6.63 NS	11.7 ± 9.7	18.3 ± 7.6 NS
Gain in Hb concentration (g/dl)	1.4 ± 0.99	1.1 ± 0.76 NS	−0.66 ± 0.33	−0.82 ± 0.28 NS

NS: No significant difference by t-test (Chiang and Selvin, 1974).

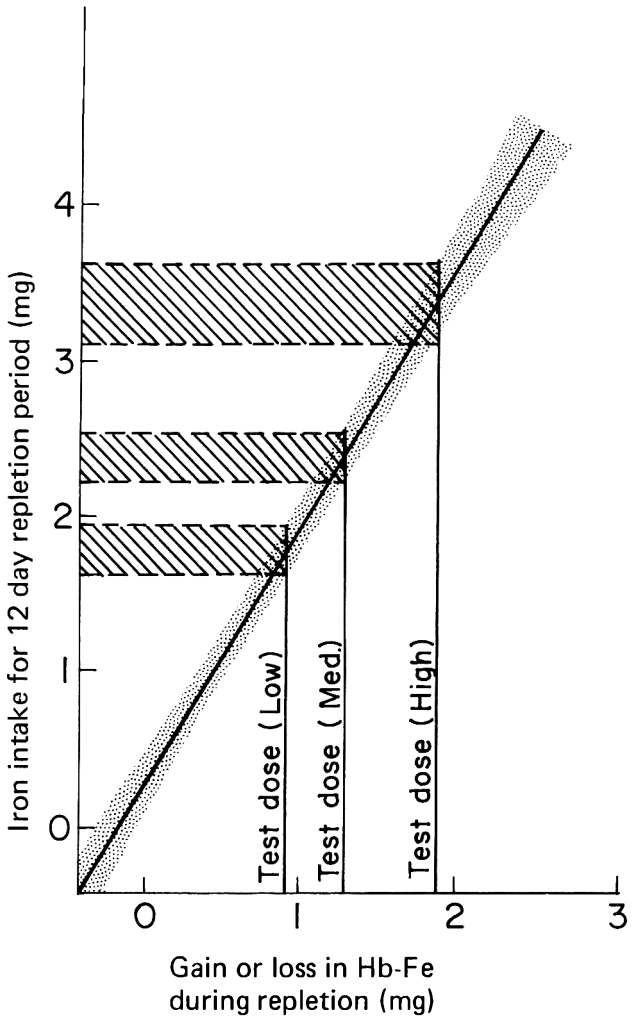


Fig. 4—Regression line with 95% confidence band for iron intake as a function of change in Hb-iron for standard ($FeSO_4$) groups. Used to calculate intake of $FeSO_4$ that showed the same gain in Hb-iron as three test doses of iron/apple.

ingestion of apples in contact with plain steel or cast iron during cooking or processing may contribute significant amounts of nutritionally available iron to the diet.

REFERENCES

AACC. 1969. "Approved Methods of the AACC," Method 40-41, approved 1960. American Association of Cereal Chemists, St. Paul, MN.

Adams, C.F. 1975. Nutritive value of American foods in common units. Agriculture Handbook No. 456, Agricultural Research Service, USDA, Washington, DC.

Burroughs, A.L. and Chan, J.J. 1972. Iron content of some Mexican-American foods. J. Am. Dietet. Assoc. 60(2): 123.

Chiang, C.L. and Selvin, S. 1974. "Biology and Public Health Statistics, 160 ABC." Dept. of Public Health, Univ. of California, Berkeley.

Christopher, J.P., Hagenauer, J.C., and Saltman, P.D. 1974. Iron metabolism as a function of chelation. In "Trace Element Metabolism in Animals—2," Ed. Hoekstra, W.G., Suttie, J.W., Ganther, H.E., and Mertz, W., p. 133. University Park Press, Baltimore, MD.

Cohen, N.L., Reyes, P., Typpo, J.T., and Briggs, G.M. 1967. Vitamin B-12 deficiency in the Golden Hamster. J. Nutr. 91(4): 482.

Crosby, W.H., Munn, J.I., and Furth, F.W. 1954. Standardizing a method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5(5): 693.

Devadas, R.P., Chandrasekhar, U., and Kumari, K.S. 1973. Availability to school children of iron from amaranthus cooked in two different utensils. Ind. J. Nutr. Dietet. 10: 223.

Everett, N.B., Simmons, B., and Lasher, E.P. 1956. Distribution of blood (Fe59) and plasma (I131) volumes of rats determined by liquid nitrogen freezing. Circ. Res. 4(4): 419.

Fritz, J.C., Pla, G.W., Roberts, T., Boehne, J.W., and Hove, E.L. 1970. Biological availability in animals of iron from common dietary sources. J. Agric. Food Chem. 18(4): 647.

MacKay, H.M.M., Dobbs, R.H., and Bingham, K. 1945. The effect of national bread, of iron medicated bread and of iron cooking utensils on the hemoglobin level of children in war-time day nurseries. Arch. Dis. Child. 20: 56.

Mahoney, A.W., VanOrden, C.C., and Hendricks, D.G. 1974. Efficiency of converting food iron into hemoglobin by the anemic rat. Nutr. Metab. 17(4): 233.

Miller, J. 1977. Study of experimental conditions for most reliable estimates of relative biological value of iron in bread. J. Agric. Food Chem. 25(1): 154.

Monsen, E.R., Kuhn, I.N., and Finch, C.A. 1967. Iron status of menstruating women. Am. J. Clin. Nutr. 20(8): 842.

Moore, C.V. 1965. Iron nutrition and requirements. Ser. Haematol. 6: 1.

Morris, E. 1974. Nutritional significance of trace elements in wheat. Bakers Digest 48(6): 32.

Motzok, I., Davies, M.I., Vermas, R.S., and Pennell, M.D. 1977. Biological availability of iron from foods and tonics containing various iron supplements. Nutr. Rep. Inter. 15(4): 459.

Motzok, I., Verma, R.S., Chen, S., Rasper, J., Hancock, R.G.V., and Ross, H.V. 1978. Bioavailability, in vitro solubility, and physical and chemical properties of elemental iron powders. J. Assoc. Off. Anal. Chem. 61(4): 887.

National Bureau of Standards. 1975-76. Catalog of NBS Standard Reference Materials. NBS Special Publication 260. Office of Standard Reference Materials, Institute for Materials Research, National Bureau of Standards, Washington, DC.

Pennell, M.D., Wiens, W., Rasper, J., and Motzok, I. 1975. Factors affecting the relative biological value of food grade elemental iron powders for rats and humans. J. Food Sci. 40(4): 879.

Pla, G.W. and Fritz, J.C. 1970. Availability of iron. J. Assoc. Off. Anal. Chem. 53(4): 791.

Pla, G.W. and Fritz, J.C. 1971. Collaborative study of the hemoglobin repletion test in chicks and rats for measuring availability of iron. J. Assoc. Off. Anal. Chem. 54(1): 13.

Pollycove, M., Saltman, P., Fish, M., Newman, R., and Tono, M. 1972. Ferric fructose absorption in man. Abstr. Western Soc. Clin. Res., Carmel, California.

Rosanoff, A. and Kennedy, B.M. 1979. Scientific efficacy of a folk remedy for iron deficiency anemia. Fed. Proc. 38(3): 454.

Sharon, G.S. 1972. Of (iron) pots and pans. Nutrition Today, March/April, p. 34.

Smith, M.C. and Otis, L. 1937. Hemoglobin regeneration in anemic rats in relation to iron intake. J. Nutr. 13(6): 573.

Walker, A.R.P. and Arvidsson, U.B. 1953. Iron "overload" in the South African Bantu. Trans. R. Soc. Trop. Med. Hyg. 47(6): 536.

Ms received 4/28/81; revised 10/9/81; accepted 10/12/81.

Presented at the 63rd Annual Meeting of the Federation of American Societies for Experimental Biology, Dallas, TX, April 1—11, 1979.

The authors thank Mr. Marc Schelstraete for excellent technical assistance on the iron analyses.

This research was supported from USDA Agricultural Experiment Station Project #3790 and a grant from the Feedstuffs Processing Company through the Univ. of California, Berkeley, CA.

Table 8—Ion concentration and nutrient density of some common foods[a]

Food	Iron conc (μg/g, wet wt)	Nutrient density[b] (mg/100 kcal)	Iron per serving (mg)
Fried liver	88	3.8	7.5
Boysenberries	12	3.3	2.0
Green peas (frozen)	20	2.8	1.4
Raisins	35	1.2	1.5
Apples	3	0.5	0.5
Milk	0.4	0.06	0.1
Iron-treated apples	10 – 100	2 – 20[c]	1 – 15

[a] Calculated from Adams (1975).

[b] $\frac{\text{♀ RDA Fe}}{\text{♀ RDA kcal}} = \frac{18 \text{ mg Fe}}{2000 \text{ kcal}} = \frac{0.9 \text{ mg Fe}}{100 \text{ kcal}}$

[c] Assumes 100g apples contains 50 Kcal.

Table 9—Relative biological value of the iron in some common foods[a]

Food	RBV in rats
Cereals enriched wth $FeSO_4$	100
Whole wheat	65, 81
Cereals enriched with electrolytic Fe	33–54
Wheat germ	53
Egg yolk	33
Cereals enriched with $FePO_4$	15
Nails-in-apples	> 93

[a] From Fritz et al. (1970); Morris (1974); Motzok et al. (1977).

Prediction of Time Correction Factor for Come-up Heating of Packaged Liquid Food

JORGE SUCCAR and KAN-ICHI KAYAKAWA

ABSTRACT

All physical properties and operational conditions which have an influence on the come-up heating correction factor (C_f), of packaged liquid food were examined and subjected to a dimensional analysis. Five dimensionless groups which uniquely define the correction factor were obtained. The influence of the parameters on the values of C_f was studied through application of a central composite experimental design and a nonlinear regression analysis, from which a regression equation for the prediction of the correction factor was obtained. It was found that the C_f values may vary between +0.86 to −0.37, having an average value of 0.31. Mathematical examination of the regression equation obtained indicates that a dimensionless group which is related to the temperature history of the heating medium during the come-up phase is the most important in determining the value of C_f, while another which is a function of the processing time has a negligible influence on this parameter. The reliability of our regression equation was verified experimentally with distilled water, 0.1% locust bean gum and tomato soup packed in cans 211 X 300 and 307 X 409. Good agreement between experimental and predicted C_f values was observed.

INTRODUCTION

DURING MOST heat sterilization procedures, the heating period includes a *Come-up time,* which is defined as the time for the temperature of the retort to reach a constant holding temperature after steam is introduced. Recommended processing times are usually defined on the basis of a negligible come-up period; therefore, determination of an adequate thermal process time requires a reliable estimation of the process lethality attained during this come-up phase. The task may be accomplished by the application of a *correction factor of the come-up heating,* C_f. Since Ball (1923) reported that the empirical C_f for the come-up heating for conduction heating was 0.42, this value has been widely presumed to be constant in industrial processes. However, Hayakawa and Ball (1971) pointed out that the C_f could depend upon operational conditions and thermal properties of the food sample, thus variations in processing conditions will yield corresponding changes in the values of C_f.

In the present study the correction factor is defined as the dimensionless larameter introduced by Uno and Hayakawa (1980):

$$C_f = \frac{t_x - (t_p - t_u)}{t_u} \qquad (1)$$

where: t_p = processing time in a process with a come-up phase; t_x = equivalent process time in a standard process with a negligible come-up phase, which makes the sterilizing value of this process identical to that of the process with come-up phase; and t_u = come-up time.

Uno and Hayakawa (1980) developed regression equations for the prediction of the C_f value of the come-up heating based on critical point in cylindrical cans of foods heating by conduction. However, there are no published procedures available for in-plant determination of the correction factor for the come-up heating of convectively heating products, including liquid products subjected to mechanical agitation.

Succar (1980) mathematically examined the physical properties and operational conditions which have an influence on the value of the correction factor for liquid foods, and established a computerized procedure to determine this parameter.

The present work was undertaken to theoretically examine the influence of these physical quantities on C_f values and to develop a theoretical regression equation for the prediction of the correction factor of packaged liquid food subjected to mechanical agitation.

Mathematical determination of C_f

As observed by Succar (1980), the mathematical procedure for the estimation of a correction factor requires the following equations (All symbols used are defined in the nomencature):

(1) Estimation of the liquid temperature T_{v2} and T_s, when the heating medium temperature T_1, remains constant:

$$T_s = T_1 - (T_1 - T_o)\exp(-t/c) \qquad (2)^1$$

$$T_{v2} = T_1 - (T_1 - T_g)\exp(-t/c) \qquad (3)$$

where

$$C = v\rho C_v/UA \qquad (3a)$$

T_s = product temperature during a standard process, as a function of time; T_o = initial product temperature; T_{v2} = product temperature during the holding period of a process with a come-up phase; and T_g = product temperature at the end of the come-up phase.

(2) Estimation of the liquid temperature when the heating medium temperature varies during the come-up:

$$\frac{dT_{vl}}{dt} = \frac{1}{C}[T_{cu} - T] \qquad (4)$$

where $$T_{cu} = (T_1 - m_o)(t/t_u)^m + m_o \qquad (4a)$$

m_o = initial heating medium temperature, and m is an empirically determined parameter for the come-up process in question.

(3) Estimation of the equivalent process time (t_x), at a reference temperature T_r, such that the variable process with a come-up heating of t_u and a total processing time of t_p, and a step functional process of time t_x, with a negligible come-up, have identical sterilizing vlaues:

$$\int_0^{t_x} 10^{(T_4 - T_r)/z}dt = \int_0^{t_u} 10^{(T_{v1} - T_r)/z}dt + \int_0^{t_p} 10^{(T_{v2} - T_r)/z}dt \qquad (5)$$

Eq (2) and (3) are solved analytically. Eq (4) may be solved analytically when the empirical constant m = 1. For $m \neq 1$ it may be solved numerically by application of integration techniques such as Runge Kutta of order fourth as is done here (Hildebrand, 1956). Eq (5) was solved numer-

Authors Succar and Hayakawa are affiliated with the Food Science Dept., Cook College, Rutgers Univ., P.O. Box 231, New Brunswick, NJ 08903.

ically by using Simpson's rule (Anonymous, 1964), for narrow time intervals while t_x is estimated through a five point inverse interpolation technique (Salzer, 1944). Once t_x is calculated, the correction factor is determined by entering this value into Eq (1).

For convenience we assumed that $T_r = T_1$ in Eq (5), where T_1 is the heating medium temperature.

Dimensional analysis

Careful examination of the system of equations shows that eight independent physical quantities are required for the estimation of a correction factor: T_1, T_o, m_o, t_u, t_p, z and the empirical factors m and C. There are three primary dimensions associated with these physical quantities: temperature (°C), temperature differential (C°), and time (min).

Through application of Buckingham-π theorem (Shack, 1965) we have:

Number of dimensionless groups required = p − r = 5 where p = number of physical quantities and r = number of primary dimensions.

Application of the algorithm for a dimensional analysis, or inspection to our system of equations, yields the following five dimensionless parameters S_{ki} which uniquely define the correction factor:

$$S_{k1} = (T_1 - m_o)/z \quad (6)$$
$$S_{k2} = C/t_u \quad (7)$$
$$S_{k3} = (T_1 - T_o)/z \quad (8)$$
$$S_{k4} = (t_p - t_u)/C \quad (9)$$
$$S_{k5} = m \quad (10)$$

Experimental design

The influence of each group on the correction factor C_f was then examined through application of a factorial central composite experimental design (Davies, 1978). For this analysis the five dimensionless groups are treated as independent variables, and C_f as a dependent value. The experimental design consists of combining factors at two levels, and star point combinations, again at two levels, with the central value point combination to the five factors. For each of these combinations a value of C_f was determined through the use of a computer program for the estimation of C_f values of packaged liquid food (Succar, 1980). In the factorial design, each S_{ki} has two levels, designated at +1 (larger value) and as −1 (smaller value), thus the number of all the possible combinations of the five factors at two levels is given by $2^n = 32$. The star point design forms a set of additional points, where the level of each parameter is selected through the use of an interval of ± A which is given by:

$$A = 2^{n/4} = 2.3784 \text{ for } n = 5 \quad (11)$$

Each star point represents a combination of factorial levels, where the level of only one factor is either +A or −A, and those of all others are at mid-level (0) of its +1 and −1 levels. Therefore the total number of combinations of these additional points is 2 n = 10.

A third and last component of the experimental design consists of one center point at which the level of each factor is at mid-level o. The total number of experimental design points is given by: $2^n + 2n + 1 = 43$ experiments.

After examining commercial heat processing as reported by Stombo, 1973; Lopez, 1975; NCA, 1976, maximum and minimum values of the eight physical quantities as well as those values most likely to be found in commercial procedures were established (Table 1).

From the values of Table 1, maximum, frequent, and minimum levels for the five dimensionless groups were determined. These values (+A, 0, −A) are shown in Table 2.

The maximum and minimum values for S_{k5} were selected through the examination of actual process histories of heating medium temperatures during come-up periods.

The maximum and minimum values of each dimensionless parameter must be equal to +A and −A respectively, in order to apply the experimental design to the region encountered in practice. Therefore, the values of the S_{ki}'s must be properly transformed. Since it is desirable to emphasize the experimental design around those values of commercial interest (0 level), the variable space was condensed by using exponential transformation for each of the five parameters.

The transformation of the dimensionless groups into the five experimental design levels is shown in Eq (11) through (15):

$$X_1 = -7.4766 \exp(-0.25\, S_{k1}) + 2.3889 \quad (11)$$
$$X_2 = -5.1233 \exp(-1.71\, S_{k2}) + 2.3783 \quad (12)$$
$$X_3 = -5.4959 \exp(-0.30\, S_{k3}) + 2.3806 \quad (13)$$
$$X_4 = -4.7568 \exp(-0.03\, S_{k4}) + 2.3784 \quad (14)$$
$$X_5 = 1.2586 \exp(1.60\, S_{k5}) - 3.8533 \quad (15)$$

The values of S_{ki} for the five experimental design levels obtained by solving Eq (11) through (15), for X_i = A, +1, 0, −1, and −A respectively are shown in Table 2.

A computer program was developed to estimate the physical property values and operational conditions which satisfy the level of the statistical design variables for each of the 43 experiments (Succar, 1980). These quantities were then used for the estimation of the correction factor for each design combination of the five dimensionless groups. The values of C_f obtained vary between +0.3070. Out of the 43 correction factors obtained, six (14%) had a negative value.

A nonlinear regression analysis (Barr et al., 1976) was applied in order to evaluate the influence of these five dimensionless parameters on C_f, as well as to obtain a regression equation for the prediction of this parameter. For our analysis we used linear, quadratic, and cubic terms. The equation obtained has a correlation coefficient of 0.999990 and sums of squared residuals of 0.3345×10^{-4} with seven degrees of freedom.

— Continued on next page

Table 1—Maximum and minimum values of dimensional parameters for determining the correction factor of liquid foods

Physical quantities	Minimum value	Most frequent value	Maximum value
T_1 (°C)	90.0	95.0	121.1
T_o (°C)	5.0	65.0	82.0
C (min)	0.4	1.3	6.5
z (C°)	4.4	10.6	16.7
m_o (°C)	4.4	45.0	60.0
t_u (min)	0.5	3.0	10.0
t_p (min)	t_u	$5 \times t_u$	$20 \times t_u$
m	0.1	0.7	1.0

Table 2—Nominal values of five dimensionless parameters which correspond to five design levels

	Values of dimensionless parameters corresponding to the following design levels				
Parameter	−A	−1	0[a]	+1	+A
S_{k1}	1.800	3.165	4.564	6.733	26.277
S_{k2}	0.043	0.244	0.449	0.768	13.029
S_{k3}	0.480	1.620	2.789	4.605	26.151
S_{k4}	0.000	11.406	23.105	41.288	459.360
S_{k5}	0.100	0.512	0.700	0.844	1.000

[a] Frequent S_{ki} values

All nonsignificant terms were eliminated for further regression analysis in order to obtain a algebraic equation with fewer terms. The final regression equation obtained is shown below:

$$\begin{aligned} C_f = {} & 0.32439 - 0.11368.X_1 - 0.00977.X_2 + 0.15583.X_3 \\ & + 0.00165.X_4 - 0.0496.X_5 - 0.03547.X_1.X_1 \\ & - 0.01213.X_2.X_2 - 0.90515.X_3.X_3 \\ & + 0.00331.X_4.X_4 + 0.03223.X_5.X_5 - 0.03713.X_1.X_2 \\ & + 0.02126.X_1.X_3 - 0.00491.X_1.X_5 \\ & + 0.05448.X_2.X_3 - 0.01249.X_2.X_5 + 0.00916.X_3.X_5 \\ & - 0.01673.X_1.X_1.X_1 + 0.00701.X_2.X_2.X_2 \\ & + 0.01413.X_3.X_3.X_3 - 0.00165.X_4.X_4.X_4 \\ & - 0.01112.X_5.X_5.X_5 + 0.01617.X_1.X_2.X_3 \\ & - 0.00591.X_1.X_2.X_5 + 0.00671.X_2.X_3.X_5 \\ & - 0.00108.X_1.X_3.X_5 \end{aligned}$$

The correlation coefficient of equation 16 is 0.999990. In order to use the equation, the vaues of S_{k1} through S_{k5} should be substituted in Eq (11) through (15) respectively, to convert them into the S_i values that appear in this expression.

EXPERIMENTAL

TWO CAN SIZES wer eused for our experiments: 211 x 300 and 307 x 409. Temperatures were sensed by using copper and constantan thermocouples, 0.02 in diameter, 0.006 in. teflon covered single wire. In order to place the thermocouple junctions inside the can, the two wires were inserted through opposite sides of the can wall, welded in place, then positioned at the desired locations. Epoxy resin was used to tightly seal the small holes in the can wall, and to fix the thermocouple junction at the prescribed location. Up to five thermocouples were placed in the 307 x 409 can and three thermocouples in the smaller size 211 x 300. The approximate location of these junctions is shown in Fig. 1. The headspace in all cans was 10% of its internal height.

The cans were filled with distilled water, 0.1% aqueous solution of locust bean gum, and content of canned tomato soup packaged by Campbell Soup Co.

Since sample consistency greatly influences convection heat transfer, a Brookfield Synchro-Electric Viscometer (Model HAT) was used to determine the apparent viscosity of the locust bean gum solution and the tomato soup. Total solids and density of tomato soup were determined by using appropriate AOAC methods.

In order to assure uniform temperature distribution throughout the can contents, a precision shaker bath, Fisher Sc. Co. (Model 25) of 17 liters capacity was utilized. The system was adjusted so that come-up times ranging from 2 to 10 minutes could be simulated (Succar, 1980). An almost step functional process was obtained by suddenly submerging a can at an initial temperature (T_o) into the water bath at a holding temperature T_1, rapidly followed by the start of the mechanical agitation. Rates of agitation selected were 120 strokes/min and 140 strokes/min for cans 211 x 300 and 307 x 409 respectively (1 stroke = horizontal displacement of a can in two opposite directions of length 3.7 cm each). At these rates of agitation, a uniform temperature distribution of the liquid product was obtained.

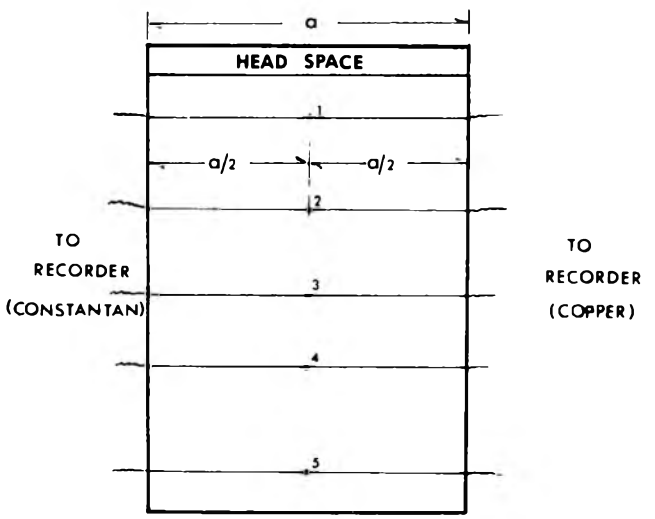


Fig. 1—Thermocouple junctions in cans 307 x 409 and 211 x 300. Height of liquid content = h. Location 1 — 0.90h; location 2 — 0.70h; Location 3 — 0.50 h. Location 4 — 0.33h; Location 5 — 0.10 h. Can size 307 x 405: Locations 1, 2, 3, 4, and 5. Can size 211 x 300, Locations 1, 3, and 5.

RESULTS & DISCUSSION

THE SHEAR-STRESS responses of 0.1% aqueous solution of locust bean gum and tomato soup are shown in Table 3. It is apparent from this table that the gum solution and tomato soup are dilatant and pseudoplastic liquids respectively. Water is known to be Newtonian.

The values of density, total solids content, and fill volume which characterize the products used in our experiment are shown in Table 4.

Each experiment consists of a come-up process and a step functional process. Although each experiment run was carefully executed, some differences between the holding temperatures of the variable and step functional processes were found, in addition to differences between initial temperature (T_o) for both processes. Since dimensionless temperature ratios are virtually independent of initial food temperature and holding medium temperature, according to our preliminary tests, the liquid temperature [$T_{s(t)}$] actually measured during the step functional process was modified so that it would match the initial temperature of the liquid and the holding temperature T_1 of the come-up heating.

This adjustment was accomplished through application of the following equation:

$$T_{sm(t)} = (T_1)_v - [(T_1)_v - (T_o)_v] \cdot \frac{(T_1)_s - T_s(t)}{(T_1)_s - (T_o)_s} \qquad (17)$$

The adjusted liquid temperatures for the step functional process together with the values obtained for the process

Table 3—Apparent viscosity[a] as a function of shear-stress of the liquid samples used

	Apparent viscosity (g/cm-S) at (rpm)					
Product	2.5	5.0	10.0	20.0	50.0	100.0
0.1% Locust bean gum	—	—	0.080	0.100	0.168	0.205
Tomato soup	19.2	11.3	6.07	3.93	2.16	1.40

[a] Measurements were performed at 23.6°C.

Table 4—Some physical constants of the sample liquids used during the experimental procedures[a]

Product	Density[b] (g/ml)	Total solids content (%)
Distilled H_2O	0.9698	0.0
0.1% locust bean gum	0.9698	0.1
Tomato soup	0.9425	9.0

[a] Fill volume in can size 211 x 300 = 220 ml and in the can size 307 x 409 = 550 ml, measured at 88°C.
[b] Determined at 83°C.

with come-up were used to determine a correction factor as described previously.

In addition T_I, T_o, m_o, t_u, t_p, and z which are directly collected form the experimental data, the theoretical prediction of a correction factor requires the estimation of m and C [Eq (7) and (10), respectively]. The parameter m is obtained through a method of least square fit, (Uno and Hayakawa, 1980), while C is determined experimentally as f_h from the plot of heat penetration data:

$$C = \frac{f_h}{\ln 10} \qquad (18)$$

Experimental correction factors were determined through linear interpolation of the equivalent process time (t_x) at which both the variable process with a come-up, and the step functional process had identical sterilizing values at a given time t_p. Once t_x is known a C_f value is determined through application of Eq (1).

Experimental values of C_f were compared against our model regression equation for the estimation of the correction factor in liquids [Eq (16)]. These values are shown in Table 5.

An overall deviation of 8.6% from that of the experimental value was observed. However, we observe that the smaller can size results in the larger deviation. Errors in the experimental measurement of the temperatures are more serious in smaller cans due to smaller temperature differences between the food and heating medium. Deviations may cause considerable errors in sterilizing values because of the exponential relation of this parameter to product temperatures (Uno and Hayakawa, 1980).

As observed in Table 5, maximum deviations of 14% and minimum of 1% were obtained. Careful analysis of the parametric values used during our experiments show that greater deviations between experimental and theoretical regression equation (predicted) C_f occurred mainly when:

1. There was difficulty in finding an appropriate experimental m value to accurately describe the heating medium temperature during the come-up.

2. The values of S_{k1} and/or S_{k3} and/or S_{k5} are not within or close to the +1, −1 levles used for the central composite experimental design. In experiment 12 for example the values of S_{ki}'s are as follows: $S_{k1} = -2.348$, $S_{k2} = -0.126$, $S_{k3} = 0.722$, $S_{k4} = -0.512$, $S_{k5} = -1.228$. In this case S_{k1} and S_{k5} are beyond the high and low levels indicated, and a deviation of +14% was observed.

The importance of the parameters S_{k1}, S_{k3} and S_{k5} in the determination of the correction factor is examined in the discussion of our error analysis. In agreement with the observations of Uno and Hayakawa (1980), the smaller values of m produced higher values of C_f.

It is observed that th experimental C_f values are found to be consistently higher than those theoretically predicted. This responds to the fact that the heating medium temperature dropped slightly, shortly after the start of the simulation of the step functional process, and this causes the equivalent process time to be greater than the one theoretically predicted. As a consequence there is a slight overestimation of the predicted liquid temperatures during the step functional processes, thus experimental t_{xe} values which yield a target sterilizing value are greater than the predicted equivalent process times. Therefore, we have that $C_{fe} > C_f$. As mentioned, the values of C_f may become negative. From Eq (1) it may be seen that a correction factor is negative only when the following is true:

$$t_x < t_p - t_u \qquad (19)$$

A necessary condition for a C_f to be negative is that $T_o > m_o$. This way the liquid temperature will decrease during a come-up, so that at the end of this phase the product temperature is below that of T_o at the beginning of the process. An additional requirement is that the lethality gained during the come-up must be negligible.

In general we observed that the values of C_f may be positive, zero or negative. If the sterilizing value gained during a come-up *is negligible*, any of the three ranges of values may be obtained if the following conditions are satisfied:

(1) $t_p - t_u = t_x$ when $T_g = T_o$. Values of C_f in this case are zero;

(2) $t_p - t_u < t_x$ when $T_g > T_o$. Values of C_f are positive;

(3) $t_p - t_u > t_x$ when $T_g < T_o$. Values of C_f are negative, where T_g is referred to the temperature of the contents at the end of the come-up phase (t_u).

From (3) above we may deduct that a correction factor may be negative only if $S_{k3} > S_{kl}$, whic reflects the fact that $T_o > m_o$.

Error analysis

The regression equation obtained, Eq (16), was mathematically examined in order to obtain maximum C_f errors most likely to occur when there are errors in the physical properties and operational conditions of a process, Table 6. For our examination, we assumed that the value of all

Table 5—Comparison of experimental and predicted correction factors in cans 211 x 300 and 307 x 409

No.	Product	t_u	t_p	Predicted	Exp.	Diff. (%)
1	Dist. water	5.5	10.0	0.3682	0.4026	8.5
2		5.0	25.0	0.4427	0.5119	13.5
3	(211 x 300)	7.0	27.5	0.3656	0.4026	9.2
4		5.5	35.0	0.3518	0.4084	13.9
5	0.1% lbg[a]	4.0	10.0	0.3441	0.3934	12.5
6	(211 x 300)	4.0	15.0	0.3409	0.3934	13.3
7		4.0	20.0	0.3425	0.3934	12.9
8	tomato	3.5	12.0	0.2298	0.2188	−5.0
9	(211 x 300)	3.5	16.0	0.2255	0.2188	−3.1
10		4.0	19.5	0.4741	0.4788	1.0
11	Dist. water	3.0	10.0	0.3917	0.4416	11.3
12	(307 x 409)	3.0	12.0	0.4394	0.5112	14.0
13		3.0	15.0	0.3901	0.4416	11.7
14	0.1% lbg	3.5	10.0	0.5004	0.5393	7.2
15	(307 x 409)	3.5	15.0	0.4962	0.5393	8.0
16		3.5	20.0	0.4971	0.5393	7.8
17	tomato	6.5	10.0	0.4217	0.4254	0.9
18	(307 x 409)	5.5	17.0	0.4420	0.4541	2.7
19		5.0	20.0	0.1915	0.2060	7.0

[a] lbg — locust bean gum

Table 6—Maximum expected errors in measurement of physical property values and operational conditions

Physical quantities	Frequent values observed	Expected error measurement	Δ value	Absolute[a] deviation
T_1	95.0°C	0.1°C	ΔT_1	0.1°C
T_o	65.0°C	0.1°C	ΔT_o	0.1°C
C	1.3 min	10%	ΔC	0.13 min
z	10.6C°	10%	ΔZ	0.106°C
m_o	45.0°C	0.1°C	Δm_o	0.1°C
t_u	180 sec	9 sec	Δt_u	9 sec
t_p	3000 sec	9 sec	Δt_p	9 sec
m	0.7	15%	Δm	0.105

[a] From column 1

design variables were equal to zero around which frequently observed values were located.

Therefore, we obtained Eq (20) differentiating Eq (16):

$$\Delta C_{f_{xi=0}} = -0.113680\Delta X_1 - 0.009772\Delta X_2 + 0.155831\Delta X_3 + 0.001652\Delta X_4 - 0.049687\Delta X_5 \quad (20)$$

Analytical expressions for the S_{ki}'s obtained from Eq (6) through (10) are given as follows:

$$\Delta S_{k1} = \frac{1}{z}\left[\Delta T_1 - \Delta m_o - \frac{T_1 - m_o}{z} \cdot \Delta z\right] \quad (21)$$

$$\Delta S_{k2} = \frac{1}{t_u}\left[\Delta C - \frac{C\,\Delta t_u}{t_u}\right] \quad (22)$$

$$\Delta S_{k3} = \frac{1}{z}\left[\Delta T_1 - \Delta T_o - \frac{T_1 - T_o}{z} \cdot \Delta z\right] \quad (23)$$

$$\Delta S_{k4} = \frac{1}{C}\left[\Delta t_p - \Delta t_u - \frac{t_p - t_u}{C} \cdot \Delta C\right] \quad (24)$$

$$\Delta S_{k5} = \Delta m \quad (25)$$

Through the differentiation of Eq (11) through (15), we obtained:

$$\Delta X_1 = 1.869149 \exp(-0.25S_{k1})\Delta S_{k1} \quad (26)$$
$$\Delta X_2 = 8.760759 \exp(-1.71S_{k2})\Delta S_{k2} \quad (27)$$
$$\Delta X_3 = 1.648762 \exp(-0.30S_{k3})\Delta S_{k3} \quad (28)$$
$$\Delta X_4 = 0.142704 \exp(-0.03S_{k4})\Delta S_{k4} \quad (29)$$
$$\Delta X_5 = -2.013715 \exp(1.60S_{k5})\Delta S_{k5} \quad (30)$$

Through the combined use of Eq (21) through (30) and Table 6, we have:

$$\Delta C_{fmax} = 0.0679\Delta S_{k1} + 0.0397\Delta S_{k2} + 0.1113\Delta S_{k3} + 0.0001\Delta S_{k4} + 0.3066\Delta S_{k5} \cong 0.040 \quad (31)$$

As mentioned, at the statistical zero level, $C_f = 0.324$, therefore at this level experimental errors may cause maximum deviations of ±12.2% on the value of a correction factor.

In agreement with the previous discussions, we observe from that error analysis that the parameters that significantly influence the value of C_f are S_{k1}, S_{k3}, and S_{k5}. As indicated by Uno and Hayakawa (1980), the value of $m(=S_{k5})$ has a considerable effect on the value of C_f.

In our analysis we observed that errors of ±15% in the estimation of m causes deviations of ±9.9% in the values of C_f. Furthermore, analysis of expression 31 indicates that expected errors in the estimation of S_{k4} have a negligible effect in the value of C_f.

NOMENCLATURE

THE FOLLOWING LIST of symbols are notations used in this article. Occasionally the same symbols have been used to designate more than one quantity. They are, however, properly defined when introduced.

- A star point value used in statistical analysis
- A exposed surface area of a can
- C constant given by: $C = v\rho C_v/UA$
- C_f correction factor of come-up heating
- C_v specific heat
- F_o sterilizing value
- f_h slope index of semilog plot of unachieved liquid temperature vs time
- h height of can contents
- m power index in come-up curves
- m_o initial retort temperature
- n number of factors in factorial central composite design of experiment
- S step functional process
- T temperature
- T_1 constant heating medium temperature
- t time
- U overall heat transfer coefficient
- V come-up heating or variable process
- V in Eq (3a) volume of contents in a package
- X transformed variable used in factorial central composite design of experiment
- z slope index of TDT curve
- ρ density
- Δ error

Subscripts

- 1,2 refer to the come-up phase or holding period phase of process V respectively
- cu heating medium temperature during come-up time
- e refers to a values obtained during experimental runs
- g values at the end of the come-up heating time
- i and 1, 2, 3 . . . referred to dummy index
- k dimensionless parameter
- m refers to modified experimental value. e.g. in Eq (17) $T_{sm(t)}$ = modified liquid temperature in process S, evaluated at time t
- o refers to values at the beginning of a process
- p values at the end of the processing time of process V
- r reference value
- s To value of step functional process S
- t time variable
- u come-up heating time
- v value at constant volume. e.g. C_v = specific heat at constant volume
- v_1 value during come-up heating time of variable process
- v_2 value during holding period of variable process
- x values at the end of a step functional process

REFERENCES

Anonymous. 1964. Calculation of process for canned foods. Technical Service Div. Memorandum, American Can Co., Maywood, Il.

Ball, C.O. 1923. Thermal process time for canned food. Bull. 7-1(37). Natl. Res. Council, Washington, DC.

Barr, A.J., Good night, J.H., Sall, J.P., and Helwig, J.Y. 1976. "A User's Guide to SAS." 76 SAS Institute, Inc., Raleigh, NC.

Davies, O.L. 1978. "The Design and Analysis of Industrial Experiments," 2nd ed. Imperial Chemical Industries Ltd., Longman Inc., London, U.K.

Hayakawa, K. and Ball, C.O. 1971. Theoretical formulas for temperature in cans of solid food and evaluating various heating processes. J. Food Sci. 36: 306.

Hayakawa, K. 1978. A critical review of mathematical procedures for determining proper heat sterilization processes. Food Technol. 32(3): 59.

Hildebrand, F.B. 1956. "Introduction to Numerical Analysis." McGraw-Hill Book Co., New York.

Horwitz, W. (Ed.) 1980. "Official Methods of Analysis." Association of Official Analytical Chemists, Washington, DC.

Leonard, S., Merson, R.L., Marsh, G.L., York, G.K., Heil, J.R., and Wolcott, T. 1975. Flame sterilization of canned foods: an overview. J. Food Sci. 40: 246.

Lopez, A. 1975. "Complete Course in Canning," Vol. 1 and 2. The Canning Trade, Baltimore, MD.

National Canners Association. 1976. Processes for low-acid canned foods in metal containers. Bull. 26-L, 11th ed. Washington, DC.

Salzer, H.E. 1944. A new formula for inverse interpolation. Bull. of the American Mathematics 50: 513.

Schack, A. 1965. "Industrial Heat Transfer." John Wiley & Sons Inc., New York.

Stumbo, C.R. 1973. "Thermobacteriology in Food Processing," 2nd ed. Academic Press, New York.

Succar, J. 1980. Correction factor for the come-up heating of packaged liquid foods. M.S. thesis, Food Science Dept., Rutgers, The State Univ., New Brunswick, NJ.

Uno, J., and Hayakawa, K. 1980. Correction factor of come-up heating based on critical point in a cylindrical can of heat conduction food. J. Food Sci. 45: 853.

Ms received 6/22/81; revised 10/9/81; accepted 10/12/81.

A paper of the Journal Series, New Jersey Agricultural Station, Cook College, Rutgers, The State Univ., Dept. of Food Science, New Brunswick, NJ 08903. This work was performed as a part of NJAES Project No. 10204, supported by the New Jersey Agricultural Experiment Station, by Hatch Act Fund, and by computer times fund provided by the Center for Computer and Information Services, Rutgers, The State Univ. of New Jersey.

Performance of a Scraped-Surface Heat Exchanger Under Ultra High Temperature Conditions: A Dimensional Analysis

ROBERTO CUEVAS, MUNIR CHERYAN, and VERNON L. PORTER

ABSTRACT

Performance characteristics of a pilot-scale scraped-surface heat exchanger (Contherm Model 6 x 2) under UHT operating conditions were evaluated. Outlet temperature and overall heat transfer coefficient (U) were significantly affected by rotational speed of the blades, mass flow rate of feed, feed inlet temperature, steam pressure and solids content of the model low-acid liquid food (soy extracts). The scraping action of the blades greatly improved the performance up to about 350 rpm; higher blade speeds had little or no effect and sometimes decreased U values. Application of dimensionless models showed that the effect of axial Reynolds number (Re_A) on the Nusselt number was more significant in the turbulent regime ($Re_A > 1800$), while rotational Reynolds number above 10^5 had a significant effect at all values of Re_A between 1200 and 3700. At very high values of the Taylor number ($> 10^{10}$) when laminar axial flow conditions prevailed, Nusselt number decreased with increase in rotational Reynolds number.

INTRODUCTION

ONE OF THE MORE IMPORTANT means of food preservation is ultra-high temperature (UHT) processing. By increasing temperature and decreasing processing time, the desired lethal effect of microorganisms may be achieved, while the loss of nutrients is reduced. Since the product temperature must be raised and lowered as quickly as possible, the rate of heat transfer must be high. One rapidly developing technological alternative when indirect heating is used is the use of the scraped-surface heat exchanger (SSHE). These units are fitted with blades that continuously scrape the heat transfer wall, thus minimizing fouling and burn-on problems and improving the rates of heat transfer by the combined effect of mechanically-induced turbulence, film removal, and mixing of the product. Scraped-surface heat exchangers are particularly useful for processing liquid foods exhibiting non-Newtonian rheological properties, containing suspended solids, or which tend to form fouling deposits during thermal processing (Cuevas-Garcia, 1981).

The heat transfer phenomena in SSHE have been studied by many workers, including among others Houlton (1944), Hosking (1962), Kool (1958), Harriot (1959), Penney and Bell (1969), Ramdas et al. (1977), Skelland (1958) and Trommelen et al. (1971). However, most studies were restricted to very low mass flow rates (where axial Reynolds numbers were in the low laminar flow region) and relatively low temperatures. There is very little published data on the use of SSHE for UHT processing, where temperatures range from 250–300°F (120–150°C), and, since very short holding times are needed, where mass flow rates tend to be much higher. This paper presents results of a study of the performance of a vertical, liquid-full pilot size SSHE used under UHT conditions, with the ultimate aim of developing models by dimensional analysis that correlate desired responses of the unit with operating parameters.

Authors Cuevas, Cheryan, and Porter are, respectively, graduate research assistant, associate professor of food engineering, and professor assistant, Dept. of Food Science, Dairy Manufactures Building, Univ. of Illinois, 1302 W. Pennsylvania Ave., Urbana, IL 61801. Address inquiries to Dr. Cheryan.

EXPERIMENTAL

Overall UHT system

The scraped-surface heat exchangers used in this study were the Contherm model 6 x 2 units (The DeLaval Separator Co., Newburyport, MA) mounted vertically on a portable-column frame, each fitted with two scraping blades of the "floating" type. No modifications were made of the units themselves for this study. Further details of the experimental set-up, instrumentation and procedures are available elsewhere (Cuevas and Cheryan, 1981).

Model working liquids

In order to compare our data to previous published work, pure water was a logical choice for preliminary studies. Water extracts of soybeans was used as the model low-acid liquid food. They were prepared as described by Omosaiye et al. (1978) by a series of unit operations that involved soaking of whole soybeans, blanching briefly, grinding hot and separating the coarse insolubles by means of a plate-and-frame filter press or a continuous disc-bowl centrifugal desludger. Solids content of the extract were varied by modifying the soybeans/water ratio in the grinding, rising and separation steps. Bacterial growth in the extracts prior to use was controlled by adding 100 ppm thimerosal (Sigma Chemical Co., St. Louis, MO). Physical properties of the working liquids such as viscosity and density were experimentally determined while thermal conductivity and specific heat were calculated using well-established models (see Cuevas and Cheryan, 1981 for details).

Experimental design

The original design for preliminary experiments was a multifactorial experiment with all factors quantitative and at different levels from each other (Davies, 1956). The literature review had indicated that the effects of product mass flow rate and blade rotational speed (acting independently and together) on heat transfer needed to be clarified and analyzed, especially in the UHT range. In addition, careful choice of operating levels would allow us to span the fluid flow range from laminar to turbulent (axial) flows. Hence a wide range of these variables (product mass flow rate and blades RPM) was selected, within the physical constraints of the system.

The five independent variables and their levels are shown below:

Mass Flow Rate (W, lb/min): 10, 15, 20, 25, 30, 35
Blades Rotational Speed (N, rpm): 0, 100, 200, 300, 400, 575
Steam Pressure (P_S, psig): 60, 110
Product Inlet Temperature (T_i, °F): 120, 160
Solids Content of Feed (S, % w/w): 0–6.14

Data presented in this paper are means of several replicates (at least 3 for the water data and 2 for the soy extracts). The data were analyzed following the standard analysis of variance procedure to test for main effects and interactions (Davies, 1956) using a statistical package available at the Computing Services Office, Univ. of Illinois, Urbana.

Heat transfer calculations and presentation of data

The basic heat transfer equation used in this research is:

$$Q = U A \Delta T_{ln} \quad (1)$$

where Q is the rate of heat transfer, A the outside heat transfer area and ΔT_{ln} the log mean temperature difference. The assumptions

made in using this equation are (i) the overall heat transfer coefficient is constant over the range of temperatures considered, (ii) no heat losses to or gains from the surrounding, and (iii) steady-state flow of heat. This results in the development of the ΔT_{ln} term, i.e., a logarithmic mean temperature drop between the working liquid and the heat transfer medium. Furthermore, the use of ΔT_{ln} implies that any axial dispersion of heat or backmixing effects have been neglected. (See later discussion for justification).

The energy contributed by the power to the motor rotating the shaft has been neglected since calculations show it is a small fraction (less than 0.5%) of that being transmitted by the condensing steam (Cuevas-Garcia, 1981).

The Q value was calculated from an enthalpy balance for the working fluid:

$$Q = W C_P (T_o - T_i) \quad (2)$$

The scraped-side (internal) heat transfer coefficient (h_i) was calculated using the resistance concept (Cuevas and Cheryan, 1981):

$$h_i = \frac{D_o/D_t}{\frac{1}{U} - \frac{1}{h_o} - \frac{D_o}{D_L h_w}} \quad (3)$$

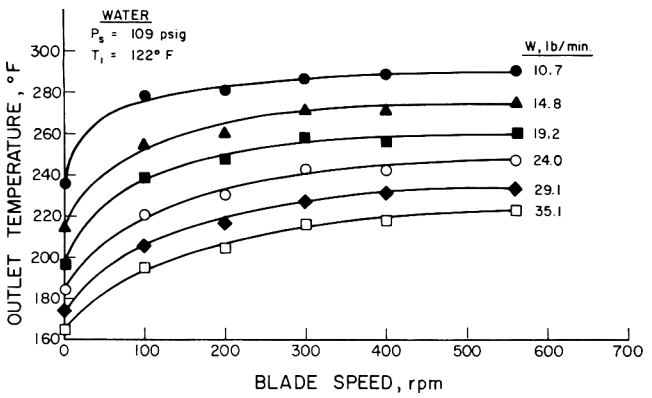


Fig. 1—Effect of mass flow rate (W) and blade rotational speed on the heater outlet temperature for water as feed.

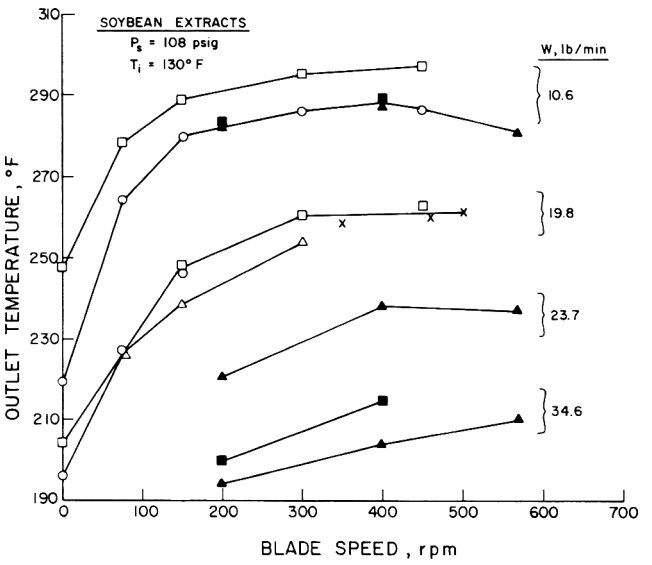


Fig. 2—Effect of mass flow rate (W) and blade rotational speed on heater outlet temperature for water extracts of soybeans: □ = 1.65% total solids w/w; ■ = 2.91%; ▲ = 3.13%; x = 3.16%, ○ = 3.97%; △ = 6.14%.

The steam-side coefficient (h_o) was estimated using the Nusselt theory, assuming film-type condensation of steam, and an iteration procedure to estimate the wall temperature, as described by Cuevas and Cheryan (1981). The wall transfer coefficient (h_w) was obtained from the thermal conductivity and thickness of the wall, as specified by the manufacturer, and was calculated as 1500 BTU/hr $ft^{2\circ}F$ (8517 $W/m^{2\circ}K$).

RESULTS & DISCUSSION

Temperature profiles of heater

The outlet temperature was the dependent variable measured in all experiments. The mean values of both dependent and independent variables are presented in graphical form in Fig. 1 and 2 which show typical effects of blade rotational speed (N) and working liquid mass flow rate (W) on the outlet temperature. Two important facts are evident from these graphs: (1) the effect of blade rotation is more significant at low blade speeds, where outlet temperature increases sharply as rpm is increased, whereas this effect is negligible or even negative at higher values of rpm; and (2) higher outlet temperatures correspond to lower mass flow rates, as may be expected from the heat transfer Eq 1 and 2. For a given steam pressure and product inlet temperature, the system response, when expressed as outlet temperatures, is more sensitive to the effect of scraping at higher flow rates, which illustrates the interaction between both variables. Other steam pressures and feed inlet temperatures resulted in similar trends of outlet temperatures for the same levels of W and N considered in Fig. 1 and 2 (not shown: See Cuevas et al., 1980; Cuevas-Garcia, 1981).

The data for soy extracts (Fig. 2) indicate that, as concentration increases, the effect of scraping becomes more uniform for the different flow rates. For a given mass flow rate, higher solids content results in lower outlet temperatures due, no doubt, to the effect of solids on physical properties.

The significance of these data on UHT processing of liquid foods must be stressed. If temperatures above 250°F (121°C) are considered as the UHT range, the data indicate that only certain mass flow rates will result in such outlet temperatures. Moreover, it was noticed in these experiments that wear of the blades (i.e., as a result of intensive use) resulted in less effective scraping and, consequently, lower outlet temperatures.

Overall heat transfer coefficients

U values as a function of blade rotational speed at different mass flow rates are presented in Fig. 3 and 4. Those curves show that the overall heat transfer coefficient increases as a result of scraping, but only up to a certain point, and further increments in blade speed do not induce any significant improvement in the transfer of heat, and indeed may actually lower the U value at very high values of blades speed. The effects of mass flow rate, blades speed and steam pressure were analysed statistically (Cuevas-Garcia, 1981); typical analysis of variance for a particular feed inlet temperature is shown in Table 1. All variables and their interactions are significant. These findings are especially significant since the heat transfer phenomenon in SSHE has frequently been analysed assuming that axial velocity effects (i.e., mass flow rate effects) are negligible (Trommelen et al., 1971; Kool, 1958; Harriot, 1959), as developed in the Penetration Theory. However, as the data in Fig. 3 show, the heat transfer coefficients are significantly affected by even relatively low values of the axial Reynolds number. The U values obtained here are comparable to those reported in the literature for water (Bolanowski, 1967; Houlton, 1944; Ghosal et al., 1967).

Visual inspection of the data in Fig. 3 and 4 suggest that there may be an "optimum" blades speed, i.e., a value of N

above which the U values actually decrease. To confirm this, the data were fitted to a polynomial of the form:

$$U = b_0 + b_1W + b_2N + b_3S + b_{11}W^2 + b_{22}N^2 + b_{33}S^2 + b_{12}WN + b_{13}WS + B_{23}NS \quad (4)$$

The coefficients of the model obtained by multiple regression analysis are shown in Table 2, together with the appropriate statistics. From the statistical viewpoint, the results indicate the models fitted are acceptable. From the phenomenological viewpoint, the models indicate that, within the expected operating range of the SSHE heater studied here, there is a true maximum in the U vs N correlation (since the value of b_{22} is negative: see Cuevas et al., 1980 and Cuevas-Garcia, 1981). Calculations using the model indicate that the optimum blades speed is 350–430 rpm, tending to be lower at higher solids content in the feed (Cuevas et al., 1980). The reasons for this optimum in the blades speed are not clear, but there exists the possibility, especially in units with relatively small diameters, that some of the material scraped away from the wall may be thrown back against the wall due to high centrifugal forces at high blade speeds (a "slingshot" effect), thus lowering the U value (Kool, 1958).

Effect of backmixing on U values

U values calculated using Eq 1 assumes that backmixing effects are negligible. Eq 1 (and the logarithmic mean temperature difference) has been used as the basis for SSHE heat transfer calculations by many workers (Houlton, 1944; Skelland, 1958; Skelland et al., 1962; Blaisdell and Zahradnik, 1959). Depending on the operating conditions, this may or may not be a valid assumption. Fluid flow in SSHE is very complex since it is controlled by the presence of two nonvanishing components of the velocity field. An indication of the mixing patterns in the overall SSHE system was obtained from a preliminary residence time distribution study conducted under operating conditions most likely to produce the greatest backmixing effects (i.e., lowest mass flow rates, 10–15 lb/min, and high blades rotational speed, > 400 RPM). The results (not shown here: see Cuevas et al., 1980: Cuevas-Garcia, 1981) indicated that, although a moderate amount of backmixing was apparent, the residence time distribution function more closely approximated plug flow behavior than a completely mixed system. Similar conclusions could be arrived at by applying the work of Chen and Zahradnik (1967), Penney and Bell (1969) and Trommelen et al. (1971), among others, to the conditions prevailing in our particular studies.

The studies of Bott et al (1968) are especially significant. They developed a theoretical model of the effect of backmixing on heat transfer in SSHE. Their conclusions were that backmixing effects could be ignored at low values of the Stanton number and high values of the Peclet (Bodenstein) number. Based on their model and our preliminary

Table 1—Analysis of variance of U values as affected by mass flow rate, blade speed, and steam pressure [Feed (water) inlet temperature of 122°F]

Source	Sum of Squares	d.f.	Mean square	F
Mass flow rate (W)	232855.22	5	46571.06	1719.76**
Blades speed (N)	443930.98	4	110982.75	4098.33**
Steam pressure (P_s)	17563.68	1	17563.68	648.58**
W x N	60800.09	20	3040.00	112.26**
W x P_s	1676.38	5	335.28	12.38**
N x P_s	10241.81	4	2560.45	94.55**
W x N x P_s	2189.76	20	109.49	4.04**
Within all	3250.00	120	27.08	
TOTAL	772508.08	179		

**Highly significant

Table 2—Regression coefficients for polynomial model for overall heat transfer coefficients [according to Eq (4)]

	Water		Soybean extracts	
	Regression coefficient	t value	Regression coefficient	t value
b_0	138.3	12.96**	174.2	2.15*
b_1	8.19	8.99**	17.07	3.14**
b_2	0.965	34.01**	0.762	4.32**
b_3	–	–	−62.45	2.31*
b_{11}	−0.189	9.80**	−0.422	4.41**
b_{22}	-1.41×10^{-3}	38.39**	-1.08×10^{-3}	4.41**
b_{33}	–	–	7.41	2.45*
b_{12}	1.31×10^{-2}	16.34**	1.03×10^{-2}	1.91 (NS)
b_{13}	–	–	−0.192	0.18 (NS)
b_{23}	–	–	1.66×10^{-2}	0.43 (NS)
R^2	0.9467		0.8295	
C.V.	5.4%		10.7%	

* Significant at 5% level; **Significant at 1% level; NS = Not significant

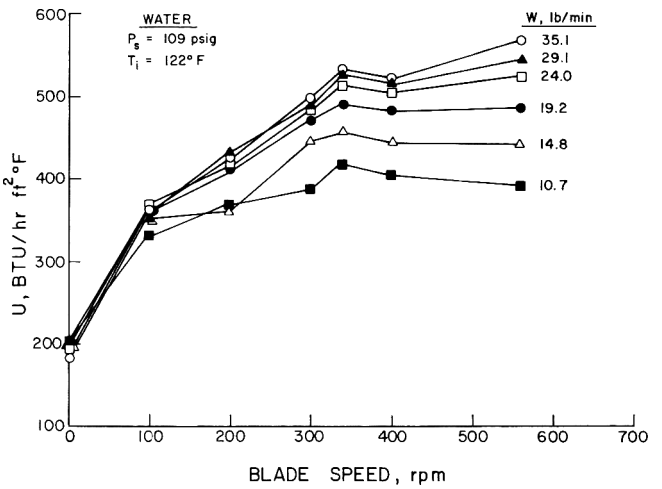


Fig. 3—Effect of mass flow rate (W) and blade speed on the overall heat transfer coefficient (U) in heater. Feed is water. [1 BTU/hr ft²°F = 5.678 W/m²°K]

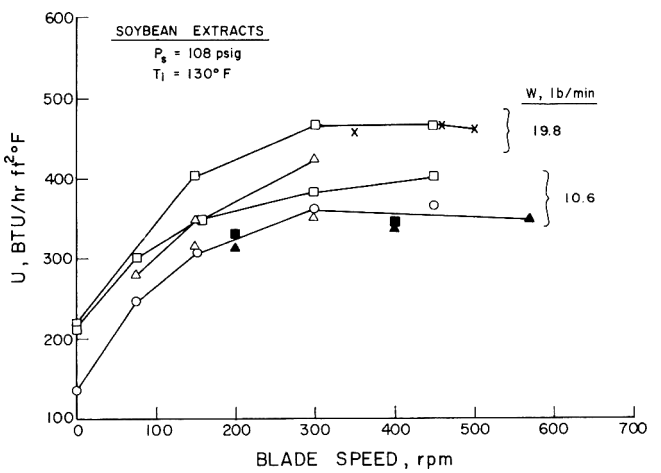


Fig. 4—Effect of mass flow rate (W), blade speed and soy extract composition on the overall heat transfer coefficient. Symbols have same meaning as in Fig. 2. [1 BTU/hr ft²°F = 5.678 W/m²°K].

residence time distribution data, and considering the range of Stanton numbers in our study (approximately 0.01–1.6), we have assumed that our system was operating close enough to the plug flow mode to neglect backmixing effects, at least as a first approximation. The studies of Penney and Bell (1969), Flower et al. (1969) and Trommelen and Beek (1971) appear to confirm this assumption. In addition, Trommelen et al. (1971) concluded that axial dispersion effects for their system were negligible above $Re_A > 41$. Our values of Re_A are in great excess of this (Table 3).

Finally, the steam-side (external) heat transfer coefficient (h_o) was calculated using the Nusselt theory and an iteration procedure to estimate the mean film temperature (Cuevas and Cheryan, 1981). The calculated values of h_o (1404–1513 BTU/hr ft^2°F; 7972–8591 W/m^2°K) are within the expected range for vertical condensation and only three iterations were needed for the method to converge, suggesting that the original assumptions and the use of log mean temperature difference are applicable in our case. There is little doubt, however, that some of the U values presented here, especially at the lower mass flow rates, may be in error due to neglecting axial dispersion effects and it may be more appropriate to refer to them as "apparent" U values.

Dimensional analysis

Previous attempts at modelling the SSHE data (Cuevas and Cheryan, 1981) showed that classic methods such as the Penetration Theory were inadequate over most of the conditions studied here except at low mass flow rates. Dimensional analysis was used here instead since it is a powerful heat transfer methodology when no satisfactory theory exists (Bird et al. 1960; McCabe and Smith 1956). In this case, as seen in Table 3, axial Reynolds numbers (Re_A) were in the high-laminar, low-turbulent regions for water and in the laminar region for soy extracts. Rotational Reynolds number was relatively high, and probably corresponds to flow regions in which Taylor vortices exist (see later). It is important to mention that in the experiments described here, any variation in the Prandtl number for water is due solely to the effect of temperature on the physical properties and therefore related to the temperature profiles in the exchanger.

The dimensional correlations were derived by the π theorem (not shown; see Cuevas-Garcia, 1981). No geometrical factors are included in an explicit manner, since they were not independent variables in the experiments. Therefore, the values of the parameters will absorb any assumptions undertaken and the effects of the variables included in the correlations. These correlations are commonly taken as logarithmic functions (power-law type) and for this reason, the effects of zero blades speed could not be included even though it was studied (Fig. 1–4). However, effects in the important low blades speed region ($N < 100$ rpm) could be studied using the polynomial correlation developed earlier [Eq (4)]. These polynomials were used to simulate temperature profiles and overall heat transfer coefficients (and thus the Nusselt number) as a function of the operating parameters (i.e., axial and rotational Reynolds numbers, Prandtl number and viscosity factor) over the entire range of experimental conditions shown in Fig. 1–4 (for values of $N > 0$). This approach served to reduce the number of experimental runs needed to confirm the dimensional analysis.

Table 3–Range of dimensionless numbers in heat transfer experiments[a]

Dimensionless number	Formula	Water	Soybean extracts
Nu	$h_i (D_t-D_s)/k$	145–1950[b]	177–774
Re_A	$W (D_t-D_s)/\mu A_f$	1197–3692	596–1949
$Re_R \times 10^{-5}$	$D_t^2 \rho N/\mu$	0.086–7.323[c]	0.351–3.808[d]
P_r	$C_p \mu/k$	1.76–2.90	3.05–5.16
μ/μ_w	——	1.15–1.75	1.30–2.37
Ta x 10^{-9}	$\frac{N^2\rho^2(D_t-D_s)^3D_s^2(2\pi)^2}{\mu^2(D_t+D_s)/2}$	0.04–27.57	0.06–7.4

[a] Physical properties estimated at bulk average temperature.
[b] Values of Nu at blades speeds $10 < N < 100$ were calculated based on h_i estimated from polynomials relating overall heat transfer coefficient (U) and temperature profile to operating parameters (See text).
[c] Blades speed (N) = 10–575 rpm.
[d] Blades speed (N) = 100–575 rpm.

Correlations of the type $Nu = f(Re_A, Re_R, Pr, \mu/\mu_w)$

Fig. 5–7 clearly show that, despite the vast differences in the order of magnitude between axial and rotational Reynolds numbers, Nusselt numbers are significantly affected by both variables. It had earlier been shown that the outlet temperature decreased as the mass flow rate was increased. For pure pipe flow, there are two regions in which this happens: as the flow rate (and hence the axial Reynolds number) is increased, the outlet temperature will first decrease until Re_A reaches approximately 2100, then increase (until Re_A is about 8000), and then decrease again. In the case of the SSHE, this could be applied in two ways: either the effect of blade rotation will interact with the axial flow and trigger transition to turbulent flow at lower values of Re_A; or exactly the opposite effect, viz., that the rotational velocity effects result in a dampening of the onset of turbulence in the axial direction. In this particular case, the state of mixing generated due to the action of the blades would seem to favor the former type of situation.

On the other hand, Fig. 5–7 also show that the Nusselt number does not increase with increases in Re_R after a certain value of the rotational speed has been reached, a trend similar to that seen in Fig. 3 and 4. The shapes of the graphs suggest that the value of Re_R for which no further improvement is obtained is slightly higher for higher axial Reynolds numbers. This would mean that the beneficial effect of scraping is enhanced by increased axial velocities.

Fig. 5 also indicates that at low blade speeds ($N < 100$ RPM) where $Re_R < 10^5$, axial Reynolds number has a small effect on Nusselt number. Axial effects appear to be first noticeable at $Re_R > 1.5 \times 10^4$ and are quite significant at $Re_R > 10^5$. Note that a particular value of Re_R in Fig. 5–7 does not mean a single value of N for all values of Re_A, since these two groups include other variables besides the axial and rotational velocities.

The parameters in the dimensionless correlation equation were obtained by multiple regression analysis rather than by the graphical method, since the former allows for simultaneous assessment of the effects of the variables taken alone and in combination with one another. The independent variables were Re_A and Re_R, while the dependent variable was $Nu \cdot P_r^{-0.33} (\mu/\mu_w)^{-0.18}$. The exponents on the Prandtl number and viscosity factor are based on well-established heat transfer models: for example, for developed flow in smooth tubes, the Sieder-Tate model (Bird et al., 1960) predicts that the Nusselt number is a function of the Prandtl number to the power of 0.33, and of the viscosity correction factor to the power of 0.14. On the other hand, for agitated vessels, an exponent of 0.18 is generally used for the viscosity correction factor (Uhl, 1966). Since the scraped-surface heat exchanger is essentially a combination of pure tube flow and pure rotational

flow, the values of 0.33 and 0.18 were used as the exponents for the Prandtl and the viscosity groups. The significance of the viscosity correction factor is discussed later.

It was found that the data fit the models better (i.e., sums of squares decreased) if the data were divided by the working liquid (water vs soybean extracts) and further subdivided according to the state of axial flow, whether it was laminar ($Re_A < 1800$) or turbulent (Cuevas and Cheryan, 1981), indicating that there could be different phenomena being exhibited in the heater depending on axial flow conditions, at least in the range considered in this study.

The following correlations were obtained for water:

$$Nu = 0.304\ Re_A^{0.504}\ Re_R^{0.322}\ Pr^{0.33}\ (\mu/\mu_w)^{0.18},$$

$$\text{for } Re_A < 1800 \qquad (5)$$

$$Nu = 4.599 \times 10^{-4}\ Re_A^{0.942}\ Re_R^{0.637}\ Pr^{0.33}\ (\mu/\mu_w)^{0.18},$$

$$\text{for } Re_A > 1800 \qquad (6)$$

Multiple correlation coefficients were in the range 0.9393–0.9476 and coefficients of variation (C.V.) 1.8–2.4%. For soybean water extracts of solids contents varying from 1.65–6.14% w/w, the following correlation best fit the data:

$$Nu = 0.0982\ Re_A^{0.468}\ Re_R^{0.400}\ Pr^{0.33}\ (\mu/\mu_w)^{0.18},$$

$$\text{for } Re_A < 1800 \qquad (7)$$

with a multiple R = 0.7784 and C.V. = 5.0%.

For pure axial tube flow, the exponent on the Re_A term is generally 0.33 in the laminar regime and 0.8 for turbulent flow (Bird et al., 1960). The values of the Re_A exponent obtained here are much higher (0.47–0.50 for laminar flow, 0.94 for turbulent flow). On the other hand, for agitated vessels, the exponent on the rotational Reynold's number should be 0.66–0.7 for $Re_R > 300$ (Uhl, 1966; Penney and Bell, 1969). Our values span a wide range, tending to be lower (0.32–0.4) for axial laminar flow conditions and in good agreement (0.637) for turbulent axial flow. In general, some deviations from the ideal cases should be expected, considering the composite-type of flow that exists in scraped-surface heat exchangers (See later).

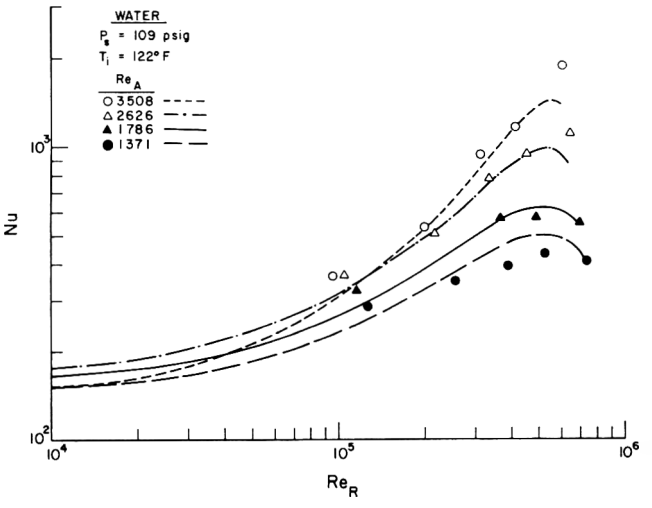


Fig. 5—Effect of axial (Re_A) and rotational (Re_R) Reynolds number on Nusselt number (Nu). Data for water at feed inlet temperature = 122° F, steam pressure = 109 psig. Points are experimental data. Lines are predicted behavior based on polynomial models for overall heat transfer coefficient and temperature rise. See text for details.

Comparison with other SSHE systems

Table 4 is a compilation of some of the models available in the literature based on the following general model:

$$Nu = A\ Re_A^a\ Re_R^b\ Pr^c\ (\mu/\mu_w)^d \qquad (8)$$

There is a wide variation in the values of the exponents, which is partly an indication of the complexity of the problem in defining parameters of importance in SSHE and partly due to the wide variation in the types of fluids, equipment and operating conditions encompassed in Table 4. A value of zero against an exponent implies either that it is insignificant or that the investigators did not explicity account for that dimensionless group. In some cases, such as the models of Skelland (1958) and Ramdas et al. (1977), the rate of heat transfer does not seem to be dominated by blades speed. In most cases, the axial flow rate tends to have a fairly significant effect on heat transfer. However,

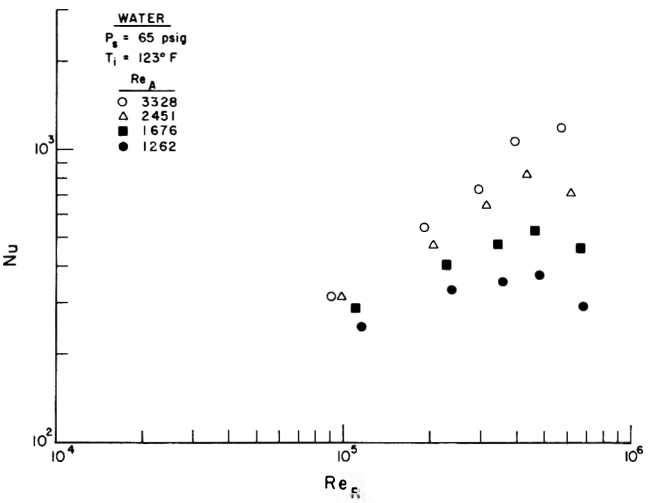


Fig. 6—Effect of axial (Re_A) and rotational (Re_R) Reynolds numbers on Nusselt number (Nu). Data for water at feed inlet temperature = 123° F, steam pressure = 65 psig.

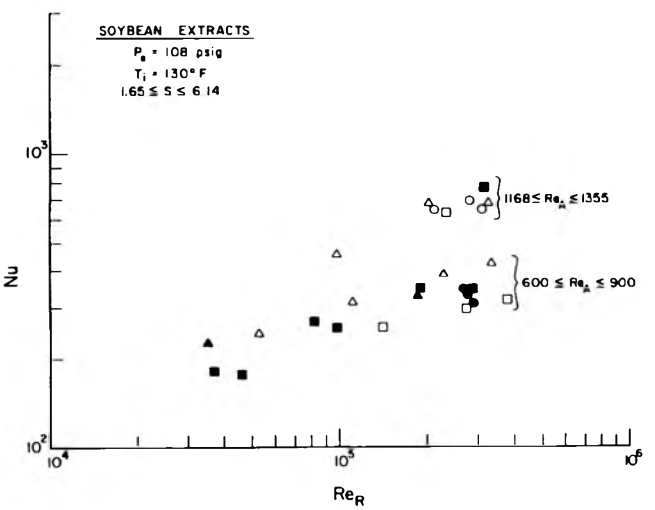


Fig. 7—Effect of axial Reynolds number (Re_A) and rotational Reynolds number (Re_R) on Nusselt number for soybean water extracts: S = solids content: □ = 1.65%, ■ = 2.91%, ▲ = 3.13%, ● = 3.16%, ○ = 3.97%, △ = 6.14%.

unlike this work, some of these data were obtained at very low flow rates and with highly viscous fluids, where the real cause may be axial dispersion or backmixing effects and not axial velocity effects.

Viscosity correction factor, μ/μ_w

In the original dimensional analysis the viscosity correction factor was included as a dimensionless group that could affect the Nusselt number. For a given set of experimental conditions a lower heater outlet temperature results in a larger radial temperature gradient ($T_w - T_b$), and therefore a larger ratio μ/μ_w. In a parallel way, a large radial temperature gradient results in a smaller Nusselt number. The outlet temperature at a fixed flow rate increases as blade speed is increased (Fig. 1 and 2) and therefore, the radial temperature gradient decreases, resulting in (i) a smaller value of μ/μ_w (as shown in Fig. 8), and (ii) a greater value of the Nusselt number (as shown in Fig. 5). Therefore, both Nu and the viscosity correction factor are functions of blade speed and mass flow rate, as shown here in Fig. 5–8.

The original purpose for which the viscosity ratio was defined was as a correction factor, to account for effects of temperature gradients on viscosity (McCabe and Smith 1956; Bird et al. 1960). Hence the viscosity correction factor was not included as one of the independent variables in the regression analysis. If that were done, a highly significant correlation between Nu and μ/μ_w, and very small nonsignificant partial regression coefficients for Re_A and Re_R would have been obtained (Cuevas-Garcia, 1981). A significant correlation between two factors y and x_1 does not provide evidence that these variables are necessarily causually related since, as in this case, a variable x_2 could produce a change in both y and x_1. Thus an exponent value of 0.18 was used here since it has been demonstrated to be appropriate under a wide variety of experimental conditions (Bird et al., 1960; McCabe and Smith, 1956).

Table 4—Comparison of literature values for the exponents in the dimensionless correlation [Eq (8)] for scraped-surface heat exchangers[a]

Data Source	A	a	b	c	d
Bott and Romero (1963)	0.013	0.46	0.60	0.87	0
Ghosal et al. (1967)	0.123	0.79	0.65	0.60	0
Penney and Bell (1969)	0.123	0	0.78	0.33	0.18
Ramdas et al. (1977)	57.0	0.059	0.113	0.063	−0.018
Skelland (1958)	3.26	0.57	0.17	0.47	0
Skelland et al. (1962)	0.0306	1.00	0.062	0.70	0
Sykora & Navratil (1966)	0.565	−0.01	0.48	0.40	0
Sykora et al. (1968)	4.09	0	0.48	0.24	0
Uhl (1966)	0.036	0	0.66	0.33	0.18
This work:					
Water[b]	0.304	0.50	0.32	0.33	0.18
Water[c]	0.0004	0.94	0.64	0.33	0.18
Soy extracts[b]	0.098	0.46	0.40	0.33	0.18

[a] Dimensionless numbers may not be defined in exactly the same manner as in this paper. Values of A reported are those that would apply if the models were applied to our system. Range of applicability of individual models vary widely.
[b] Laminar flow ($Re_A < 1800$)
[c] Turbulent flow ($Re_A > 1800$)

Correlations of the form $Nu = f_2(Ta, Re_A)$

The flow phenomenon in SSHE is best described as helical or spiral flow, where the path traced out by individual fluid particles are circular helices. When a certain value of blades rotational speed is reached, there appear pairs of counter-rotating vortices, and the flow is said to become unstable. Helical flow is usually characterized in terms of the axial Reynolds number and the Taylor number. Taylor numbers for the systems studied in this work are given in Table 3.

Axial flow will tend to "stabilize" the rotational flow, which implies that the onset of the formation of the so-called Taylor vortices will be delayed. Therefore the "critical" Taylor number Ta_{cr} (the value of Ta at which vortices first appear) will be a function of the axial Reynolds number (Kaye and Elgar 1958; Simmers and Coney 1979; Hasoon and Martin 1977). In general, the functional relationship between Re_A and Ta_{cr} seems to follow a power-law type of behavior; Table 5 presents some functions obtained by applying nonlinear regression methods to data gathered from the literature.

A plot of Nusselt number vs Taylor number at different axial Reynolds numbers for our system is shown in Fig. 9. Based on relationships as shown in Table 5 the critical Taylor number for the water runs is of the order of $10^5 - 10^6$. The Nusselt number appears to remain practically constant up to slightly above this value. When the flow changes from laminar to laminar-plus-vortices (or tubulent to turbulent-plus-vortices), which occurs at $Ta > Ta_{cr}$, the Nusselt number increases gradually, and then sharply with Taylor number at $Ta > 10^9$. Similar phenomenon have been reported by Kaye and Elgar (1958), Becker and Kaye (1962), among others. Apparently, the vortices act as a highly efficient mixing means to reduce any temperature differences in the bulk. As a result of this mixing action, the temperature gradients at the walls are considerably larger than those without vortices. The scraping blades would improve the effect of the vortices even further by reducing the thickness of the stagnant layer close to the wall, and therefore increasing the relative importance of convection with respect to conduction mechanisms.

CONCLUSIONS

DIMENSIONAL ANALYSIS of the heat transfer phenomena in a vertical, liquid-full scraped-surface heat exchanger (Contherm) indicated that under the normal operating conditions expected for UHT processing of liquid foods, the scraped-side heat transfer coefficient is a function of

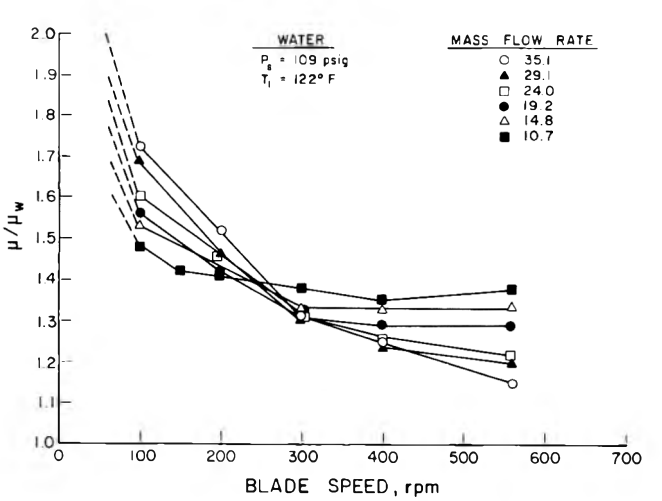


Fig. 8—Dependence of the viscosity correction factor on blades rotational speed and mass flow rate.

Table 5—Critical Taylor number (Ta_{cr}) as a function of axial Reynolds number

Range of Re_A	D_t/D_s[a]	Function[b]	R^2	Source of data
300–600	0.955	$Ta_{cr} = 162.87\ Re_A^{0.916}$	0.988	Simmers and Coney (1979)
400–2000	0.900	$Ta_{cr} = 41.79\ Re_A^{1.18}$	0.999	Hasoon and Martin (1977)
400–2000	0.500	$Ta_{cr} = 106.24\ Re_A^{1.09}$	0.999	Hasoon and Martin (1977)

[a] Ratio of outer cylinder diameter to shaft diameter
[b] Obtained by nonlinear regression of the experimental data in the source

both rotational and axial velocity components. The effect of axial Reynolds number was more significant in the turbulent regime ($Re_A > 1800$). Rotational Reynolds numbers $> 10^5$ had a significant effect on Nusselt number regardless of the value of Re_A. At very high values of rotational Reynolds numbers, the Nusselt number no longer increases and in fact may actually decrease, the nature of the decrease being a function of axial Reynolds number. The existence of Taylor vortices in SSHE affects not only the heat transfer characteristics, but may also affect residence time distributions in the unit, which will have to be considered in the design of thermal processes.

NOMENCLATURE

A	=	area for heat transfer
A_f	=	annular area for flow
b	=	regression coefficient
C_p	=	specific heat (heat capacity at constant pressure)
C.V.	=	coefficient of variation
D_e	=	equivalent diameter = $D_t - D_s$
D_L	=	logarithmic mean diameter
D_o	=	outside diameter of heat transfer tube
D_s	=	shaft diameter
D_t	=	heat transfer tube diameter (inside diameter)
f	=	mathematical functions
h_i	=	scraped-side heat transfer coefficient
h_o	=	steam-side heat transfer coefficient
h_w	=	wall heat transfer coefficient
k	=	thermal conductivity
N	=	blade speed, revolutions per minute (rpm)
Nu	=	Nusselt number
Pr	=	Prandtl number
P_s	=	steam pressure
Q	=	rate of heat transfer
R, R^2	=	correlation coefficient
Re_A	=	axial Reynolds number
Re_R	=	rotational Reynolds number
S	=	solids content (% w/w)
Ta	=	Taylor number
Ta_{cr}	=	critical Taylor number
T_b	=	bulk average temperature
T_i	=	working liquid inlet temprature
T_o	=	working liquid outlet temperature
T_w	=	wall temperature of heater
ΔT_{lm}	=	logarithmic mean temperature difference
U	=	overall heat transfer coefficient
W	=	mass flow rate

Greek Letters

μ	=	viscosity of working liquid at bulk average temperature
μ_w	=	viscosity of working liquid at wall temperature
ρ	=	density of working liquid at bulk average temperature

REFERENCES

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

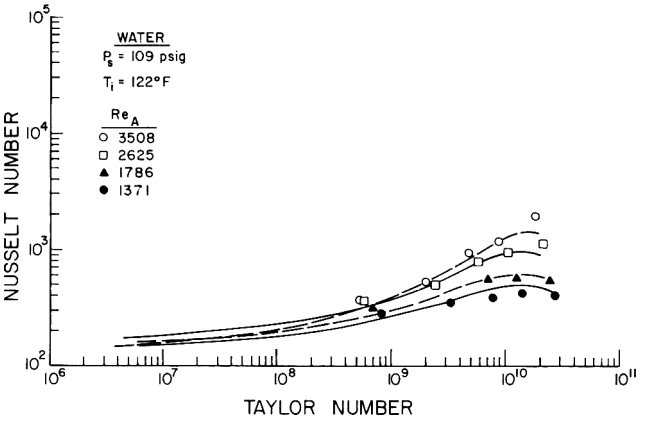


Fig. 9—Effect of axial Reynolds number (Re_A) and Taylor number (Ta) on Nusselt number. Points are experimental data, predicted lines obtained from polynomial models as described in text.

Becker, K.M. and Kaye, J. 1962. Measurements of diabatic flow in an annulus with an inner rotating cylinder. Trans ASME (J. Heat Transf.) 84: 97.

Bird, R.B., Stewart, W.E., and Lightfoot, E.N. 1960. "Transport Phenomena." John Wiley and Sons, Inc., New York, NY.

Blaisdell, J.L. and Zahradnik, J.W. 1959. Longitudinal temperature distribution in a scraped-surface heat exchanger. Food Technol. 13: 659.

Bolanowski, J.P. 1967. What you should know about heat exchangers. Food Engr. 39(12): 68.

Bott, T.R., Azoory, S., and Porter, K.E. 1968. Scraped surface heat exchangers. 2. The effects of axial dispersion on heat transfer. Trans. Instn. Chem. Engr. (London) 46(2): T37.

Bott, T.R. and Romero, J.J.B. 1963. Heat transfer across a scraped surface. Can. J. Chem. Eng. 41: 213.

Chen, A.C.Y. and Zahradnik, J.W. 1967. Residence time distribution in a swept-surface heat exchanger. Trans ASAE 10: 508.

Cuevas, R., Cheryan, M., and Porter, V.L. 1980. Heat transfer and thermal process design in scraped-surface heat exchangers. 89th Annual Meeting, Am Inst Chem Engr., Portland, OR, Aug. 17—20. Paper No. 25b. To be published in AIChE Symposium Proceedings.

Cuevas, R. and Cheryan, M. 1981. Heat transfer in a vertical, liquid-full scraped-surface heat exchanger. Application of the Penetration Theory and Wilson Plot models. J. Food Process Engineering. 5(1): 1.

Cuevas-Garcia, R. 1981. Heat transfer and thermal process design in scraped-surface heat exchangers. Ph.D. thesis, Univ. of Illinois, Urbana, IL.

Davies, O.L. 1956. "The Design and Analysis of Industrial Experiments." Oliver and Boyd, London, UK.

Flower, J.R., Macleod, N., and Shahbenderian, A.P. 1969. The radial transfer of mass and momentum in an axial fluid stream between coaxial rotating cylinders. 1. Experimental measurements. Chem. Eng. Sci. 24: 637.

Ghosal, J.K., Srimani, B.N., and Ghosh, D.N. 1967. Study of the heat transfer rate in a steam-heated Votator. Ind. Chem. Engr. 9(2): T53.

Harriot, P. 1959. Heat transfer in scraped-surface exchangers. Chem. Eng. Prog. Symp. Ser. 55(29): 137.

Hasoon, M.A. and Martin, B.W. 1977. The stability of viscous axial flow in an annulus with a rotating inner cylinder. Proc. Roy. Soc. London. A. 352: 351.

Hosking, A.P. 1962. Votator heat exchangers. The Chem Engr. (Bull. Instn. Chem. Engrs.) No. 161: A97.

Houlton, H.B. 1944. Heat transfer in the Votator. Ind. Eng. Chem. 36(6): 522.

Kaye, J. and Elgar, E.C. 1958. Modes of adiabatic and diabatic fluid flow in an annulus with an inner rotating cylinder. Trans ASME 80: 753.

Kool, J. 1958. Heat transfer in scraped vessels and pipes handling viscous materials. Trans. Instn. Chem. Engr. 36: 253.

—Continued on page 641

Comparison of Formula Methods for Calculating Thermal Process Lethality

TRUDI SMITH and MARVIN A. TUNG

ABSTRACT

Accuracy of five formula methods for determining process lethality in conduction heating foods was examined. Finite-difference simulation was used to generate temperature history curves for a range of cans and processing conditions. Delivered lethality was evaluated using the formula methods and compared to the lethality calculated using a numerical general method. Deviations exhibited trends related to container dimensions and unaccomplished temperature difference (g), the largest errors occurring when g was large and the height-to-diameter ratio was close to unity. Errors were mostly on the "safe" side, but the energy use implications could be significant for high retort temperature processes.

INTRODUCTION

SINCE PUBLICATION of the first general method for thermal process evaluation by Bigelow and coworkers (1920), much has been written about the determination of safe processes for thermally sterilized foods. Several reviews and comparisons of process evaluation methods have appeared (Hayakawa, 1977, 1978; Merson et al., 1978; Stumbo and Longley, 1966), although none offers extensive numerical evaluations.

In the last decade, more attention has been given to the effects of processing on nutritional and sensory quality. The desire to improve quality and the need to control processing costs have resulted in a move to reduce unnecessary overprocessing. Packaging changes, in container types, sizes and shapes, and process condition changes, such as elevated retort temperatures and higher filling temperatures, are becoming important. The introduction of computer technology for retort control is making possible more precise control of processing conditions. Because of these developments, there is an increasing interest in the accuracy and reliability of the currently available thermal process evaluation methods. Thus, the objective of this study was to prepare an evaluation of the selected formula methods using a wide range of conditions that would include traditional and novel thermal processing applications.

EXPERIMENTAL

FIVE FORMULA METHODS for center point lethality determination were examined. These were chosen to be representative of currently applied methods as well as the most recent developments in process evaluation.

Process calculation methods

Ball's table method. This formula method was a great milestone in thermal processing and has been the industry standard since it was first introduced (Ball, 1923). Ball developed tables of process value (U) with respect to heating rate index (f_h) and temperature difference between the product cold spot and the retort at the end of the heating cycle (g). The straight-line heating and cooling portions of the time-temperature history curves were evaluated mathematically using exponential integrals. The cooling lag factor (j_{cc}) was assumed to be 1.41 and the curve was approximated by an hyperbola. The start of straight-line cooling was determined empirically through observation of experimental data, and heating and cooling rates were assumed to be equal. The heating lag factor was used for the calculation of g but its lethal effect was not accounted for. Merson et al. (1978) provide a good description of the principles of this method.

The American Can Company has since developed more detailed tables, interpolating and extrapolating the tables that were published by Ball. These tables were used for the evaluation of this method.

Ball's equation method. While trying to develop a method for implementing Ball's formula method without using the tables, it was discovered that the values in his tables did not agree with the equations that were used to develop them (Smith and Tung, 1979). This was confirmed by the findings of Steele et al. (1979). The second method investigated, therefore, was Ball's method using the equations developed for the production of the tables.

Stumbo's method. Stumbo and Longley (1966) published tables for process evaluation taking into account the variability of j_{cc} values. The values in these tables were obtained through planimeter measurements of hand-drawn temperature histories plotted on lethal rate paper, and subsequent interpolation of graphs. Revised tables (used in this evaluation) were developed through use of computer integration of thermal histories generated from heat transfer equations, using finite difference simulations (Stumbo, 1973). In all other particulars, the method is similar to Ball's.

Steele and Board's method. Ball and Olson (1957) developed tables for evaluation of processes exhibiting broken heating curves or unequal heating and cooling rates. These tables were based on the same concepts as Ball's original tables except that the heating and cooling portions of the process were kept separate. This method was improved upon by Griffin et al. (1971) through the use of a relationship between the curved and straight-line portions of the cooling curve. Steele and Board (1979) adapted this method for calculation using sterilizing ratios, to simplify calculations. This method was evaluated using the equations developed rather than the tables provided.

Hayakawa's method. Hayakawa (1970) developed a method of lethality evaluation similar to those previously described except that circular functions were used to estimate the curved portions of the heating and cooling curves. The process was divided into four sections for evaluation: curved heating, straight-line heating, curved cooling, and straight-line cooling. The lengths of the curved portions were estimated using an empirical relationship between f and j.

During testing of the procedures developed for computer solution, errors in the published tables were found. A correction to the tables was found elsewhere (Downes and Hayakawa, 1977). This method was evaluated using the equations developed rather than the tables.

Reference method. The reference method to which the others were compared was a numerical general method with data points taken every 0.05 min. The arithmetic mean temperature over each time interval (Δt) was determined and the lethal rate (L) for this temperature was considered to apply for the duration of the interval (Teixeira et al., 1969). A z value of 10C° (18 F°) and a reference temperature of 121.1°C (250°F) were used for lethal rate calculations. Thus,

$$L = 10^{(T-121.1)/10} \quad (1)$$

Lethalities were calculated as $L\Delta t$ and summed to determine the total process lethality (F_o). Because of the small time intervals (0.05 min), this method was assumed to estimate the continuous process curve and hence the graphical general method, and its lethality was used to judge the accuracy of the alternate calculation methods.

Authors Smith and Tung are with the Dept. of Food Science, Univ. of British Columbia, Vancouver, B.C., Canada V6T 2A2.

Generation of heat penetration data

Thermal history curves, for conduction heating foods in cylindrical cans of various shapes and sizes, were generated using a FORTRAN language simulation program adapted from Teixeira et al. (1969). The University of British Columbia Amdahl 470/V8 computer was used for all simulations and calculations in this study.

The finite-difference model was based on the Fourier simplification of the differential heat conduction equation for a finite cylinder:

$$\frac{\partial^2 T}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial y^2} = \frac{1}{\alpha}\frac{\partial T}{\partial t} \quad (2)$$

where α is thermal diffusivity, T is temperature, t is time, r is radial distance from the central axis and y is vertical distance from the center plane. A numerical solution using the finite-difference form of this equation was the basis of the simulation program. Carlaw and Jaeger (1959) indicated that a sufficient condition for stability of the finite difference solution is:

$$M = \frac{\alpha \Delta t}{\Delta x^2} \leq \frac{1}{2} \quad (3)$$

where Δx is the grid spacing in either radial or axial direction. In this study, time increments of 0.05 min and grid spacings of 0.3–0.4 cm were used, corresponding to M values of 0.02–0.07 for the conditions tested, well below the requirements for stability of the solution. The number of vertical and radial elements used ranged from 6–25 and was determined for each simulation by the container shape and size.

For most of the processes simulated the retort temperature was 120°C, but some tests at 140°C were performed to test whether the relative errors in process lethality calculation were affected by retort temperature. Cooling water temperature was 100C° below retort temperature, to be consistent with the assumptions of Ball's and Stumbo's f_h/U to g tables. Instant come-up to retort temperature and instant environment temperature collapse to cooling water temperature were assumed, as would be the case for a continuous rather than batch processing situation. Thermal properties were held constant within each simulation, and the initial temperature distribution was uniform. Resistance to heat transfer at the surface was considered to be negligible, and the effect of headspace on heat transfer was not considered.

Thermal diffusivity values ranging from 0.075–0.125 cm^2/min were used, covering the range of thermal properties encountered commercially for conduction heating foods (Rha, 1975). Various initial temperature differences (retort temperature - product temperature) from 15–95C° and g values (temperature difference at steam off) from 0.05–15C° were investigated. Height-to-diameter (H/D) ratios from 0.1–3 were studied. For most of the study can diameters of 8 cm were used, except for cans with very small H/D ratios (0.1 and 0.25) for which larger diameters were used, to allow a minimum height of 2 cm to be used.

Adaptation for computer solution

Adaptation of the various formula methods for solution solely by computer required two major systems. One was a table accessing system and the other a system for determining f and j values from the time-temperature input data. Table access was accomplished by setting up files organized so that the line number corresponded to the g-value for each part of the table. The information on the file-line varied slightly from one method to another. In cases where tables were to be accessed in the course of a lethality determination, g values were converted from C° to F° to be compatible with available tables.

Determination of f and j values was accomplished using an iterative regression technique to locate the start of the straight-line portion of the heating and cooling curves (log g vs t and log m vs t, respectively). Linear regression was performed iteratively, deleting points up to the crossover of the fitted straight line and the data curve. This procedure was continued until the fitted line crossed the data curve close to the first point included in the regression (maximum relative difference 0.1%).

Each temperature history curve was evaluated for process lethality (F_o) using the reference method and each of the formula (test) methods. Deviations between F_o values of the reference and test methods were calculated as percentages of the reference F_o using:

$$\text{Error} = \frac{F_o(\text{ref}) - F_o(\text{test})}{F_o(\text{ref})} \times 100\%. \quad (4)$$

A positive percentage difference would indicate that the test method underestimated the actual process lethality.

RESULTS & DISCUSSION

INITIAL STUDIES were conducted to determine which factors had the greatest effect on the accuracy of the various process calculation methods in an attempt to reduce the number of experiments required to compare their performance. Even after this reduction, over 200 thermal history curves were evaluated. The initial studies showed that the temperature difference at the end of the cook (g) and the height-to-diameter ratio (H/D) were the most significant factors, resulting in a wide variation of error magnitude, with consistent error patterns. Varying the initial temperature difference resulted in small differences, mostly due to the effect on the evaluation of f_h and j_{ch}. A range of initial temperature differences from 20–95C° was included in the study; but data from initial temperature differences of 20 C° were later excluded because estimates of f_h were poor, especially when g was large, since straight-line heating behavior was not well established before the end of the heating cycle.

Can size, thermal diffusivity and f_h did not have large effects on error magnitudes. Some variability was noted (Tables 1 and 2) but the effects were small compared to the effects of other factors being studied.

Parallel thermal histories for retort temperatures of 120° C and 140° C were evaluated and the calculated percentage errors were found to be comparable (Table 3). All subsequent thermal histories were generated using a retort temperature of 120° C but the percentage errors presented can be considered to apply for higher retort temperatures as well. This is of particular significance when considering large g values since these are of greater importance for higher retort temperature processes than for processes at 120° C.

Effects of can shape and g value

Fig. 1 through 5 show the errors, relative to the reference method, that resulted from calculation of process lethality using each of the five test methods. Each point represents an average error for initial temperature differences of 35, 65, and 95 C°. The deviations are functions of both can shape (H/D) and g. The shapes of the error curves are

Table 1—Errors in calculated lethalities using five formula methods for various can sizes and values of g (H/D=1.35)

g (C°)	Radius (cm)	Percent error: Ball's tables	Ball's equation	Steele & Board	Hayakawa	Stumbo
15	3	59.4	67.8	56.6	46.8	17.1
15	4	60.3	68.6	57.3	47.5	15.9
15	5	60.7	69.0	57.7	47.5	13.8
5	3	31.3	40.4	29.2	20.9	10.1
5	4	32.1	41.2	30.0	21.8	10.7
5	5	32.4	41.4	30.0	21.4	9.2
1.5	3	18.0	22.7	15.1	9.5	4.9
1.5	4	18.4	23.0	15.6	10.2	5.9
1.5	5	18.5	23.1	15.7	10.2	5.5
0.5	3	12.1	14.5	9.4	5.7	2.1
0.5	4	12.3	14.7	9.6	5.8	2.1
0.5	5	12.3	14.7	9.7	6.0	2.2
0.15	3	9.1	9.8	6.3	3.8	1.9
0.15	4	9.2	9.8	6.4	3.8	1.6
0.15	5	9.2	9.7	6.4	3.8	1.5

Table 2—Errors in calculated lethalities using five formula methods for various thermal diffusivities and f_h (H/D=1.0; g=5 C°)

f_h (min)	α (cm^2/min)	Percent error: Ball's tables	Ball's equation	Steele & Board	Hayakawa	Stumbo
30	0.075	35.2	43.8	32.6	24.2	11.4
30	0.100	35.6	44.2	32.9	24.4	11.4
30	0.125	35.9	44.5	33.2	24.7	11.6
50	0.075	35.7	44.3	33.1	24.7	11.8
50	0.100	36.2	44.7	33.7	25.5	12.8
50	0.125	36.4	44.9	33.9	25.7	13.1
70	0.075	36.1	44.7	33.3	24.7	11.0
70	0.100	36.4	44.9	33.6	25.1	11.1
70	0.125	36.7	45.2	33.9	25.4	11.7
90	0.075	36.4	44.9	33.7	25.2	11.7
90	0.100	36.6	45.1	33.9	25.4	11.9
90	0.125	36.8	45.2	34.1	25.6	12.2

Table 3—Errors in calculated lethalities using five formula methods for various values of g and two retort temperatures (H/D=1.35)

g (C°)	Retort (°C)	Percent error: Ball's tables	Ball's equation	Steele & Board	Hayakawa	Stumbo
15	120	60.3	68.6	57.3	47.5	15.9
15	140	60.3	68.7	57.5	47.6	16.1
5	120	32.1	41.2	30.0	21.8	10.7
5	140	32.1	41.2	30.0	21.8	10.7
1.5	120	18.4	23.0	15.6	10.2	5.9
1.5	140	18.5	23.2	15.8	10.5	6.4
0.5	120	12.3	14.7	9.6	5.8	2.1
0.5	140	12.5	14.9	9.8	6.1	2.5
0.15	120	9.2	9.8	6.4	3.8	1.6
0.15	140	9.4	10.0	6.6	4.0	1.9
0.05	120	5.8	7.0	4.7	2.9	0.9
0.05	140	6.1	7.4	4.8	2.9	0.8

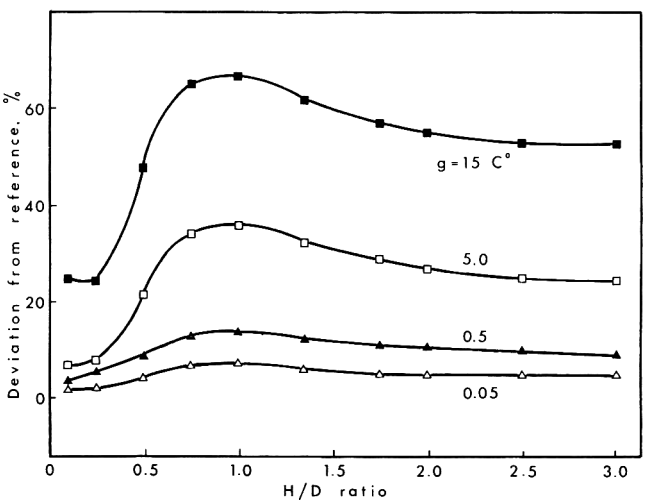


Fig. 1—Errors in process lethality determinations using Ball's tables.

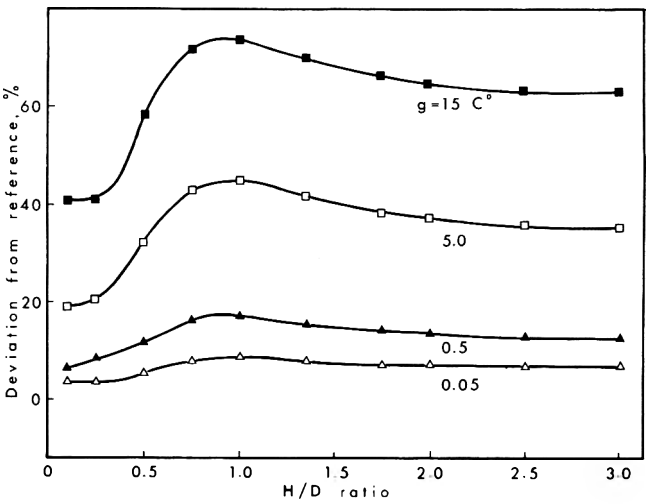


Fig. 2—Errors in process lethality determinations using Ball's equation.

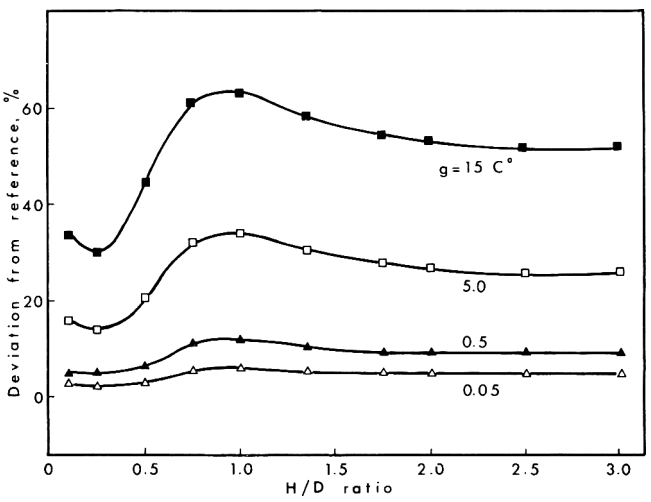


Fig. 3—Errors in process lethality determinations using Steele and Board's method.

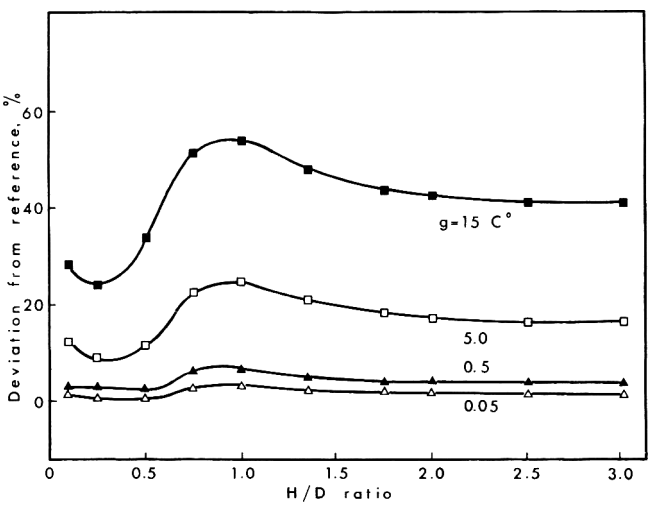


Fig. 4—Errors in process lethality determinations using Hayakawa's method.

similar for Ball's table method, Ball's equation method, Steele and Board's method and Hayakawa's method, although the magnitudes of the errors differ. The error curves for Stumbo's method have similar characteristics, but do not appear to be simple functions of H/D, especially for large values of g. In almost all cases these errors were underestimations of process lethality, that is, errors on the "safe" side.

These deviations were smallest for small H/D, increased to a maximum when H/D was near unity, then decreased slowly to an intermediate value as H/D became large. It might be expected that the errors influenced by can shape would be a function of the cooling lag factor, since a plot of j as a function of H/D has a shape similar to the plot of error magnitude (Ball and Olson, 1957). Fig. 6 shows that although the errors tended to be greater for larger j_{cc} values, the trend was not a smooth function. Therefore, some other factor or factors, must also be influencing the error magnitude, and these would be expected to be related to the H/D ratio, since the plot of error magnitude as a function of H/D is a smooth and continuous curve in most cases. The shape effect is believed to be a result of differences in cooling curve shape resulting from the temperature gradient through the can at the end of the heating cycle.

Errors in lethality determination were also found to be a function of g, increasing as the value of g increased. The effect of g value on lethality determination errors may be a result of the temperature gradient from the surface to the center of the container at the end of the heating cycle. During the heating cycle, the temperature near the surface of the can is higher than that near the center. If the heating cycle is continued until g is small, this temperature gradient becomes insignificant. However, if the process is stopped when g is large, the gradient causes the temperature at the center of the can to continue to rise for a period of time after the start of the cooling cycle and the center temperature of the can may not begin to drop for several minutes. This effect is not accounted for in any of the formula methods tested.

Comparison of methods

Fig. 7 shows a comparison of the errors associated with the five formula methods tested. Errors for only one value of g are shown, but the trends were similar for other values of g, although the error magnitudes differ (Fig. 1–5). The value of g=5C° used in Fig. 7 is in the range of the largest g values that would be encountered in conventional processing.

Calculation of process lethality using Ball's table method resulted in relatively large errors, indicating that the formula method approach has been significantly improved by more recent modifications. Steele and Board's method, having eliminated the assumptions of only one value for j_{cc} and equal heating and cooling rates, performed slightly better than Ball's table method under most conditions. Hayakawa's method, which estimated the cooling lag using circular functions, resulted in smaller errors than either of these methods. The largest error for a g value of 5 C° using Hayakawa's method was 25% compared to 36% using Ball's tables and 33% using Steele and Board's method. Use of Ball's equation method resulted in the largest errors (up to 44% for g=5 C°).

Stumbo's method appears to be the most accurate formula method of those tested. However, when g was very small, this method overestimated the process lethality slightly, in some cases. Stumbo's method also resulted in highly variable accuracy when g was large (Fig. 5).

– Continued on next page

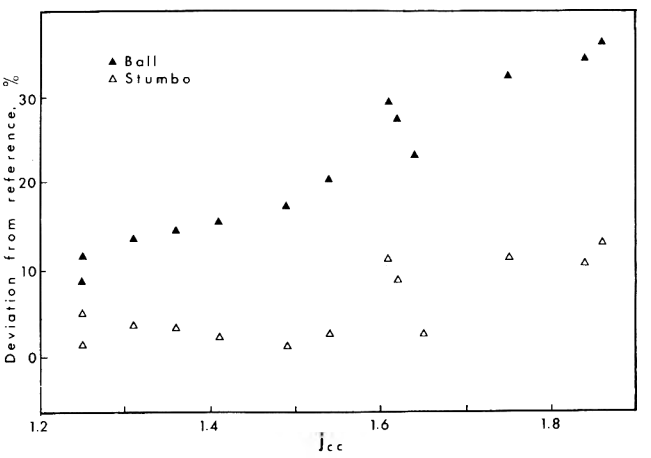


Fig. 6—Errors in process lethality determinations as related to the cooling lag factor (g=5C°).

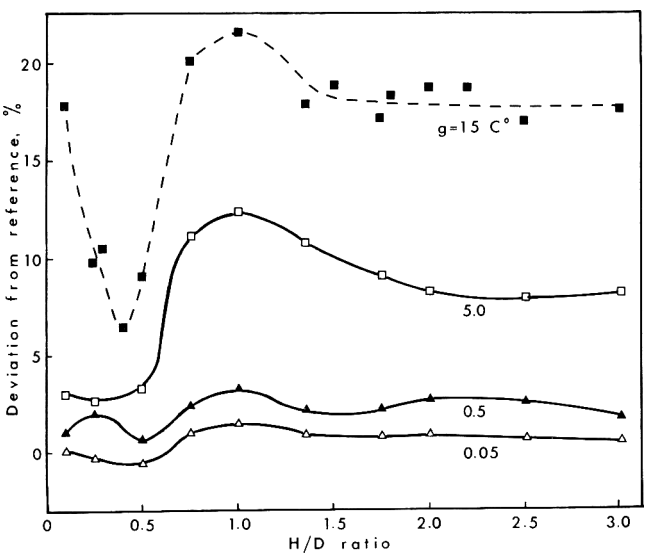


Fig. 5—Errors in process lethality determinations using Stumbo's method.

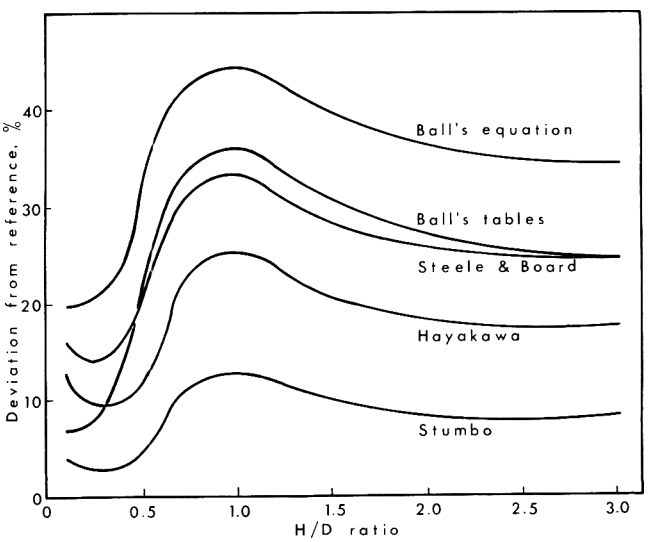


Fig. 7—Errors in process lethality determinations using five formula methods (g=5C°).

Calculation errors in terms of processing time

Since all of the methods underestimated the lethality of thermal processes for conduction heating foods, the process times calculated using these methods would be longer than required to achieve a specified target process lethality. Fig. 8 shows that Ball's method overestimated required process times by 6 to 7 min for a process of one hour or more. Although this figure shows data for only one can size and initial temperature, errors were similar for other conditions. Stumbo's method overestimated required process times by only about 2 min, which is approaching the accuracy of process control for manually operated retorts.

Although these errors could be considered to be extra safety factors, they are influenced by can shape and processing conditions and therefore do not provide a constant margin for error. Safety margins are certainly necessary, but should be well defined, to help to assure product safety. Statistical analysis of variability, as suggested by Lund (1978) may be a reasonable approach to this problem.

Convection heating products

It must be noted that the results reported here apply to foods that heat and cool by conduction only. Products or processing methods for which natural or forced convection are significant factors in heat transfer will experience a shortened lag before cooling begins. Thus the formula methods can be expected to estimate lethality more accurately for convection heating products than for conduction heating products. In order to achieve similar accuracy in process estimation for both conduction and convection heating products, different evaluation methods may be required.

CONCLUSIONS

THE FIVE FORMULA METHODS for determining process lethality showed deviations from the reference general method for conduction heating foods in cylindrical containers under a wide range of conditions. Deviations were greatest when g values were large, as might be encountered in high retort temperature processing. Errors were also found to vary according to the shape (H/D) of the can, with the greatest errors corresponding to H/D close to unity. Can size, thermal diffusivity, heating rate (f_h), initial temperature difference and retort temperature did not greatly affect error magnitudes. Under all conditions examined, Stumbo's method was found to give the best estimates of process lethality, but the method was more sensitive to slight variations in f_h and j_{cc} than were the other methods.

Use of these formula methods to calculate process times would result in slight overestimates of required processing time for conduction heating products. This overprocessing represents an extra safety margin, but could also be significant in terms of energy use and plant throughput.

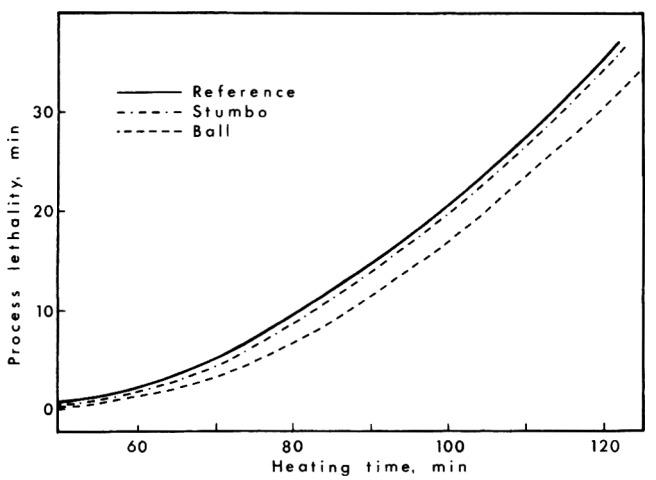


Fig. 8—Calculated lethal effect relative to processing time (H/D= 1.0, I_h=95C°).

REFERENCES

Ball, C.O. 1923. Thermal process time for canned food. Bull. 7-1 (37) Nat'l. Res. Council, Washington, DC.
Ball, C.O. and Olson, F.C.W. 1957. "Sterilization in Food Technology," McGraw-Hill Book Company, Inc. New York, NY.
Bigelow,W.D., Bohart, G.S., Richardson, A.C., and Ball, C.O. 1920. Heat penetration in processing canned foods. Bulletin No. 16-L. Res. Lab. Nat'l. Canners Ass'n., Washington, DC.
Carslaw, H.S. and Jaeger, J.C. 1959. "Conduction of Heat in Solids," 2nd ed. Oxford University Press, U.K.
Downes, T.W. and Hayakawa, K.-I. 1977. A procedure for estimating the retention of components of thermally conductive processed foods. Lebensm.-Wiss. u. Technol. 10: 256.
Griffin, R.C., Herndon, D.H., and Ball, C.O. 1971. Use of computer derived tables to calculate sterilizing processes for packaged foods. 3. Application to cooling curves. Food Technol. 25(2): 134.
Hayakawa, K.-I. 1970. Experimental formulas for accurate estimation of transient temperature of food and their application to thermal process evaluation. Food Technol. 24(12): 1407.
Hayakawa, K.-I. 1977. Mathematical methods for estimating proper thermal processes and their computer implementation. Adv. Food Res. 23: 75.
Hayakawa, K.-I. 1978. A critical review of mathematical procedures for determining proper heat sterilization processes. Food Technol. 32(3): 59.
Lund, D.B. 1978. Statistical analysis of thermal process calculations. Food Technol. 32(3): 76.
Merson, R.L., Singh, R.P., and Carroad, P.A. 1978. An evaluation of Ball's formula method of thermal process calculations. Food Technol. 32(3): 66.
Rha, C. 1975. Thermal properties of food materials. In "Theory, Determination and Control of Physical Properties of Food Materials," Ed. C. Rha. D. Reidel Publishing Company, Boston, MA.
Smith, T. and Tung, M.A. 1979. Unpublished data.
Steele, R.J. and Board, P.W. 1979. Thermal process calculations using sterilizing ratios. J. Food Technol. 14: 227.
Steele, R.J., Board, P.W., Best, D.J., and Willcox, M.E. 1979. Revision of the formula method tables for thermal process evaluation. J. Food Sci. 44: 954.
Stumbo, C.R. 1973. "Thermobacteriology in Food Processing," 2nd ed. Academic Press, Inc., New York, NY.
Stumbo, C.R., and Longley, R.E. 1966. New parameters for process calculation. Food Technol. 20(3): 341.
Teixeira, A.A., Dixon, J.R., Zahradnik, J.W., and Zinmeister, G.E. 1969. Computer optimization of nutrient retention in the thermal processing of conduction heating foods. Food Technol. 23(6): 848.

Ms received 6/22/81; revised 9/8/81; accepted 9/11/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, Ga., June 7—10, 1981.
This study was supported in part by the Natural Sciences & Engineering Research Council of Canada.

Rheological Evaluation of Maturing Cheddar Cheese

LAWRENCE K. CREAMER and NORMAN F. OLSON

ABSTRACT

A number of Cheddar cheese samples of different age, pH and moisture content have been examined rheologically and electrophoretically to determine whether the progressive changes in cheese texture were related to casein proteolysis. The force-compression curves obtained by crushing cubes of cheese between small flat plates at constant speed were different for the different cheese samples and were affected by the moisture content, pH and extent of α_{s1}-casein proteolysis that had taken place in the cheese. These results support a model of cheese microstructure in which an extensive network involving α_{s1}- casein molecules traverses the cheese and as the cheese ripens, chymosin cleavage of α_{s1}-casein weakens the protein network. Such a model explains the rapid decrease in Cheddar cheese yield-force that occurs during the early stages of ripening.

INTRODUCTION

THE TEXTURE OR BODY of a cheese is one of the important characteristics that determines the identity and the quality of a cheese. Early studies, which have been summarized by Baron (1952) and Baron and Scott Blair (1953) concentrated on the relationship between manufacturing parameters and the rheological quality, or acceptability, of the finished cheese. Since that time cheeses having a surface "rind" which exhibited uneven and time-dependent moisture contents have virtually disappeared from the market, pasteurization of cheese milk has become widespread, some of the major steps in casein proteolysis during cheese ripening have been elucidated (Creamer, 1979) and textural analysis has been simplified by the use of constant-speed materials testing instruments leading to the development of the texture profile analysis (TPA) technique (Szczesniak, 1975). Although these latter methods have been applied to a large range of cheese samples and cheese varieties in recent years (Shama and Sherman, 1973; Culioli and Sherman, 1976; Lee et al., 1978; Vernon-Carter and Sherman, 1978; Chen et al., 1979; Imoto et al., 1979; Dickinson and Goulding, 1980; Emmons et al., 1980), surprisingly few studies on the relationship between manufacturing parameters, compositional data and rheological properties of cheese have been reported.

The present study has been restricted to a single cheese variety, Cheddar, although some of the samples were deliberately manufactured so that their compositions were outside the normal range for good quality cheese. In this way it has been possible to make a preliminary examination of how cheese composition and casein proteolysis in Cheddar cheese is related to the rheological behavior of the cheese at room temperature.

MATERIALS & METHODS

CHEDDAR CHEESE was manufactured in the University of Wisconsin Dairy Plant as required using 160 liter vats, purchased from a single commercial factory or was taken from store as surplus from other (unrelated) experiments. Lower pH in the manufactured cheese was achieved by attaining a lower curd pH when the whey was drained from the curd and conversely a higher pH was achieved by rinsing the curd prior to salting to remove some of the lactose. Some compensation in the other manufacturing parameters was made to attain a more nearly constant moisture content in the finished cheeses (Table 1).

Cheese samples were cut into 2 cm cubes with the cheese cutter shown in Fig. 1. Some of these samples were weighed and sealed into individual polyethylene plastic bags together with small (0.2–0.8 ml) quantities of 4% (w/v) NaCl solution to adjust moisture content of these samples. Although these solutions were fully absorbed into the cheese samples within 36 hr at room temperature (20–25°C), the samples were held for about 72 hr at room temperature prior to compression testing.

Samples for compositional analysis were taken when the cheeses were ten days old or when they were purchased. Samples for electrophoresis were taken at the time of compression testing and were stored frozen.

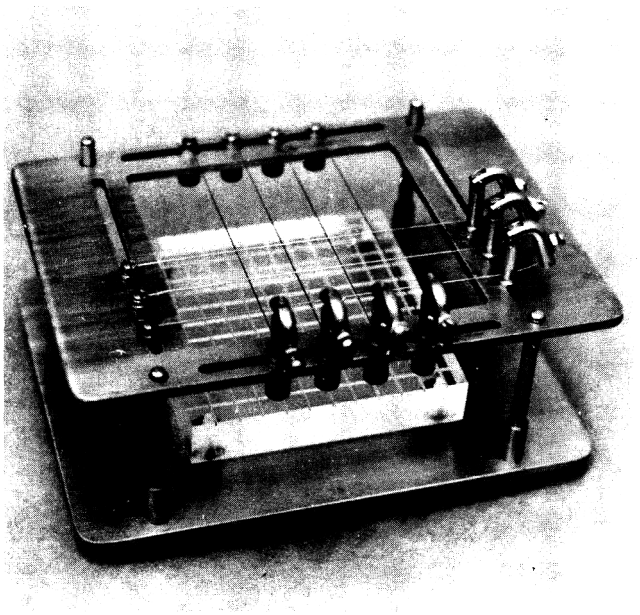


Fig. 1—Photograph of the cheese cutter used to obtain the 2 cm cubes of cheese. All samples were cooled to between 0 and 4°C before cutting to prevent them being distorted.

Table 1—Manufacturing conditions used for preparing a series of Cheddar cheese

	VAT 1[a]	VAT 2[b]	VAT 4
Initial milk pH	6.60	6.60	6.60
Starter added, %	.6	1.0	1.5
Cooking temp, °C (°F)	40 (104)	40 (104)	36.7 (98)
Draining pH	6.35	–	6.08
Draining time, hr:min	3:00	3:00	3:20
Milling pH	5.90	5.55	5.08
Milling time, hr:min	4:15	4:15	5:15

[a] One-third of the whey in Vat 1 was replaced with water at 40°C at the beginning of cooking and the quantity of whey reduced. The curd was also washed with 46°C (115°F) water at milling.
[b] The curd in Vat 2 was rinsed with 47.8°C (115°F) water at milling.

Author Olson is with the Dept. of Food Science and the Cheese Research Institute of the Univ. of Wisconsin, Madison, WI 53706. Author Creamer is on leave from the New Zealand Dairy Research Institute, Palmerston North, New Zealand.

Electrophoresis and densitometry were carried out essentially as outlined earlier (Creamer, 1970; Richardson and Creamer, 1974).

The compression testing was carried out using the T5002 MTS Tensile Testing Machine manufactured by J.J. Lloyd Instruments Ltd. (Southhampton, England), and the data recorded on a Hewlett Packard 7045A X-Y recorder. The tensile tester was fitted with a compression cage and the recorder was generally run at a speed (X-axis) of 0.254 cm/sec. The MTS tester was usually run in its cycling mode with the compression plates opening to 2.1 cm and closing to 0.4 cm to give a compression ratio (on a 2 cm piece of cheese) of 80% and the crosshead speed was usually adjusted to 5 cm/min in both directions. Two small blocks were made so that each had a 2 cm square face and these could be used in the compression cage to provide 2 cm square compression plates (Fig. 2).

RESULTS

Preliminary examination

A 2-wk and a 3-yr old Cheddar cheese of essentially the same fat and moisture contents were examined using the MTS tester with the compression cage. The small blocks were also used to provide 2 cm square faces to crush some of the 2 cm cubes of cheese. Fig. 3 shows the force-compression curves obtained when the plates were brought as close as possible to each other, i.e. to the greatest practical compression. In all cases the cheese deformed on compression and the force on the cheese increased until the yield point was reached. As the compression increased beyond the yield point, the force decreased to give a trough or plateau and then rose sharply again when the compression plates approached closely. This effect was greatest as illustrated in Fig. 3A when the normal compression plates were used because at high compressions the cheese was being compressed over an increasingly wide area while the maximum area being compressed with the small blocks was 4 square cm as shown in Fig. 3B. Thus the curves obtained with the small blocks were less distorted than when the normal compression plates were used. In some instances a yield point could not be discerned when the normal compression plates were used. In some instances a yield point could not be discerned when the normal compression plates were used. The remainder of the data reported were obtained using the small blocks to provide small compression plates.

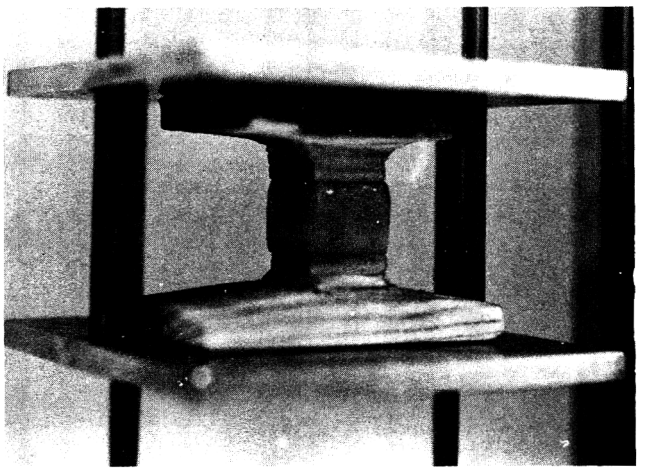


Fig. 2—Compression cage of the MTS tester with the small blocks in position at the beginning of the compression cycle.

Crosshead speed

The effect of rate of compression on the force required to compress the cheese to the yield point was measured. The results for a sample of young cheese are presented, in log-log form, in Fig. 4. The compression at the yield point was the same at all crosshead speeds although the force increased with increased crosshead speed. The recorder used was especially designed for high speed plotting of complex signals and the pen was capable of a speed of 97 cm/sec. Consequently the pen speed of the recorder was unlikely to be a limitation at the highest crosshead speed used. Similar results, but with lower yield compressions and forces, were obtained with a sample of older cheese. The remainder of the data reported were obtained using a crosshead speed of 5 cm/min.

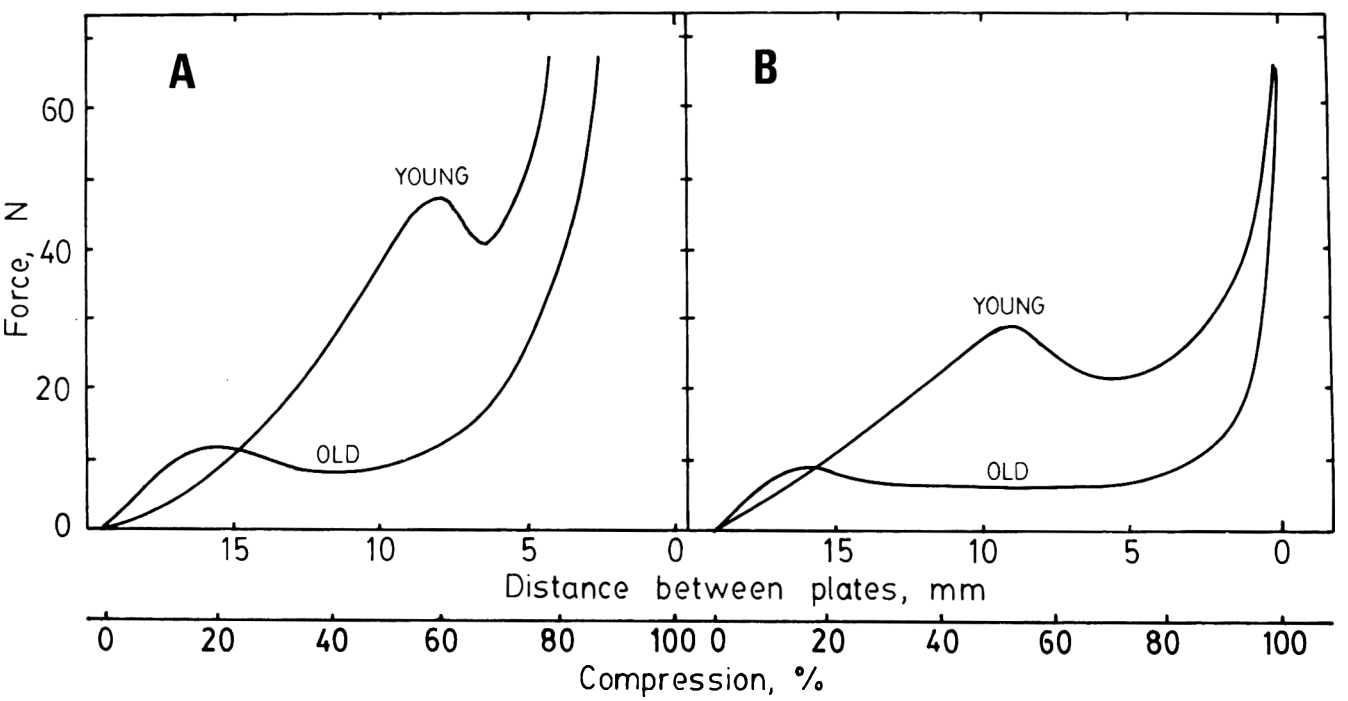


Fig. 3—Force-compression curves for young and old cheese using the MTS tester with the compression cage. In (B) two small blocks are placed above and below the cheese cube so that it is compressed over a constant area of 4 square cm.

Effect of age and extent of proteolysis

Fig. 5 shows the force-time curves for the 14-day and 107 wk cheese samples (Table 2) using the MTS tester in the constant-speed cycling mode (the double "bite" of the texture profile analysis method; see Szczesniak, 1975). It can be seen that the initial upward slope of the force-time curve was concave for the younger cheese but steeper and convex for the older cheese. However, the yield point occurred at both greater force and greater compression for the younger cheese. Examination of the second "bite" curves show that the younger cheese recovered more after the first compression and the ratio of the areas under the second peak to the first peak was greater for the younger cheese (i.e. the younger cheese was more springy and more cohesive). All these results are consistent with the younger cheese being more elastic than the older cheese. Samples 3 to 8 (Table 2) in this series all had force-time curves that were intermediate between those shown in Fig. 5 (samples 2 and 9). The principle characteristics of the yield points are listed in Table 2. The yield forces were much higher for the two youngest cheese and much lower for all the other samples. However the yield compression was more nearly related to the age of the cheese. The electrophoretic patterns of casein proteolysis (Fig. 6) and the derived casein contents of these cheese samples (Table 2) show that samples 3 to 7 are quite similar with most of the α_{s1}-casein degraded to α_{s1}-I but with the β-casein essentially intact while samples 1 and 2 show very little proteolysis and sample 9 has very little intact casein remaining. The yield force results for these cheese samples seem to be related to the content of intact α_{s1}-casein in the cheese while the changes in compression at the yield point seem to be related to NPN content of the cheeses.

Effect of pH and moisture content

Two attempts were made to manufacture cheeses with the same fat, salt and moisture contents but with different pHs. The analyses of these cheese are shown in Table 3. The first attempt produced cheese of moderately different pH but markedly different moisture contents. This presented an opportunity to examine the effect of added salt

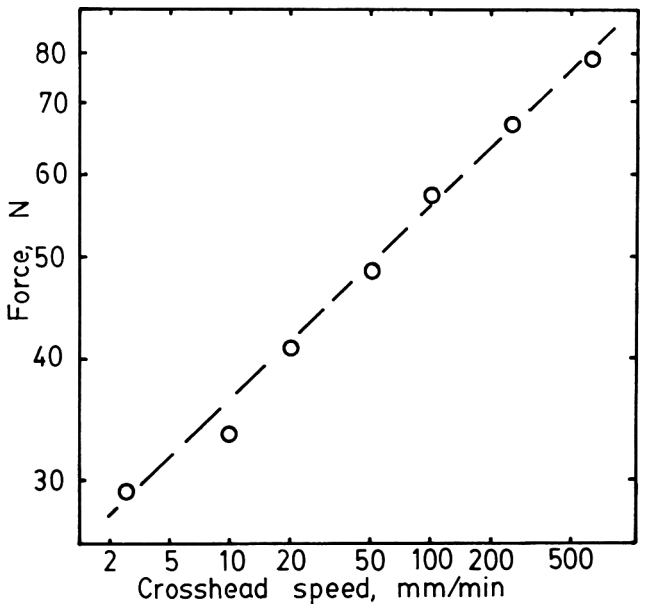


Fig. 4—Plot of the variation in the force at the yield point versus the rate of compression. The correlation coefficient of the regression line was 0.997.

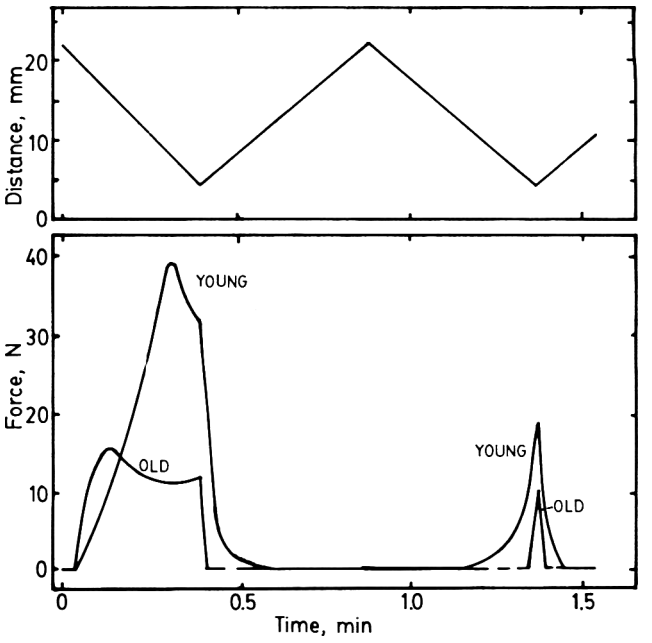


Fig. 5—Variation in compression force with time over 2 cycles of compression. The young and old cheeses are samples 2 and 9 of Table 2. The upper curve shows the variation in the distance between the faces of the compression plates.

Table 2—Composition and rheological data on commercial Cheddar cheeses of various ages purchased from one manufacturing plant

Cheese sample	Age at analysis	pH	Fat (%)	Moisture (%)	NaCl (%)	NPN[a] (mg/g cheese)	NCN[a] (mg/g cheese)	TN[a] (mg/g cheese)	Casein content (% of fresh curd β-casein content)[b] α_{s1}–	α_{s1}–I	β–	Compression at yield point (%)	Force at yield point (N)
1	6 d	5.23	32.5	37.6	1.88	2.29	5.46	41.8	95	5	100	72	45.0
2	14 d	5.26	34.5	34.5	1.88	3.12	6.72	43.5	85	15	105	64	37.0
3	10 wk	5.07	32.5	36.3	1.89	4.91	8.24	42.6	10	60	95	50	14.7
4	18 wk	5.07	34.5	35.9	1.91	4.13	7.89	41.9	45	50	100	42	19.5
5	28 wk	5.07	34.5	35.9	2.02	6.10	9.48	39.6	15	40	90	46	14.6
6	35 wk	5.04	34.5	35.7	2.06	6.06	10.41	40.3	15	35	85	32	18.1
7	44 wk	5.04	35.5	35.1	2.05	6.48	10.03	41.8	15	40	80	36	20.5
8	49 wk	5.10	33.5	36.6	1.89	7.52	10.79	42.3	5	20	50	30	18.8
9	109 wk	5.08	33.5	36.0	2.00	10.84	14.40	40.2	5	5	30	24	13.4

[a] NPN—nonprotein nitrogen; NCN—noncasein nitrogen; TN—total nitrogen content. All these were determined by the Kjeldahl method.
[b] Each cheese sample was the same weight and each sample (2% w/v) used for electrophoresis was the same size (20 μl). The densitometer data were rounded to the nearest 5%.

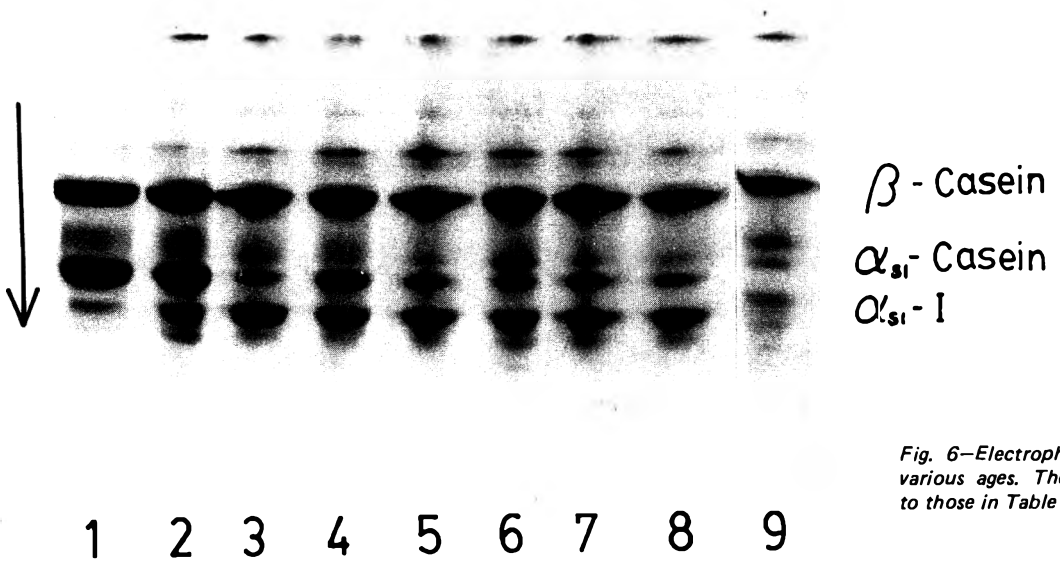


Fig. 6—Electrophoretic patterns of cheeses of various ages. The sample numbers correspond to those in Table 2.

Table 3—Composition of both series of Cheddar cheese manufactured for rheological study

Vat no.	pH	Fat (%)	Moisture (%)	Salt (%)	MNFS[a]	S/M[a]
			Manufactured Nov., 1980			
1	5.12	32.0	39.7	1.24	58.4	3.1
3	4.93	34.5	34.9	1.79	55.0	5.1
4	5.00	33.5	37.2	1.38	55.9	3.7
			Manufactured Feb., 1981			
1	5.40	32.5	39.1	1.30	57.9	3.3
2	5.15	33.0	37.5	1.34	56.0	4.1
4	4.88	33.0	37.7	1.20	56.3	3.2

[a] MNFS—Moisture in the nonfat substance; S/M—Salt in moisture

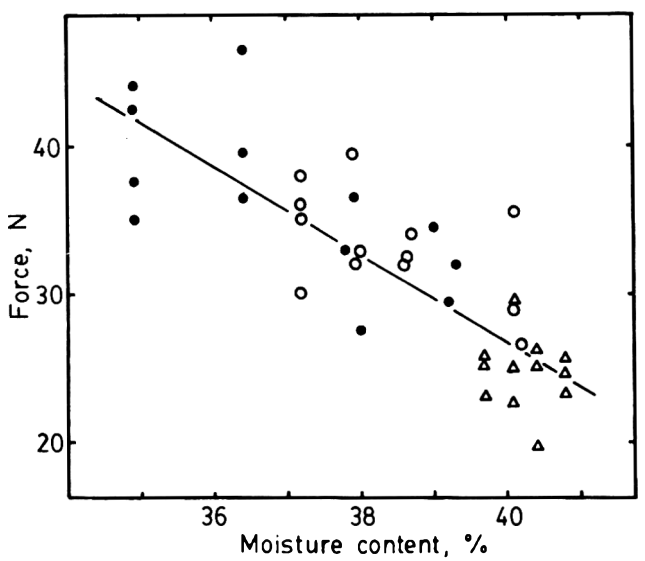


Fig. 7—Variation in the force at the yield point for three cheeses whose samples had had small quantities of 4% (w/v) NaCl added to them. •, low pH (Vat 3; Nov., 1980); ○, medium pH (Vat 4; Nov., 1980); △, high pH (Vat 1; Nov., 1980). See Table 3 for cheese composition. The correlation coefficient for the regression line was 0.820.

or water (as 4% w/v NaCl solution) on the properties of the cheese samples. It was found that up to 0.5% (w/w) added salt (either as solid crystals, or as concentrated solutions with 4% NaCl solution as a control) did not have an observable effect on the force-time curves of the cheese. However, added moisture in the form of 4% (w/v) NaCl solution decreased the force at the yield point but, with a few exceptions, the compression at the yield point was the same for each cheese sample regardless of the quantity of added water.

Fig. 7 shows the yield force versus moisture content for all three cheese samples whose compositions are shown in the upper part of Table 3. It can be seen that all the points are close to a single straight line. Electrophoretic examination of the three cheese samples showed that close to 50% of the α_{s1}-casein had been converted to α_{s1}-I in all three cheeses. Consequently it seems likely that within experimental error Cheddar cheese of similar pH and at the same extent of proteolysis have yield forces that are influenced by moisture content alone. Compression at the yield point however, was perceptibly influenced by some factor (possibly pH) that varied with the cheese samples (Table 4) but was not affected by moisture content of the cheese.

The second set of cheese samples was manufactured to obtain closer moisture contents and a wider range of pH. The compositions of samples are also shown in Table 3. Unfortunately it was not possible to make cheeses with this pH range and with a lower moisture content. Typical force-time curves for the three cheese samples are shown in Fig. 8. It can be seen that these are markedly different. The low pH cheese has a steep convex force-time curve with a low yield-point force and compression. The high pH cheese has the opposite characteristics while the third cheese is intermediate. The yield forces and moisture contents are plotted for all the samples at 7 wk in Fig. 9 while Fig. 10 shows the yield compressions versus moisture content for the same samples. It can be seen that the yield force decreases with increasing moisture content while the compression ratio is virtually invariant. It can also be seen that at each pH the yield force decreases as the cheeses age (Table 4) but compression increases with the high pH cheese whereas it decreases with the other two cheeses (Table 4).

One feature of the behavior of the high pH cheese was the greater variation from sample to sample (Fig. 9). This was caused, in part, by the difference in response of the high and low pH cheese to compression. The high pH cheese stretched until a sudden split in the cheese occurred

and thus the force attained for any individual sample was dependent upon the number of minor defects in the consistency of the cheese. The low pH cheese, however, was gradually crushed between the compression plates with no sudden change in response of the cheese to the applied force.

Fig. 11 shows the electrophoretic patterns of the cheeses manufactured in February 1981 at the times when they were examined rheologically. It can be seen that the low pH cheese had the lowest content of α_{s1}-casein at each age and that the high and medium pH cheeses were similarly degraded at each age.

DISCUSSION

THE PRESENT RESULTS of this preliminary investigation into the compositional factors that might affect the texture of cheese, have shown that moisture content, pH and casein proteolysis are all important.

Cheddar cheese is particularly interesting because it is intermediate (Shama and Sherman, 1973) in its textural properties between the crumbly cheeses such as Cheshire or Feta (Vernon-Carter and Sherman, 1978; Dickinson and Goulding, 1980) and the soft, plastic cheeses such as Gouda or Colby (Culioli and Sherman, 1976). The present study has shown that it is possible to use the Cheddar process to produce either of these two types of cheese by altering the

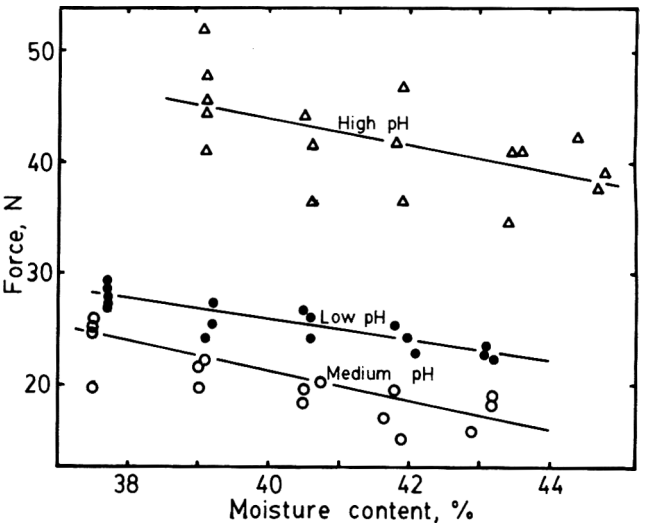


Fig. 9—Plots of variation in force at the yield point for 7 wk old cheese samples with added NaCl solution •, low pH (Vat 4; Feb., 1981); ○, medium pH (Vat 2; Feb., 1981); △, high pH (Vat 1; Feb., 1981). See Table 3 for cheese composition. The correlation coefficients of the regression lines were 0.849, 0.811, and 0.592 for the low, medium and high pH cheese respectively.

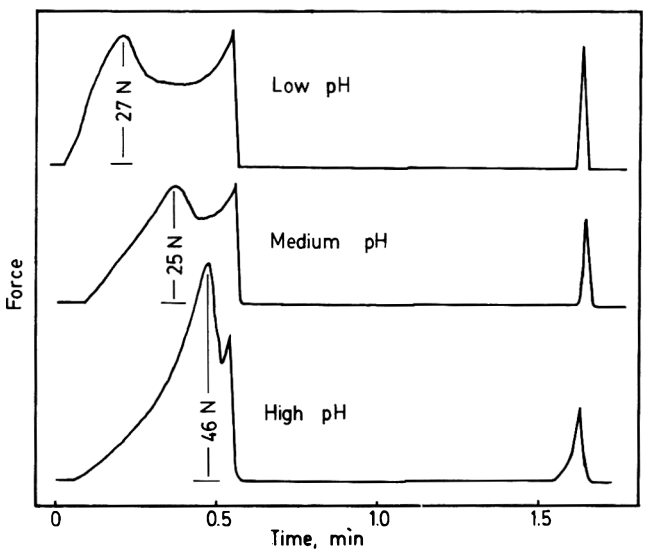


Fig. 8—Force-time compression curves at 7 wk of age for the low (upper curve), medium (middle curve), and high (lower curve) pH cheeses manufactured Feb.,1981.

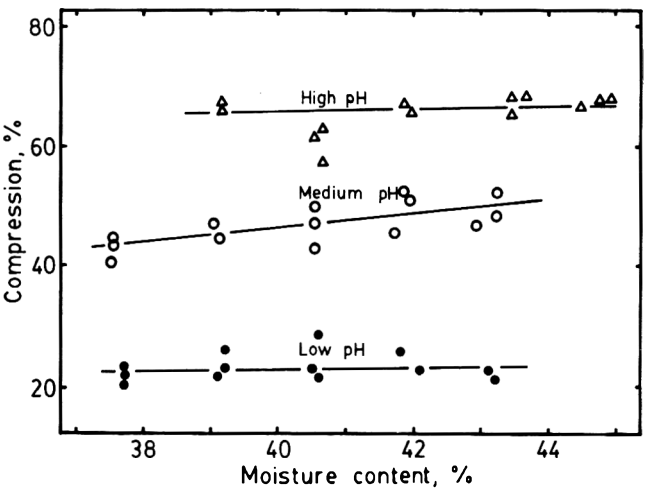


Fig. 10—Plots of variation in compression with moisture content at the yield point for 7 wk old cheese samples. See Fig. 9 for symbols.

Table 4—Yield point data for the specially manufactured Cheddar cheese[a]

Vat Number	pH	Compression (%) 1 wk	Compression (%) 3 wk	Compression (%) 7 wk	Force (N) 1 wk	Force (N) 3 wk	Force (N) 7 wk
			Manufactured Nov., 1980				
3	4.93			26 ± 0.7			42 ± 0.3
4	5.00			39 ± 4.0			36 ± 0.9
1	5.12			55 ± 0.7			25 ± 0.1
			Manufactured Feb., 1981				
4	4.88	32 ± 0.1	27 ± 0.7	22 ± 1.0	37 ± 2.2	32 ± 0.5	28 ± 0.8
2	5.15	48 ± 2.0	48 ± 2.0	42 ± 1.8	47 ± 4	35 ± 2.5	24 ± 1.5
1	5.40	56 ± 1.2	61 ± .7	67 ± .7	60 ± 6	55 ± 3	44 ± 2.5

[a] Each result is the average of 3 or 4 individual measurements; standard deviations are shown.

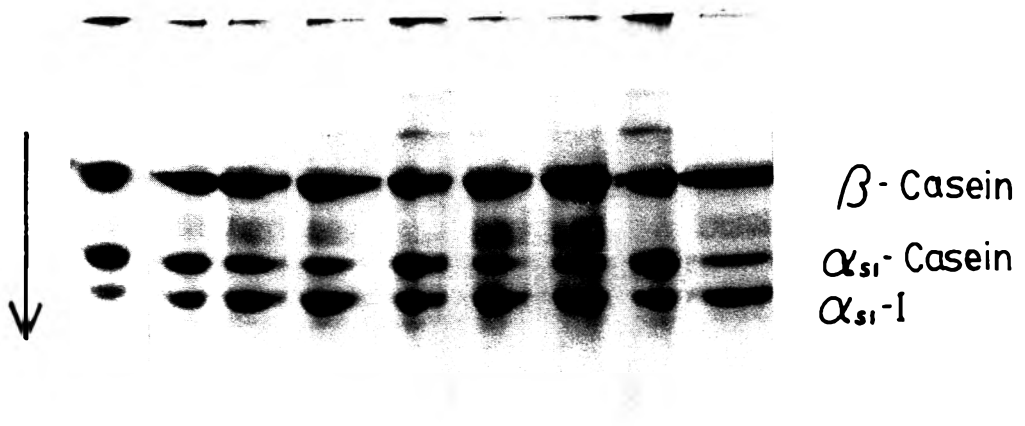


Fig. 11—Electrophoretic patterns of the cheeses made in Feb., 1981, at each time a rheological study was made.

parameters that affect the final pH of the cheese. A low pH cheese has a steep convex force-compression curve and crumbles at the yield point while a high pH cheese has a less steep, concave, force-compression curve (Fig. 8) and at the higher yield point it splits into large fragments. Young Cheddar cheese of normal pH has the curve shape characteristics of the high pH cheese but these change to be more similar to those of a low pH cheese as the Cheddar matures, Fig. 3.

Baron (1949) examined the rheological properties of a group of Cheddar cheese over a period of nearly a year. She found that the hardness increased and the elasticity decreased with the age of the cheese with the greatest changes occurring during the first 30 days. The elasticity results support the present conclusions but the hardness increase seems to be in direct contradiction to the present results (Table 2). However, consideration of the methods used in the two studies suggests an explanation. Baron used a weighted ball and measured its depth of penetration into large pieces of cheese, whereas the present study measured the force at the yield point of a piece of cheese. Consequently Baron's measure of hardness should be more closely related to the initial slope of the force-compression curve, which indeed increased with the age of the cheese (see Fig. 5). Thus there is concurrence between the earlier and the present results.

At first sight it is not easy to see why many cheeses should have either of two distinctly different textures; "plastic" or "granular." the high-pH plastic cheeses are elastic but flow readily while the low pH granular cheeses are less easily deformed by shatter at their yield point.

Cheese is made up from the fat and protein (casein) of milk and clearly the fat globules are unlikely to be affected by the pH of their surroundings. The major casein fractions have isoelectric points near 4.5 which is close to that of low pH cheese (Table 3). At their isoelectric points the casein fractions form compact aggregates which are held together with strong ionic and hydrophobic intra-aggregate forces while the inter-aggregate forces are weaker. Most of the water in such a system is inert, interstitial and not distributed evenly throughout the curd mass. By contrast, at a higher pH, the casein molecules acquire a net negative charge and while the hydrophobic interactions persist the ionic interactions change from attraction between the protein molecules to repulsion. Thus the tight protein aggregates absorb water partly to solvate the un-neutralized ionic charges and, if the pH is high enough and there is enough water present, the proteins can then dissolve. In cheese, however, dissolution is prevented both by the presence of calcium which binds tightly to the casein and reduced its solubility and by the limited quantity of water available. Nevertheless the tendency to absorb water into the protein matrix is strong and there is unlikely to be interstitial water in the high pH cheese. Thus, the picture of high pH cheeses as concentrated protein emulsions and low pH cheeses as porous masses of casein and fat particles is an aid to our understanding of the rheological properties of Cheddar cheese which is intermediate between these two extremes described above.

Now why should aging affect Cheddar cheese as it does, altering its structure to be less elastic and pliant? It seems likely that α_{s1}-casein can interact strongly with two, or possibly more, casein molecules (either α_{s1}- or β-casein) and can thus be a link in a protein network. (This proposal has been suggested (Lin et al., 1972) as the basis of one model for the casein micelle.) Consequently if the α_{s1}-casein molecule is cleaved so that it loses its ability to act as a link in the protein network, then the network would lose its strength. This model explains why cleavage of a few peptide bonds can cause a relatively large change in the yield force of young Cheddar cheese (Table 2). At higher and lower pH the effect of proteolysis would also be to decrease the extent of the protein network and hence the yield force. It is tempting to extend this model of the rheological behavior of young Cheddar cheese to other varieties, especially cheeses such as Mozzarella and Swiss in which chymosin destruction has occurred during cheese manufacture (Matheson, 1981), but it would be premature to do this before the hypothesis had been tested further.

The other feature of the changes in the force-compression curves with age of cheese that requires comment is the decreasing compression at the yield point, an increasing brittleness or fragility in the cheese. In part it is caused by the loss of elastic structural elements but another feature of proteolysis is probably important. Notably, as each peptide bond is cleaved two new ionic groups are generated and each of these will compete for the available water in the system. Thus the water previously available for solvation of the protein chains will become tied up with the new ionic groups making the cheese harder and less easily deformed. The effect in combination with loss of an extensive protein network gives the observed effect.

—Continued on page 646

Interaction Between Ovomucoid and Lysozyme

TSUKASA MATSUDA, KENJI WATANABE, and YASUSHI SATO

ABSTRACT

The interaction between ovomucoid and lysozyme was investigated by precipitation experiments, polyacrylamide gel electrophoresis and inactivation experiments on ovomucoid. The ovomucoid-lysozyme mixture produced turbidity at low salt concentrations over the pH range 6–11. Although both ovomucoid and lysozyme solutions (0.2%, pH 8.5) yielded no precipitation when heated separately at 80°C for 10 min, about 40% of the proteins in the 0.2% solution of the ovomucoid-lysozyme mixture (2:3, in weight; 1:3, in mole) was precipitated by the same treatment. Ovomucoid was found to form complexes with lysozyme, some of which were precipitated by centrifugation (2000 × *g*, 20 min), and it was presumed that ovomucoid and lysozyme molecules were brought close together by the electrostatic attractive force, unfolded by heating, and then aggregated through intermolecular forces such as hydrophobic forces, hydrogen bonds and disulfide bonds.

INTRODUCTION

IN COMMERCIAL FOOD PROCESSING, the interaction of two or more kinds of proteins is an important factor in determining the structure and texture of end products, and the capacity of proteins to aggregate or coagulate under practical conditions is a usual functional property in many food systems.

Ovomucoid is a glycoprotein with a trypsin inhibitory activity which accounts for about 10% of the protein in egg white (Rhodes et al., 1960). It has been reported as interacting neither with itself nor other proteins. For example, ovomucoid in dilute solution is not precipitated by hot 5% trichloroacetic acid; nor does it aggregate at the isoelectric point when its activity is completely destroyed by heat (Lineweaver and Murray, 1947). Moreover, even after most of the protein in egg white was precipitated by heating, ovomucoid remained in the supernatant (Matsuda et al., 1981b).

Lysozyme is a highly basic protein which accounts for 3% of the protein in egg white (Osuga and Feeney, 1974). It is known to form electrostatic complexes with other proteins, such as ovomucin (Cotteril and Winter, 1955; Garibaldi et al., 1968), ovotransferrin (Ehrenpreis and Warner, 1956), ovalbumin (Forsythe and Foster, 1950; Nichol and Winzor, 1964) and bovine serum albumin (Steiner, 1953).

Although heat-induced aggregation or coagulation of egg white proteins has been investigated in single-protein systems (Nakamura et al., 1978; Hegg et al., 1978, 1979; Egelandsdal, 1980), few reports are available on systems containing two or more proteins (Sato et al., 1977). In this study, we examine the interaction between ovomucoid and lysozyme, and describe the heat-induced aggregation of ovomucoid with lysozyme.

MATERIALS & METHODS

OVOMUCOID AND LYSOZYME (6X crystallized) were purchased from Worthington Biochemical Corporation and Seikagaku Kogyo Co., Ltd. (Tokyo), respectively. Trypsin (2X crystallized) was obtained from the Shigma Chemical Company. All materials were used without further purification.

Authors Matsuda, Watanabe, and Sato are with the Dept. of Food Science & Technology, Faculty of Agriculture, Nagoya Univ., Chikusaku, Nagoya 464, Japan.

Heat treatment

Four mg of ovomucoid or lysozyme was dissolved in 2 ml of distilled water. Equal volumes of the 0.2% solutions containing each protein were mixed. Two hundred μl of the protein solution containing ovomucoid or lysozyme or both, pH of which was adjusted to the desired value with 20 mM NaOH or 20 mM HCl, were placed in test tubes (0.8 × 14 cm). No correction for ionic strength of the solution was made. The test tubes were positioned in a rack and immersed in a controlled temperature water bath, shaken gently for 30 sec, and then kept at definite temperatures for various periods of time. Each sample was cooled immediately after heat treatment by placing the tubes in ice water.

Analysis of the precipitated protein amount

Measurement of the precipitation was performed after centrifugation of samples at 2000 × *g* for 20 min. The protein and neutral hexose concentrations of the supernatant were measured according to the method of Lowry et al. (1951) and the phenol/sulfuric acid method (Dubois et al., 1956), respectively. The percentage of precipitated protein (P) was calculated as $[P = (1-C/C_o) \times 100]$, where C is the protein concentration of the supernatant and C_o is that measured before heat treatment. The percentage of precipitated neutral hexose, which is the constituent of ovomucoid carbohydrate chain, was also calculated in a similar manner as above by using the neutral hexose concentration instead of the protein concentration, and this percentage was taken as that of precipitated ovomucoid, because only ovomucoid contains the neutral hexose (17 residues/mole) (Osuga and Feeney, 1968).

Measurement of turbidity

Turbidity of ovomucoid-lysozyme mixture was measured immediately after the pH of the mixture was adjusted to the desired value. It was expressed as the absorbance at 600 nm of the sample solution which was measured with a Shimadzu double beam spectrophotometer model UV-200S.

Polyacrylamide gel slab electrophoresis

Gel sheets (0.1 × 13 × 13 cm) of 7.5% polyacrylamide and electrophoresis buffer of tris-glycine were prepared as described by Davis (1964). Sodium dodecylsulfate ($NaDodSO_4$) polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). The gel sheets were stained with a solution of 0.25% Coomassie Brilliant Blue R-250 in water/isopropanol/acetic acid (5:5:1, v/v/v) and destained with 7% acetic acid containing 3% methanol overnight.

Measurement of trypsin inhibitory activity

Trypsin inhibition was assayed by measuring the initial rate of increase in absorbance at 420 nm with α-N-benzoyl-L-arginine p-nitroanilide as described by Waheed and Salahuddin (1975).

RESULTS

THE OVOMUCOID-LYSOZYME MIXTURE produced turbidity over the pH range 6–11, and the maximum turbidity was observed at pH 9.2 as shown in Fig. 1. The solution containing ovomucoid alone produced no turbidity at any pH between 2 and 12 and that containing lysozyme alone produced turbidity at above pH 10.

–Continued on next page

Fig. 2 shows the effect of pH on the heat-induced precipitation in the sample solution containing ovomucoid, lysozyme or both. Ovomucoid could not be precipitated over the pH range 2–12 when heated without lysozyme, and lysozyme was not precipitated either at below pH 9 when heated separately. On the other hand, a considerable amount of precipitation was observed in the ovomucoid-lysozyme mixture when the mixture was heated at the pH range between 7.2–11.5. The amount of precipitated hexose in ovomucoid-lysozyme mixture was increased with a rise above pH 7, and its maximum value was observed for th sample heated at pH 9.9.

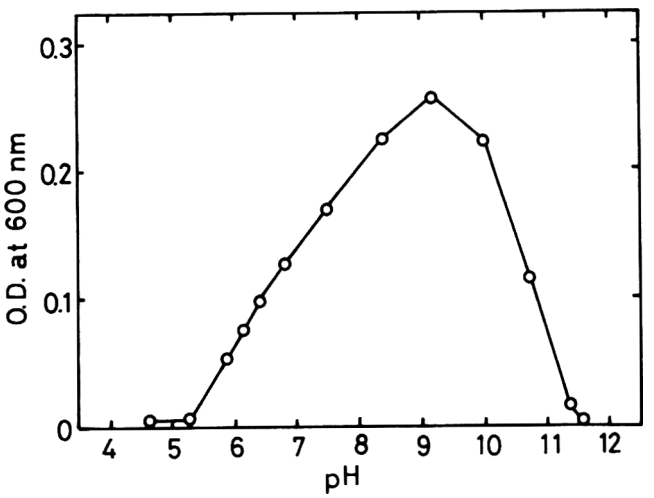


Fig. 1–Effect of pH on the turbidity of ovomucoid-lysozyme mixture (1:1, in weight). Equal volumes of 0.2% solutions containing each protein were mixed, and pH of the solutions was adjusted at the desired values.

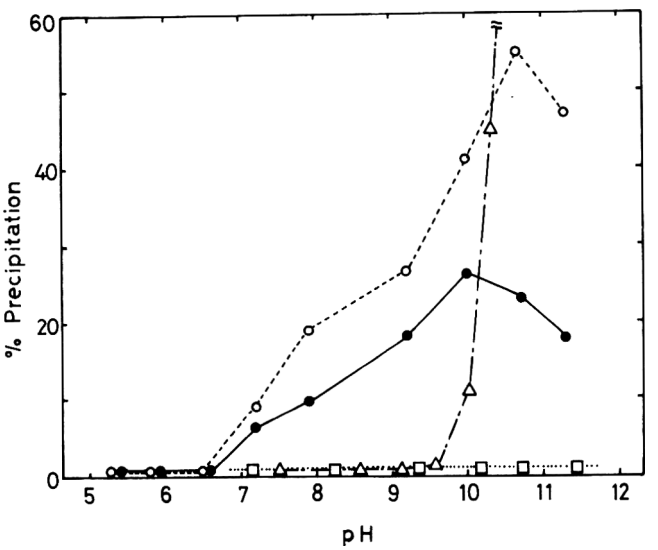


Fig. 2–Effect of pH on the precipitation of ovomucoid, lysozyme or ovomucoid-lysozyme mixture (1:1, in weight) heated at 80°C for 10 min: - - ○ - - Relative amount of precipitated proteins in the ovomucoid-lysozyme mixture; — ● — Relative amount of precipitated hexose, or precipitated ovomucoid in the ovomucoid-lysozyme mixture; - — - △ - — - Relative amount of precipitated lysozyme when lysozyme was heated separately; · · · □ · · · Relative amount of precipitated ovomucoid when ovomucoid was heated separately.

As seen in Fig. 3, the ovomucoid-lysozyme mixture (pH 8.3) yielded little precipitation when heated at below 60°C, and the amount of precipitation was gradually increased with an elevation in heating temperature above 60°C. A similar tendency was observed with the precipitated hexose or precipitated ovomucoid amount.

Fig. 4 shows the effect of heating time on the precipitation induced by heating at 80°C in the ovomucoid-lysozyme mixture (pH 9.2). Both precipitated protein and hexoses amounts were increased by increasing heating time up to 5 min. Moreover, both were virtually independent of heating time over 5 min.

Fig. 5 shows the effect of the mixing ratio of ovomucoid to lysozyme on the precipitation caused by heating the mixture. The total protein concentration of the ovomucoid-lysozyme mixture was fixed at 0.2%, and the two protein solutions of the same concentrations were mixed in various ratios. Maximum amount of precipitated protein and hexose were both observed at the mixing ratio of 2:3 (ovomucoid:lysozyme) in weight.

The pH of ovomucoid-lysozyme mixture was varied by adding NaCl. Therefore, after the addition of NaCl to the mixture, pH was re-adjusted to 9.2 with 20 mM NaOH or HCl. As shown in Fig. 6, the amount of heat-induced precipitation was decreased rapidly upon addition of a small amount of NaCl (less than 10 mM). However, when NaCl of more than 10 mM was added to the mixture, the amount of precipitated protein was increased with increase in the NaCl concentration and little or no additional precipitation took place in 0.2M NaCl. The amount of precipitated hexose was rapidly decreased by adding NaCl (less than 10 mM), and increased NaCl concentrations had little effect on the amount of hexose precipitated. When lysozyme was heated separately, the amount of precipitated protein was increased with increase in the NaCl concentration (more than 0.1 M). On the other hand, no precipitation was observed in any concentration of NaCl ($0 \approx 1.0$M) when ovomucoid was heated separately.

Ten μl of the supernatant obtained by centrifuging the heat-treated samples was applied to the polyacrylamide gel electrophoresis. As seen in Fig. 7, in the pH range between 7.8 and 10.1, the intensity of the stained band of ovomucoid clearly decreased, and the large-particle complexes which migrated only with difficulty into the small pore gel were observed at the top of the gel. Some weak bands were seen at the post region of the ovomucoid band in the pat-

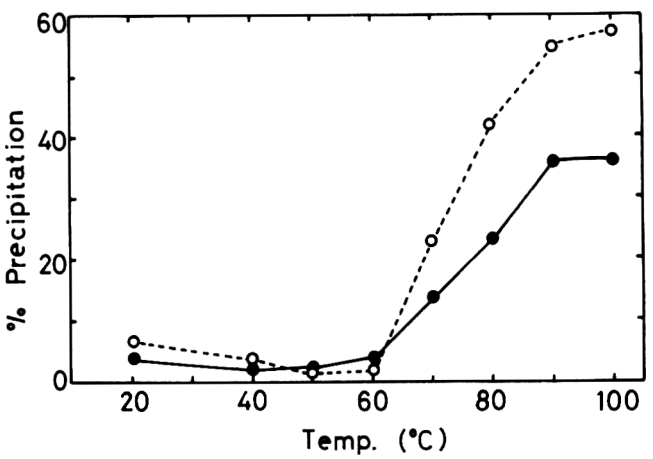


Fig. 3–Effect of heating temperature on the precipitation of ovomucoid-lysozyme mixture (1:1, in weight) heated at pH 8.3 for 10 min: - - ○ - - Relative amount of precipitated proteins; — ● — Relative amount of precipitated hexose.

tern of several samples. Proteins in these bands were regarded as ovoinhibitor (Matsuda et al., 1981b), which is frequently a contaminant of commercial ovomucoid.

After the precipitate obtained by the centrifugation was washed twice with distilled water in order to prevent the contamination with non-precipitated proteins, the precipitate was completely dissolved in 50 μl of 0.1M tris-HCl buffer (pH 6.8) containing 2% $NaDodSO_4$, 5% 2-mercaptoethanol, 10% glycerol and 0.001% bromophenol blue as the dye by heating at 100°C for 5 min. Twenty μl of the solution was applied to the $NaDodSO_4$-electrophoresis. The electrophoretogram is shown in Fig. 8. The ovomucoid band was only slightly detected at below pH 6.9, but between pH 7.8 and 10.1 the strong band corresponding to the ovomucoid was clearly observed in the stained gel. A strong band corresponding to the lysozyme was also observed at above pH 7.8. Some bands which were not detected in the unheated sample appeared in the pattern of samples heated at between pH 7.8 and 10.7.

Fig. 9 shows the effect of lysozyme on the inactivation of ovomucoid by heating and the relative amount of ovomucoid which remained in the supernatant. Trypsin inhibitory activity was assayed for the supernatant obtained by centrifuging the heat-treated samples. The activity was not decreased by heating at below 80°C. Moreover, no less than 50% of the activity remained even after heating at 90°C for 10 min, when ovomucoid was heated without lysozyme. In the presence of lysozyme, however, ovomucoid was completely inactivated by heating at 80°C for 10 min.

DISCUSSION

OVOMUCOID is a highly acidic protein (pI = 4.1), and lysozyme is a highly basic protein (pI = 10.7) (Osuga and Feeney, 1974). Lysozyme and ovalbumin are known to produce electrostatic complexes with a relatively low degree of aggregation, and both showed a high degree of association (solution visibly turbid) at a low salt concentration (Donovan and Beardslee, 1979). From these observations, ovomucoid and lysozyme would presumably have been attracted and associated mutually with each other by the electrostatic forces in the pH range between the isoelectric points of the two proteins.

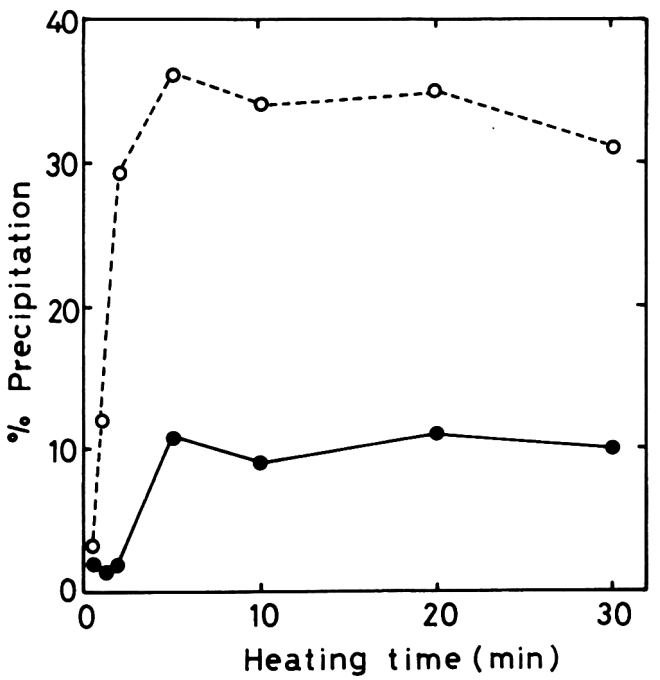


Fig. 4—Effect of heating time on the precipitation of ovomucoid-lysozyme mixture (1:1, in weight) heated at pH 8.2 at 80°C: - - ○ - - Relative amount of precipitated proteins; — ● — Relative amount of precipitated hexose.

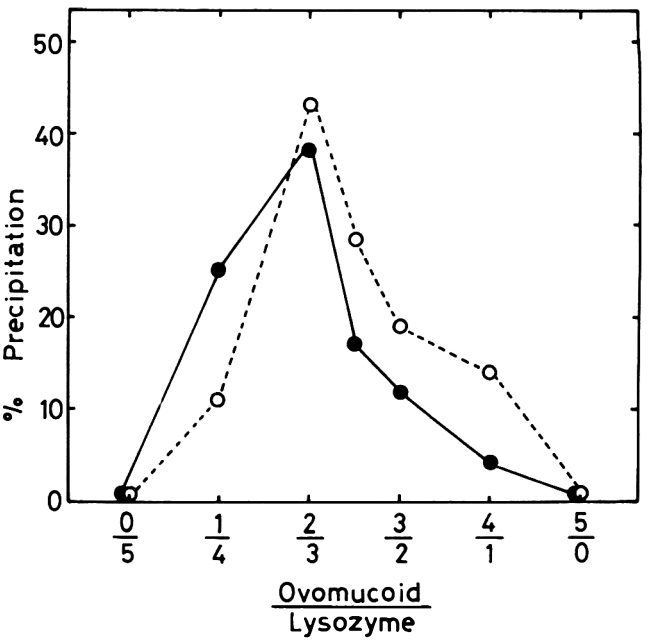


Fig. 5—Effect of mixing ratio on the precipitation of ovomucoid-lysozyme mixture (pH 9.0) heated at 80°C for 10 min: - - ○ - - Relative amount of precipitated proteins: — ● — Relative amount of precipitated hexose.

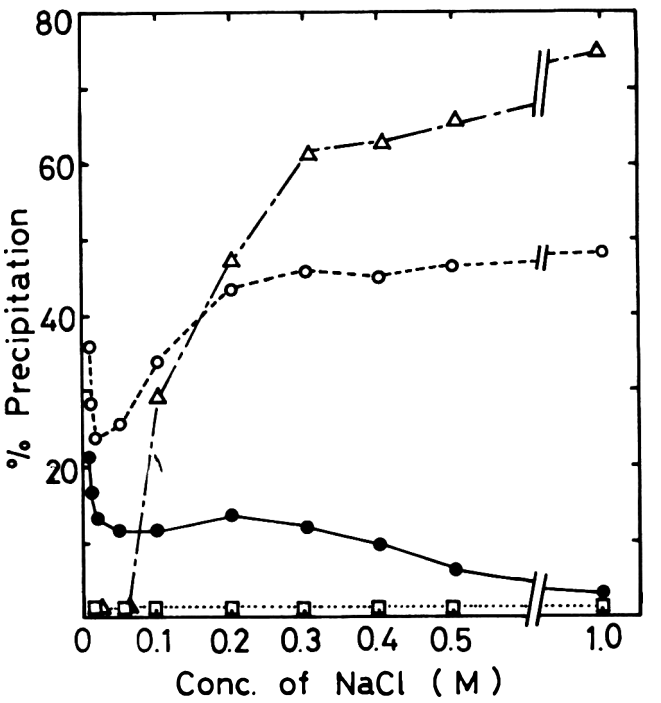


Fig. 6—Effect of NaCl concentration on the precipitation of ovomucoid, lysozyme or ovomucoid-lysozyme mixture (1:1, in weight) heated at pH 9.2 at 80°C for 10 min: - - ○ - - Relative amount of precipitated proteins in the ovomucoid-lysozyme mixture; — ● — Relative amount of precipitated hexose, or precipitated ovomucoid in the ovomucoid-lysozyme mixture; - — △ - — Relative amount of precipitated lysozyme when lysozyme was heated separately; · · · □ · · · Relative amount of precipitated ovomucoid when ovomucoid was heated separately.

The present authors have already reported that the conformational changes of the ovomucoid molecule were caused by heating in the temperature range between 60 and 90°C, and these structural changes were almost completely reversible (Matsuda et al., 1981a). Ovomucoid was precipitated by heating at above 60°C in the presence of lysozyme (Fig. 3). The coincidence of the precipitation temperature with the conformational change-temperature suggested that ovomucoid which unfolded at temperatures above 60°C interacted with lysozyme and resulted in the formation of larger particle complexes.

The amount of precipitated protein was more or less constant when the mixture was heated at 80°C for over 10 min (Fig. 4). The protein concentration in the supernatant solution was decreased by the elimination of associated proteins as the precipitation and, moreover, the supernatant no longer contains a 1:1 (weight) mixture because the precipitate contains unequal quantities of each protein precipitated from a 1:1 weight mixture (Fig. 2, 3, 4). Therefore, the concentration and ratio of proteins would be less favorable than those of the original protein solution (0.2%, 1:1 weight mixture).

The coincidence of the optimum mixing ratio for the total protein precipitation to that for the ovomucoid precipitation (Fig. 5) suggested that the ovomucoid-lysozyme interaction proceeded stoichiometrically and the optimum molar ratio of ovomucoid to lysozyme for the interaction was nearly 1:3.

Various neutral salts have generally been known to diminish the repultive force between the proteins which predominantly have the same kind of charge and to diminish the attractive force between the proteins which predominantly have the opposite charge. The decrease of heat-induced precipitation of total protein or ovomucoid by addition of small amount of NaCl indicated that the electrostatic interaction between ovomucoid and lysozyme was weakened and, moreover, the increase of total protein precipitation by further addition of NaCl was well explained by the decrease of the repultive force among lysozyme molecules. The NaCl would also decrease the repultive force among ovomucoid molecules but ovomucoid appeared not to associate even when ovomucoid molecules were brought close together by decreasing the repulsive force (Fig. 6). Though it is uncertain why the observed break in effects of increasing NaCl concentration occurred at 10 mM, the amount of precipitated protein seemed to relate to the effect of NaCl on both of the ovomucoid-lysozyme interaction and the lysozyme-lysozyme interaction. From the effect of pH and NaCl on the interaction between ovomucoid and lysozyme, it is presumed that ovomucoid and lysozyme molecules are brought close together by the electrostatic attractive force, unfolded by heating, and then aggregated through intermolecular forces such as hydrophobic forces, hydrogen bonds and disulfide bonds.

The pH at which the weakest intensity of the stained band of ovomucoid was observed in the electrophoretogram (Fig. 7) was not consistent with the optimum pH for the ovomucoid precipitation (Fig. 2). This suggests that there are some complexes too small to be precipitated by the centrifugation (2000 × g, 20 min) and, simultaneously, too large to migrate into the separation gel under the electrophoresis. It is natural that lysozyme did not migrate

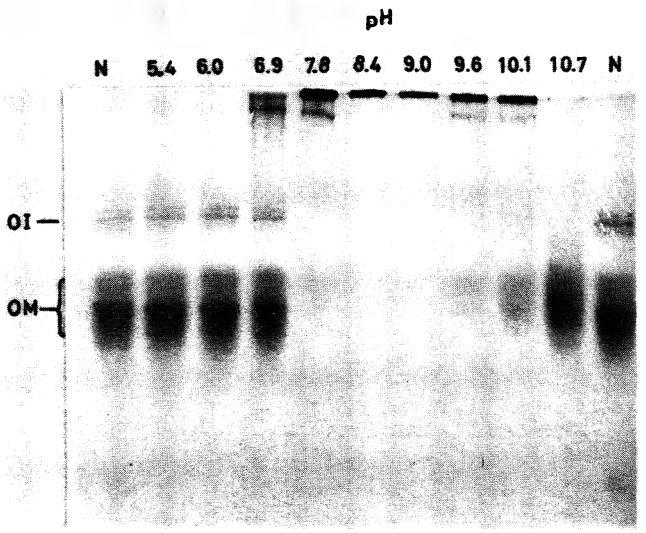


Fig. 7—Polyacrylamide gel electrophoresis of the supernatant obtained by centrifuging the ovomucoid-lysozyme mixture heated at 80°C for 10 min: (N) Unheated ovomucoid; (OM) ovomucoid; (OI) ovoinhibitor.

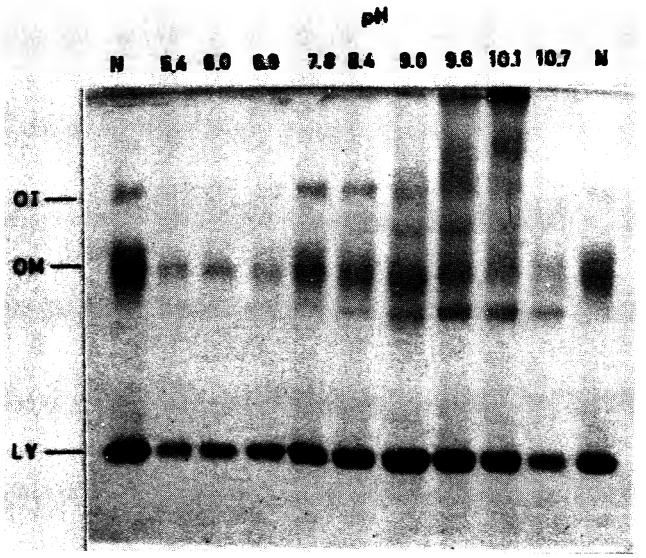


Fig. 8—NaDodSO$_4$-polyacrylamide gel electrophoresis of the precipitation obtained by centrifuging the ovomucoid-lysozyme mixture (1:1, in weight) heated at 80°C for 10 min: (N) Unheated proteins; (OM) ovomucoid; (OI) ovoinhibitor; (LY) lysozyme.

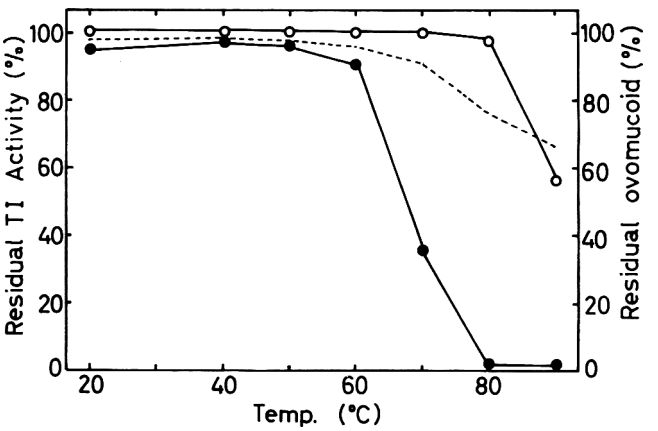


Fig. 9—Effect of lysozyme on the inactivation of ovomucoid caused by heating at various temperatures at pH 8.6 for 10 min: — ○ — Inactivation profile of ovomucoid heated alone; — ● — Inactivation profile of ovomucoid heated in the presence of lysozyme (ovomucoid : lysozyme = 1:1); - - - - - Relative amount of the residual ovomucoid in the supernatant when ovomucoid was heated in the presence of lysozyme.

into the gel (Fig. 7), because lysozyme (pI = 10.7) had a predominantly positive charge in the electrophoresis buffer (pH 8.6).

The stained bands corresponding to ovomucoid and lysozyme in the $NaDodSO_4$-electrophoretogram demonstrated the existence of both proteins in the precipitate. The stained bands other than those corresponding to ovomucoid or lysozyme suggested that the proteins remain partially undissociated even after the dissolving treatment described above.

More than 70% of the ovomucoid remained in the supernatant even when the ovomucoid-lysozyme mixture was heated at 80°C. Nevertheless, the supernatant had no trypsin inhibitory activity (Fig. 9). This result supports the above described suggestion that ovomucoid formed complexes with lysozyme which could not be precipitated by the centrifugation (2000 × *g*, 20 min). Moreover, it is presumed that ovomucoid could be reversibly unfolded by heating separately at 80°C but the reversibility of unfolding was lost by the presence of lysozyme.

In this study, the interaction between ovomucoid and lysozyme was investigated mainly from the viewpoint of the heat-induced aggregation or precipitation, and the heat-induced aggregation of ovomucoid was found to be enhanced by the presence of lysozyme. However, the excessive aggregation of proteins in food systems may not necessarily result in the desirable structure and texture of final products. Studies on the effect of heat-induced aggregation of proteins on the properties of the protein solution or protein coagulum are now in progress.

REFERENCES

Cotteril, O.J. and Winter, A.R. 1955. Egg white lysozyme. 3. The effect of pH on the lysozyme-ovomucin interaction. Poultry Sci. 34: 679.

Davis, B.J. 1964. Disc electrophoresis. 2. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: Art. 204.

Donovan, J.W. and Beardslee, R. 1975. Heat stabilization produced by protein-protein association. J. Biol. Chem. 250: 1966.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Ehrenpreis, S. and Warner, R.C. 1956. The interaction of conalbumin and lysozyme. Arch. Biochem. Biophys. 61: 38.

Egelandsdal, B. 1980. Heat-induced gelling on solutions of ovalbumin. J. Food Sci. 45: 570.

Forsythe, R.H. and Foster, J.F. 1950. Egg white proteins. 1. Electrophoretic studies on whole white. J. Biol. Chem. 184: 377.

Garibaldi, J.A., Donovan, J.W., Davis, J.G., and Cimino, S.L. 1968. Heat denaturation of the ovomucin-lysozyme electrostatic complex — A source of damage to the whipping properties of pasteurized egg white. J. Food Sci. 33: 514.

Hegg, P.O., Martens, H., and Lofquist, B. 1978. The protective effect of sodium dodecylsulphate on the thermal precipitation of conalbumin. A study on thermal aggregation and denaturation. J. Sci. Fd. Agric. 29: 245.

Hegg, P.O., Martens, H., and Lofquist, B. 1979. Effect of pH and neutral salts on the formation and quality of thermal aggregates of ovalbumin. A study on thermal aggregation and denaturation. J. Sci. Fd. Agric. 30: 981.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680.

Lineweaver, H. and Murray, C.W. 1947. Identification of the trypsin inhibitor of egg white with ovomucoid. J. Biol. Chem. 171: 565.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265.

Matsuda, T., Watanabe, K., and Sato, Y. 1981a. Independent thermal unfolding of ovomucoid domains. Biochim. Biophys. Acta. 669: 109.

Matsuda, T. Watanabe, K., and Sato, Y. 1981b. Heat-induced aggregation of egg white proteins as studied by vertical flat-sheet polyacrylamide gel electrophoresis. J. Food Sci. 46: 1829.

Nakamura, R., Sugiyama, H. and Sato, Y. 1978. Factors contributing to the heat-induced aggregation of ovalbumin. Agric. Biol. Chem. 42: 819.

Nichol, L.W. and Winzor, D.T. 1964. The determination of equilibrium constants from transport data on rapidly reacting systems of the type $A+B \rightleftharpoons C$. J. Phys. Chem. 68: 2455.

Osuga, D.T. and Feeney, R.E. 1974. Avian egg whites. In "Toxic Constituents of Animal Food Stuffs," I.E. Liener (Ed.). Academic Press, New York.

Rhodes, M.B., Bennet, N., and Feeney, R.E. 1960. The trypsin and chymotrypsin inhibitors from avian egg whites. J. Biol. Chem. 235: 1686.

Sato, Y., Iwatsuki, K., and Hayakawa, M. 1977. Interaction between ovalbumin and κ- or β-casein due to heating. Agric. Biol. Chem. 41: 1331.

Steiner, R.F. 1953. Reversible association processes of globular proteins. 2. Electrostatic complexes of plasma albumin and lysozyme. Arch. Biochem. Biophys. 47: 56.

Waheed, A. and Salahuddin. A. 1975. Isolation and characterization of a variant of ovomucoid. Biochem. J. 147:139.

Ms received 7/27/81; revised 10/16/81; accepted 10/19/81.

SCRAPED-SURFACE HEAT EXCHANGER . . . From page 625

McCabe, W.L. and Smith, J.C. 1956. "Unit Operations of Chemical Engineering." McGraw-Hill, New York, NY.

Omosaiye, O., Cheryan, M., and Matthews, M.E. 1978. Removal of oligosaccharides from soybean water extracts by ultrafiltration. J. Food Sci. 44: 1027.

Penney, W.R. and Bell, K.J. 1969. The effect of backmixing on the mean temperature difference in an agitated heat exchanger. Chem. Eng. Progr. Symp. Ser. 65(92): 21.

Ramdas, V., Uhl, V.W., Osborne, M.W., and M.W., and Ortt, J.R. 1977. Heat transfer to viscous materials in a continuous-flow, scraped-wall, commercial-size heat exchanger. Preprints, AIChE National Heat Transfer Conference, 17th, p. 24.

Simmers, D.A. and Coney, J.E. R. 1979. A Reynolds analogy solution for the heat transfer characteristic of combined Taylor vortex and axial flows. Intnl. J. Heat Mass Transfer 22: 679.

Skelland, A.H.P. 1958. Correlation of scraped-film heat transfer in the Votator. Chem. Eng. Sci. 7: 166.

Skelland, A.H.P., Oliver, D.R., and Tooke, S. 1962. Heat transfer in a water-cooled scraped-surface heat exchanger. Brit. Chem. Eng. 7: 346.

Sykora, S. and Navratil, B. 1966. Heat transfer on scraped walls. Collection Czechoslov. Chem. Commun. 31(8): 3299.

Sykora, S., Navratil, B., and Karasak, O. 1968. Heat transfer on scraped walls in the laminar and transitional regions. Coll. Czechoslov. Chem. Commun. 33(2): 518.

Trommelen, A.M. and Beek, W.J. 1971. Flow phenomena in scraped-surface heat exchanger (Votator-type). Chem. Eng. Sci. 26: 1933.

Trommelen, A.M., Beck, W.J., and van de Westelaken, H.C. 1971. A mechanism for heat transfer in a Votator-type scraped-surface heat exchanger. Chem. Eng. Sci. 26: 1987.

Uhl, V.W. 1966. Mechanically aided heat transfer. In "Mixing Theory and Practice," Vol. 1, p. 279. Academic Press, New York, NY.

Presented at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 7—10, 1980. This material forms part of a doctoral dissertation submitted by RC to the Graduate College, Univ. of Illinois. Funds were provided in part by the Agricultural Experiment Station, Univ. of Illinois, and by Research Corporation, New York.

Correction Factor of Deviant Thermal Processes Applied to Packaged Heat Conduction Food

ERNESTO B. GIANNONI-SUCCAR and KAN-ICHI HAYAKAWA

ABSTRACT

A procedure was developed to estimate the values of correction factor, Cf, for deviant thermal processes applied to canned or other packaged heat conduction food. Sterilizing values at the thermal center of the food were used as a criterion for this estimation. In this procedure empirical heat transfer parameters, f and j values, were used to estimate Cf values. The devleoped procedure is based on the use of a regression equation, which was obtained through the dimensional and statistical analyses of theoretically determined Cf values. According to a series of experiments which were performed by using 307 X 409 and 211 X 300 cans of 8% bentonite suspension, there was reasonably good agreement in Cf values estimated theoretically and determined experimentally.

INTRODUCTION

HEATING MEDIUM TEMPERATURES deviate significantly from specified levels in the heating phases of some thermal processes. Since these deviations could lead to under sterilization, there is an important need for examining the influence of temperature deviations on process lethality.

According to our literature survey there is a scarcity of published studies on the influence of heating medium temperature deviations on process lethality. Houtzer and Hill (1977) investigated experimentally the lethalities of deviant thermal processes having the temperature drops of different magnitudes at different locations in the heating cycles of thermal processes.

According to the code of Federal Regulations Title 21 a can conveying system should be stopped promptly when the temperature of a continuous sterilizer drops significantly below one specified in a normally scheduled process. Therefore, Houtzer and Hill stopped the rotation of cans when a temperature drop was experimentally produced until it was restored to a normal level. Their results show that the lethality of deviant process is greater than that of a normal process regardless of whether the temperature drop occurs in an early or late part of the heat process. The results also show that can sizes do not appear to influence an increase in the process lethality observed with the deviant process.

It would be useful to develop a reliable procedure for determining a proper corrective processing schedule when there is a deviation in the heating medium temperature. Therefore the present investigation was initiated to attain this objective.

DEVELOPMENT OF METHOD FOR PREDICTING CORRECTION FACTOR

FOR OUR INVESTIGATION, a correction factor is introduced to estimate a proper corrective schedule for a deviant heat process. This correction factor is defined as follows:

Author Hayakawa is with the Food Science Dept. Cook College, Rutgers Univ., P.O. Box 231, New Brunswick, NJ 08903. Author Giannoni-Succar, formerly affiliated with Rutgers Univ., is currently at Universidad Nacional Agraria, Apartado 456, La Molina, Lima, Peru.

$$Cf = t_x/t_b \quad (1)$$

(All symbols used are defined in the Nomenclature.)

For a normal process, a proper heating time, t_b, may be obtained from recommended heat processing schedules (Anon. 1976; Lopez, 1975) or may be estimated by using any reliable mathematical procedure for thermal process evaluation when a desired process lethality is given. For a deviant process, we assume a step functional drop and step functional restoration of heating medium temperature in the heating phase of a thermal process (Fig. 1). When temperature deviation is not step functional, as for many commercial processes, a Cf value estimated by the present investigation may be treated as an upper limit of an actual Cf value.

To determine a Cf value, the lethalities of normal and deviant processes are estimated. These lethalities are applicable to the slowest heating point of packaged heat conduction food. A proper heating time of the deviant process, t_x, is estimated from food temperatures of the same process, T_d, and from those of a normal process, T, or a desired process lethality, F_p, Eq (2).

$$F_p = \int_o^{t_b} 10^{(T - T_r)/z}\,dt + \int_{t_b}^{t_e} 10^{(T - T_r)/z}\,dt = \int_o^{t_x} 10^{(T_d - T_r)/z}\,dt + \int_{t_x}^{t_{ed}} 10^{(T_d - T_r)/z}\,dt \quad (2)$$

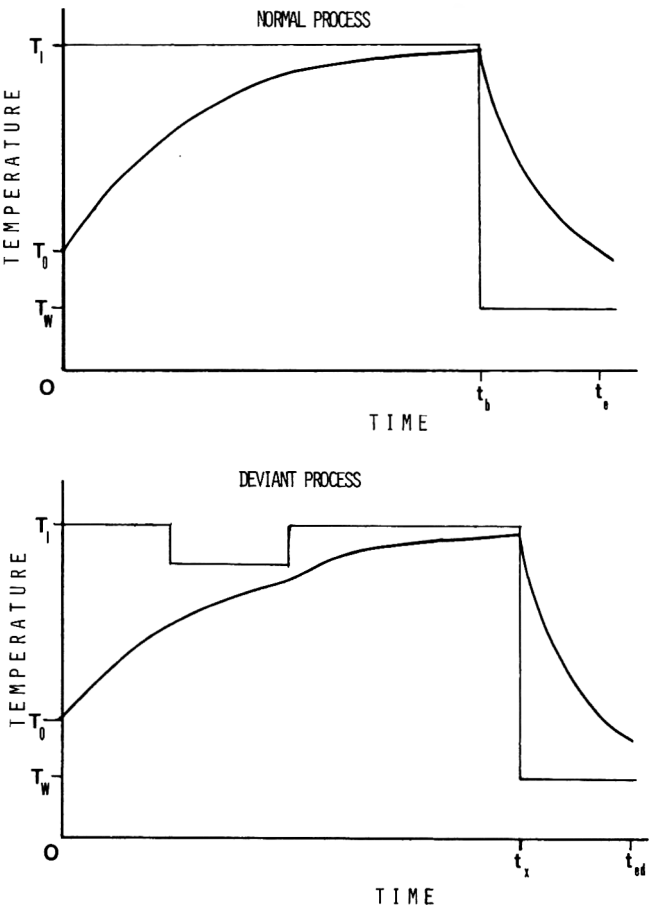


Fig. 1—Graphical representation of idealized normal and deviant heat processes.

For the present investigation, at t_x value is determined by using a computer program package developed in our laboratory (Giannoni et al., 1980). In this program, modified Duhamel's theorem (Hayakawa, 1971) as well as a slightly modified version of the same theorem are applied to estimate food temperature. This slightly modified version may be used when there are changes in surface heat conductance during a thermal process. Since these theorems are based on the use of empirical heat transfer constants, f and j values, the results of our analysis presented below are applicable to any cans or any packages of heat conduction food.

Through the careful examination of computational processes for estimating Cf values, we found that the following twelve independent parameters were required for the estimation: T_o, T_1, T_w, ΔT_d, t_1, t_2, t_b, f_h, f_c, j_h, j_c, and z. When a dimensional analysis (Kreith, 1973) was applied to these independent parameters, we obtained the following nine dimensionless parameters, which were required to uniquely define Cf:

$$\begin{aligned} P_1 &= T_o/T_1 & P_6 &= \Delta T_d/z \\ P_2 &= t_b/t_2 & P_7 &= j_h \\ P_3 &= f_h/t_2 & P_8 &= j_c \\ P_4 &= f_c/t_2 & P_9 &= t_1/t_2 \\ P_5 &= T_w/T_1 \end{aligned} \qquad (3)$$

The influence of each dimensionless parameter on Cf was evaluated by using a fractional central composite experimental design (Davies, 1978). For this usage, the dimensionless parameters were treated as independent variables and Cf as a dependent variable. The fractional design used was a quarter replicate of a full design, with which all main effects and all quadratic effects were measurable.

The values of Cf were estimated for all combinations of the dimensionless groups, which were specified in the selected experimental design. These combinations are represented by points in a multi-dimensionless design space. The points consist of those defined in a 2^n factorail design (Anon., 1957), star-points, and of a central point. The fractional points were obtained from a quarter replicate of 2^9 factorial design, the total number of points being: $2^{(n-r)} = 2^{(9-2)} = 128$. The star-points are determined through use of an s value (Davies, 1978) given by: $s = 2^{(n-r)/4} = 2^{(9-2)/4} = 3.3636$. The star-points were generated by assigning a value of +s and −s to one independent variable while all others were kept at zero level. The total number of star-points is 2n = 18. For the central point the level of each factor is kept at a zero level (mid-level). Therefore, the experimental design consisted of 147 combinations of dimensionless parameters.

Table 1—Maximum and minimum values of dimensional parameters

Physical quantity	Minimum values	Most frequent values	Maximum values
T_o	0.0	37.0	93.3
T_1	105.0	117.0	125.0
T_w	5.0	20.0	40.0
f_h	5.0	30.0	250.0
f_c	5.0	40.0	300.0
j_h	0.6	2.3	4.0
j_c	0.6	2.8	5.0
t_b	10.0	60.0	400.0
t_1	0.0	30.0	392.0
t_2	0.2	39.0	400.0
ΔT_d	0.0	4.0	22.0
z	4.0	9.5	15.0

Table 2—Values of dimensionless parameters corresponding to the five design levels

Dimensionless parameter	Values of dimensionless parameters corresponding to the following design levels				
	−s	−1	0	+1	+s
P_1	0.0	0.2029	0.32	0.4580	0.8888
P_2	1.0	1.1376	1.54	3.0748	50.00
P_3	0.0125	0.0959	0.77	6.8712	1250.0
P_4	0.0125	0.1292	1.03	8.9592	1500.0
P_5	0.04	0.1257	0.1713	0.2238	0.3810
P_6	0.0	0.1805	0.42	0.9232	5.5
P_7	0.6	1.7946	2.3	2.8054	4.0
P_8	0.6	2.1454	2.8	3.4541	5.0
P_9	0.0	0.6699	0.77	0.8486	0.98

The following five levels are assigned to each design variable for estimating Cf values: −s, −1, 0, +1, +s. To use the selected experimental design, each dimensionless parameter should be transformed to a proper design variable so that the lower and upper limits of the transformed variable become equal to −s and +s respectively. In addition, the three other design coordinates, −1, 0, +1, should be placed within the range of parametric values observed frequently. Since the range of values of each dimensional parameter is required to obtain a proper transformation function, it was determined through literature survey (Anon., 1976; Houtzer, 1980; Lopez, 1975; Stumbo, 1973) as shown in Table 1. From the tabulated values, the lower and upper limits and most frequently observed values of all dimensionless parameters were estimated, Table 2. The limits are listed under design coordinates −s and +s and the most frequently observed values under 0. To locate the design coordinates around the frequently observed values, many different equations were tried to transform dimensionless parameters to design variables. Among them, the following equations were found to be best.

$$\begin{aligned} x_1 &= 14.1453\,[\log_{10}(P_1 + 0.46)] + 1.5255 \\ x_2 &= 1.7227 \log_{10}(P_2 - 0.9939) + 0.4517 \\ x_3 &= 1.0455 \log_{10}(P_3 - 0.01209) + 0.1256 \\ x_4 &= 1.0626 \log_{10}(P_4 - 0.0118) - 0.0113 \\ x_5 &= 16.5979 \log_{10}(P_5 + 0.18) + 7.5176 \\ x_6 &= 3.1027 \log_{10}(P_6 + 0.0379) + 1.0535 \\ x_7 &= 1.9786\, P_7 - 4.5508 \\ x_8 &= 1.5289\, P_8 - 4.2809 \\ x_9 &= 0.3387 \exp(3.1\, P_9) - 3.7023 \end{aligned} \qquad (4)$$

Table 2 shows the parametric values which correspond to all design coordinates, which were estimated by using Eq (4).

The values of Cf were computed for all combinations of parametric values specified by the design of experiments. The computed Cf values range from 1.0000 to 1.3390. These values were subjected to nonlinear regression analyses by using a program in a statistical computer program package (Barr et al., 1976). When Cf is correlated with a quadratic equation of all nine design variables, we obtained a regression formula whose r^2 value is 0.899. This quadratic equation produced some Cf values less than unit for some combinations although they should all be greater than unity according to the definition of Cf.

To obtian a better regression formula, several different functions were used to transform Cf to new dependent variables before the regression analysis. Among them, the following regression equation of a transformed dependent variable yielded the best results.

$$\begin{aligned} Y = {} & 1.073772 - 0.024692X_2 - 0.058306X_3 \\ & + 0.033920X_6 - 0.022598X_9 - 0.006917X_2^2 \\ & + 0.009977X_6^2 + 0.027350X_2X_3 - 0.011302X_2X_6 \\ & + 0.008141X_2X_9 - 0.029534X_3X_6 + 0.018966X_3X_9 \\ & - 0.008449X_6X_9 \end{aligned} \qquad (5)$$

$$Cf = 1 + |y - 1|^{10/8.58} \qquad (6)$$

The value of r^2 of Eq (5) is 0.904. This equation yielded Cf values greater than unity for all combinations of parametric values tested. According to the comparison of Cf values estimated by Eq (5) and (6) against those determined by using the computer program (Giannoni et al., 1980), the maximum deviation of the former Cf values from the latter ones was less than 3.5% when the values of design variables are within −1 and +1 and less than 11.5% when they are within −s and +s. Parametric values for most thermal processes are within two limiting values, which correspond to ±1 in design coordinates. Therefore, Eq (5) and (6) likely produce satisfactory results for most commercial processes.

EXPERIMENTAL VERIFICATION

EXPERIMENTAL correction factors were determined by using three 307 X 409 and three 211 X 300 cans of 8% (w/w) bentonite suspension. Before filling this suspension in the cans, three copper constantan thermocouple junctions, which were made of Teflon-coated 0.0127 mm diameter wires, were placed on the central axis of each can. One junction was placed at the center of the suspension when it was filled and two others at off central positions. The three

junctions were installed to locate a slowest heating location in each sample.

The bentonite suspension was filled up to a proper level of each can so that there was 10% head space in each sample. The filled cans were properly sealed with a vacuum seamer and heated twice for 2.5 hr at 1219°C to thermally stabilize the bentonite suspension (Townsend et al., 1949). The prepared samples were then subjected to normal processes or to deviant processes. For all processes, come-up times at the beginning of heating cycles were less than 30 sec and go-down times at the beginning of cooling cycles were less than 1 min. During each thermal process, the temperatures of samples and of surrounding media were recorded with a strip-chart recorder. From temperature data recorded for the normal processes, f and j values at the thermal center of each sample were determined by using a computer program developed in our laboratory, Table 3. We found that the thermal center was virtually at the geometrical center of canned content. As expected, the f values of cooling curves were significantly greater than the f values of corresponding heating curves.

According to preliminary experiments, 8.5 C° was the maximum limit for nearly step-functional deviation in retort temperature, which we could produce without any control problem. The preliminary experiments show further that it took about 10 sec to step down retort temperature by 3 C° and about 20 sec to drop by 8.5 C° and also that it took as long as three-quarters of step-down time to restore the retort temperature from a deviated level to a normal level.

Based on the preliminary experiments, deviant processes shown in Table 4 were simulated by using an experimental retort. Run numbers 1 and 2 have relatively small temperature drops for relatively short time in the initial and later parts of heating phases respectively. Run numbers 3 and 4 are similar to run numbers 1 and 2, respectively, although they have the longer duration of temperature deviations. In run numbers 5 and 6, temperature deviations are located in the median part of the heating phases. Run number 6 has the largest temperature drop, 8.3 C°.

The sterlizing values of each deviant process were computed by using central sample temperatures collected experimentally. These values are given in Table 4.

To estimate Cf values, the heating times, t_b, of normal processes are required. These processes should produce sterilizing values identical to respective deviant processes. The determination of t_b values was accomplished by performing two normal processes for each can size, whose sterilizing values provided means for interpolating the heating time which produced the sterilizing value of each deviant process. Table 5 shows the normal processes and their sterilizing values. Shiga's method (Shiga, 1976) was used to obtain the t_b values shown in Table 4. Finally, Cf values were computed by using the t_x and t_b values, Table 6. The values of Cf, which were predicted by Eq (5) and (6) are also included in the same table.

Results given in Table 6 clearly show that there is reasonably good agreement in Cf values predicted with those deterined experimentally especially if one realizes that data obtained from three separate thermal processes were used to determine one Cf value.

DISCUSSION

WE OBSERVE from Table 6 that the theoretically estimated Cf values of 307 X 409 cans are slightly less than those determined experimentally. However, the comparative relationship of theoretical and experimental Cf values is reversed with 211 X 300 cans. To examine cause for this reversal relationship, we performed the following analyses.

Let Δt_x and Δt_b be errors in experimentally determined t_x and t_b values; then an experimental Cf value becomes:

$$Cf_p = (t_x + \Delta t_x)/(t_b + \Delta t_b)$$
$$\cong t_x/t_b - (t_x/t_b)\,\Delta t_b/t_b + \Delta t_x/t_b$$

Therefore an error in Cf_p becomes:

$$\Delta Cf = Cf - Cf_p = (\Delta t_b/t_b)\,(t_x/t_b - 1) \qquad (7)$$

Table 3—f and j values at the thermal center of a 307 x 409 and 211 x 300 can sizes

Can size	Can no.	Experimental parameter f_h (min)	f_c (min)	j_h	j_c
307 x 409	1	48.25	61.37	2.146	1.480
	2	50.74	63.57	1.955	1.441
	3	51.56	63.51	1.933	1.450
	mean	50.18	62.82	2.011	1.457
211 x 300	1	29.49	35.51	2.116	1.583
	2	29.34	35.71	2.123	1.571
	3	29.27	35.14	2.158	1.520
	mean	29.37	35.451	2.132	1.558

Table 5—Operational conditions for standard processes

Can size	Run no.	T_o (°C)	T_1 (°C)	T_w (°C)	t_b (min)	F_o (min)
307 x 409	1	21.1	121.1	19.3	83	3.998
	2	20.9	121.1	19.3	75	1.918
211 x 300	1	25.0	121.1	19.3	52	4.047
	2	23.9	121.1	19.3	46	1.621

Table 4—Operational conditions for deviant Processes

Can size	Run no.[a]	T_o (°C)	T_1 (°C)	T_w (°C)	t_x (min)	t_1 (min)	t_2 (min)	ΔT_d (C°)	Δt_d (min)	F_o (min)	t_b (min)
307 x 409	2	22.8	121.1	19.3	83	65	70	5.6	5	3.84	82.45
	3	22.9	121.1	19.1	83	10	25	5.6	15	3.72	82.11
	4	22.7	121.1	19.3	86	55	70	5.6	10	3.49	81.10
	5	23.9	121.1	19.4	84	36	46	2.8	10	3.70	81.86
	6	20.1	121.1	19.1	86	36	46	8.3	10	3.73	81.96
211 x 300	1	23.3	121.1	19.4	52	10	15	3.3	5	3.65	51.06
	2	24.2	121.1	19.3	54	35	40	5.6	5	3.66	51.08
	3	25.1	121.1	18.9	53	10	25	5.6	15	2.85	49.15
	4	24.1	121.1	19.3	58	30	45	5.6	15	3.41	50.50
	5	24.7	121.1	19.2	55	21	31	2.8	10	4.42	52.92
	6	23.6	121.1	19.1	57	21	31	8.3	10	3.80	51.49

[a] Run No. 1 was not included due to a failure in the retort controller.

Data on the temperatures of sample cans were collected at uniform time intervals through the linear interpolation of those recorded on the strip-chart recorder. The interpolated data are underestimated in the heating phase of a process because the temperature history curve of the sample concaves downward. The magnitude of this underestimation decreases with increases in processing time because the curvature of the temperature history curve becomes less as heat processing progresses.

There is a different relationship on errors applicable to the temperature data in the cooling phase of a heat process. Since the temperature history curve of the sample concaves upward during this phase, the interpolated temperature data are overestimated. The magnitude of this overestimation is largest in the early part of the cooling phase and decreases as cooling time increases.

With the smaller can, the sample temperature quickly reaches a level close to the heating medium temperature in the heating phase. Therefore, an error in a sterilizing value due to the interpolation error is relatively small in the heating phase since sample temperatures in the initial portion of the temperature history curve in this phase contribute negligibly to the sterilizing value although the curve in the same portion concaves greatly downward. However, the temperature of the small can drops rather quickly during the cooling phase of a process—the temperature history curve of the smaller can concaves greatly downward. Therefore, the sterilizing value of the cooling phase is overestimated. A magnitude for this overestimation is greater than that for the underestimation of the sterilizing value of the heating phase. Therefore we have a negative error in the t_b, $\Delta t_b < 0$. Since $t_x/t_b > 1$, we have a slightly underestimated Cf value according to Eq (7).

With the larger can the situation is reversed. Since the sample temperature changes relatively slowly during the heating and cooling phases, the interpolation errors of temperature data applicable to the former phase become greater than those applicable to the latter phase insofar as their contributions to a sterilizing value are concerned. Therefore, the use of these temperature data causes an underestimated sterilizing value, which results in a positive error in t_b, $\Delta t_b > 0$. This yields a slightly overestimated Cf vlaue according to Eq (7).

Table 6—Comparison between theoretical and experimental Cf values

Can size	Run no.	Theoretical	Experimental
307 x 409	2	1.018	1.007
	3	1.031	1.012
	4	1.068	1.060
	5	1.027	1.026
	6	1.055	1.049
211 x 300	1	1.015	1.018
	2	1.036	1.057
	3	1.071	1.078
	4	1.098	1.148
	5	1.040	1.040
	6	1.081	1.108

Table 7—Maximum expected errors in measurement of physical property values and operational conditions

Physical quantity	Frequently observed values	Deviations
f_h	30.0 min	3 min
t_b	60 min	0.3 min
t_1	30 min	0.15 min
t_2	39 min	0.19 min
ΔT_d	4 C°	0.2 C°
z	9.5 C°	0.95 C°

From Eq (5), one observes that there are only four dimensionless parameters, which significantly influence the correction factor out of the nine parameters obtained through the dimensional analysis. According to the same equation, a Cf value should decrease with increase in the value of t_b/t_2, f_h/t_2, or t_1/t_2 and increase with increase in $\Delta t_d/z$. These relationships are clearly observable from the results given in Tables 4 and 6. For example, we observe from these tables that the greater the value of ΔT_d, the greater the Cf value.

It is of interest to examine the influence of errors in dimensional parameters on a Cf value. Therefore an error analysis was performed by using Eq (5). Since most frequently observed values are located around zero in the design variables, we assumed the values of all design variables to be equal to zero.

The maximum expected errors of all dimensional parameters included in x_2, x_3, x_6, and x_9 are given in Table 7. These errors were used in the following analysis. From Eq (5) we have:

$$|\Delta y|_{max} = 0.024692\Delta x_2 + 0.058306\Delta x_3 + 0.033920\Delta x_6 + 0.22598\Delta x_9 \quad (8)$$

It should be noted that in Eq. (8) all error components are added (8) since we are interested in the maximum error in Cf and since Δx_1 may be either positive or negative. Errors associated with the quadratic terms of Eq (5) are nil since all x_1's are zero.

The relationship between errors in the dimensional parameters and P_1's are:

$$\begin{aligned} \Delta P_2 &= \Delta t_b/t_2 + (t_b/t_2^2)\,\Delta t_2 \\ \Delta P_3 &= \Delta f_h/t_2 + (t_h/t_2^2)\,\Delta t_2 \\ \Delta P_6 &= \Delta(\Delta T_d/z) + (\Delta T_d/z^2)\,\Delta z \\ \Delta P_9 &= \Delta t_1/t_2 + (t_1/t_2^2)\,\Delta t_2 \end{aligned} \quad (9)$$

The relationships between errors in P_i's and x_i's are:

$$\begin{aligned} \Delta x_2 &= [0.14817/(P_2 - 0.9939)]\,\Delta P_2 \\ \Delta x_3 &= [0.45406/(P_3 - 0.01204)]\,\Delta P_3 \\ \Delta x_6 &= [1.34967/(P_6 + 0.0379)]\,\Delta P_6 \\ \Delta x_9 &= 1.0500 \exp(3.1\,P_9)\,\Delta P_9 \end{aligned} \quad (10)$$

When we substituted the values given in Table 7, we obtain:

$$|\Delta y|_{max} = 0.0116$$

Therefore, we finally have:

$$|\Delta Cf|_{max} = (10/8.58)|y - 1|^{1.42/8.58} \cdot |\Delta y|_{max} = 0.00878 \text{ or } \pm 0.82\%$$

Since the above estimated error is negligible, we may conclude that the assumed magnitudes of errors in the dimensional parameters do not result in an appreciable error in the Cf value. We observed that the differences between experimental and predicted Cf values, Table 6, are 4.5% or less. These greater differences, compared with the error estimated above, are caused likely by errors associated with the linear interpolation of temperature data recorded on a strip chart as stated previously.

CONCLUSIONS

IDEALIZED deviant thermal processes were simulated by a computerized procedure to determine the correction factors of these processes applied to packaged, heat-conductive foods.

Through a dimensional analysis it was found that there are nine dimensionless parameters that uniquely define cor-

rection factors. The influence of each parameter was determined through nonlinear regression analysis which was based on a fractional central composite experimental design. Out of the nine dimensionless parameters the ones that showed a significant influence on the correction factor were those containing process time, drop location, f_h, z, and drop magnitude. Those with less influence were initial food temperature, retort temperature, cooling water temperature, f_c, and j values.

A reasonably good agreement was obtained between theoretical and experimental correction factors for thermal processes applied to canned bentonite suspension.

NOMENCLATURE

- Cf Correction factor
- F_p Sterilizing value (min)
- f Slope index of heating or cooling curve of food (min)
- j Intercept coefficient of heating or cooling curve of food
- n Number of independent parameters
- P Dimensionless parameters
- r Power of two, which represents a fraction of a full factorial design. For example, r = 1 for a half replicate of a full design and r = 2 for a quarter replicate of the same.
- s Value related to the coordinates of star points
- T Temperature; T without any subscript represents food temperature at any time (°C)
- t Time (min)
- X Design variable obtained by tranforming P
- y Dependent variable obtained by transforming Cf
- z Slope index of thermal death time curve (C°)

subscripts

- b End of heating phase of normal process
- d Deviant process or temperature deviation
- e End of cooling phase of normal or deviant process
- max Maximum value
- o Initial value
- p Experimentally observed value
- x End of heating phase of deviant process
- w Cooling medium
- 1 Heating medium used as T_1
- 1, 2 Beginning and end of temperature deviations respectively
- 1 through 9 Different P or X variables
- Δ Difference in two quantities or error in a quantity. For example $\Delta T_d = T_1 - T_d$ and Δt_b = error in t_b

REFERENCES

Anon. 1957. Fractional factorial experiment designs for factors at two levels. Statistical Engineering Laboratory, U.S. Dept. of Commerce, National Bureau of Standards, Applied Mathematics Series 48 issued April 15. U.S. Government Printing Office, Washington, DC.

Anon. 1976. Processes for low-acid foods in metal containers. National Canners Association Research Laboratory, Bull. 26-L, Washington, DC.

Barr, A.J., Goodnight, J.H., Sall, J.P., and Helwig, J.Y. 1976. "A User's Guide to SAS' 76. SAS Institute Inc., Raleigh, NC.

Davies, O.L. 1978. "The Design and Analysis of Industrial Experiments," 2nd ed. Imperial Chemical Industries Ltd., Longman Group Ltd., London and New York.

Giannoni, E., Hayakawa, D., Kimball, R., and Lewis, L.D. 1980. Unpublished data.

Hayakawa, K. 1971. Estimating food temperatures during various processing or handling treatments. J. Food Sci. 36: 378.

Hayakawa, K. 1972. Estimating temperature of foods during various heating or cooling treatments. ASHRAE J. 14(9): 65.

Hayakawa, K. and Giannoni, E. 1980. Unpublished data.

Houtzer, R.L. and Hill, R.C. 1977. Effect of temperature deviation on process sterilization value with continuous agitating retorts. J. Food Sci. 42: 775.

Houtzer, R.L. 1980. Personal communication.

Kreith, F. 1973. "Principles of Heat Transfer." Intext Press, Inc., New York.

Lopez, A. 1975. "A Complete Course in Canning." The Cannning Trade, Baltimore, MD.

Shiga, I. 1976. A new method of estimating thermal process time for a given F value. J. Food Sci. 41: 461.

Stumbo, C.R. 1973. "Thermobacteriology in Food Processing." Academic Press, New York.

Townsend, C.T., Reed, J.M., McCornell, J., Powers, J.J., Dwyer, J.J., and Ball, C.O. 1949. Comparative heat penetration studies on jars and cans. Food Technol. 3: 213.

Ms received 8/17/81; revised 10/19/81; accepted 10/22/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 7—10, 1981.

A paper of the Journal Series, New Jersey Agricultural Experiment Stations, Cook College, Rutgers, The State Univ. of New Jersey, Dept. of Food Science, New Brunswick, NJ 08903. This work was performed as a part of NJAES Project No. 1024, supported by the New Jersey Agricultural Experiment Sation and by Hatch Act Fund. Computer time fund for conducting the investigation was provided by the Center for Computer and Information Services, Rutgers, The State Univ. of New Jersey.

RHEOLOGICAL EVALUATION OF CHEDDAR CHEESE . . . From page 636

REFERENCES

Baron, M. 1949. Further studies of rheological properties of cheese during manufacture and ripening. Dairy Ind. 14: 146.

Baron, M. 1952. "The Mechanical Properties of Cheese and Butter." United Trade Press, London.

Baron, M. and Scott Blair, G.W. 1953. Rheology of cheese and curd. In "Foodstuffs Their Plasticity, Fluidity and Consistency," Ed. Scott Blair, G.W., p. 124. North Holland, Amsterdam.

Chen, A.H., Larkin, J.W., Clark, C.J., and Irwin, W.E. 1979. Textural analysis of cheese. J. Dairy Sci. 62: 901.

Creamer, L.K. 1970. Protein breakdown in Gouda cheese. N.Z. J. Dairy Sci. Tech. 5: 152.

Creamer, L.K. 1979. Degradation of casein components during cheese maturation. In "Proceedings of the Fifth International Congress of Food Science and Technology," Ed. Chiba, H., Fujimaki, M., Iwai, K., Mitsuda, H., and Morita, Y., Elsevier, New York.

Culioli, J. and Sherman, P. 1976. Evaluation of Gouda cheese firmness by compression tests. J. Texture Stud. 7: 353.

Dickinson, E. and Goulding, I.C. 1980. Yield behavior of crumbly English cheeses in compression. J. Texture Stud. 11: 51.

Emmons, D.B., Kalab, M., Larmond, E., and Lowrie, R.J. 1980. Milk gel structure. 10. Texture and microstructure in Cheddar cheese made from whole milk and from homogenized low-fat milk. J. Texture Stud. 11: 15.

Imoto, E.M., Lee, C.H., and Rha, C. 1979. Effect of compression ratio on the mechanical properties of cheese. J. Food Sci. 44: 343.

Lee, C.H., Imoto, E.M., and Rha, C. 1978. Evaluation of cheese texture. J. Food Sci. 43: 1600.

Lin, S.H.C., Leong, S.L., Dewan, R.K., Bloomfield, R.K., and Morr, C.V. 1972. Effect of calcium ion on the structure of native bovine casein micelles. Biochemistry 11: 1818.

Matheson, A.R. 1981. Determination of chymosin by radioimmune assay. N.Z. J. Dairy Sci. Tech. 16: 33.

Richardson, B.C. and Creamer, L.K. 1974. Comparative micelle structure. 4. Similarity between caprine α_s-casein and bovine α_{s3}-casein. Biochim. Biophys. Acta 365: 133.

Shama, F. and Sherman, P. 1973. Evaluation of some textural properties of foods with the Instron Universal Testing Machine. J. Texture Stud. 4: 344.

Szczesniak, A.S. 1975. General Foods texture profile revisited—Ten years perspective. J. Texture Stud. 6: 5.

Vernon Carter, E.J. and Sherman, P. 1978. Evaluation of the firmness of Leicester cheese by compression tests with the Instron Universal Testing Machine; J. Texture Stud. 9: 311.

Ms received 5/11/81; revised 9/23/81; accepted 9/25/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologits, Atlanta, GA, June, 1981.

We gratefully acknowledge financial support from the Cheese Research Institute and the College of Agricultural and Life Sciences of the University of Wisconsin. L.K. Creamer is grateful for the award of a Study Leave Bursary by the New Zealand Dairy Research Institute.

Purification and Properties of Carp Muscle Cathepsin D

YASUO MAKINODAN, TAKESHI AKASAKA, HARUHIKO TOYOHARA, and SHIZUNORI IKEDA

ABSTRACT

A carp muscle cathepsin was purified as an electrophoretically homogeneous preparation. The preparation represented about 2,000-fold purification and about 4% yield against the crude extract. The activity against hemoglobin was maximal at pH 2.6–2.8 with 0.6M buffer and near 3.2 with 0.12M buffer and at 50°C. The molecular weight was found to be 41,000 and the isoelectric point to be pH 5.4. From the effect of various inhibitors on the enzyme activity, the enzyme was identified as cathepsin D under the classification of Barrett. Carp muscle cathepsin D hydrolyzed myofibrils optimally at pH 3–4, but did not above pH 6.0. The participation of the enzyme in autolysis is very doubtful.

INTRODUCTION

THERE ARE at least three kinds of proteinases in fish muscle (ordinary muscle): acid, neutral, and alkaline proteinases (Makinodan, 1981). Acid proteinase has been investigated from relatively early on, and it may be said to be almost the only fish muscle proteinase recognized before 1960.

From the fact that the optimum pH for proteolysis of fish muscle homogenate is about pH 4.8 (Oya and Shimada, 1923), the existence of a protease active in the acid range has been recognized for a long time. However, enzymological study with enzyme preparations separated from muscular tissue is relatively new (Siebert, 1958; Saito and Sameshima, 1958). Since then, the acid proteinase has been studied enzymologically and in relation to degradation of muscular protein (Siebert et al., 1965; Ting et al., 1968; Makinodan and Ikeda, 1969a,b; Takahashi and Yamasawa, 1969; Mush and Siebert, 1971; Wojtowicz and Odense, 1972; Reddi et al., 1972; Geist and Crawford, 1974; Doke et al., 1980).

Siebert et al. (1965) purified a cathepsin from cod muscle about 3,000-fold against crude extract and determined the enzyme was very similar to cathepsin D. Fish muscle cathepsin D, or acid proteinase, seems to exist in lysosomes, like cathepsin D of many other animal tissues (Bird et al., 1969; Reddi et al., 1972). Reddi et al. (1972) reported the hydrolysis of the sarcoplasmic fraction by the muscular lysosomal fraction of winter flounders at pH 3.0–7.0, and Doke et al. (1980) showed that optimal hydrolysis of sarcoplasmic fraction by crude muscle extract of *Tilapia mossambica* occurs at pH 5.0. However, there is no evidence that the hydrolysis depends on the protein degradation by cathepsin D.

Cathepsin D is physiologically considered to take part in intracellular digestion of proteins (Barrett, 1977c). On the other hand, the relation between fish muscle cathepsin D and proteolysis after the death of fish is uncertain. To clarify this relationship, it is necessary to purify the enzyme and examine its action on fish muscle proteins.

In the present study, we have purified an acid proteinase from carp muscle as a homogeneous preparation in disc electrophoretic analysis. Then we determined that the present enzyme was cathepsin D, and examined whether the enzyme participates in fish muscle proteolysis.

Authors Makinodan, Akasaka, Toyohara, and Ikeda are with the Dept. of Fisheries, Faculty of Agriculture, Kyoto Univ., Kitashirakawa, Kyoto 606, Japan.

MATERIALS & METHODS

Materials

Cultured carp (*Cyprinus carpio*) of approximately 900g were obtained live from commercial suppliers. The fish was killed by decapitation, eviscerated and washed by city water immediately. Only ordinary muscle was collected in an iced beaker. DEAE-Sephadex A-50 and Sepharose 6B were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden; hemoglobin was a product of Difco Laboratories, Detroit, MI; bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome c were obtained from Sigma Chemical Co., St. Louis, MO; casein, synthetic substrates, and all other chemicals were purchased from Nakarai Chemicals Co., Kyoto, Japan. Carp muscle myofibrils were prepared by the method of Tokiwa and Matsumiva (1969).

Measurement of enzyme activity

Activities of cathepsins A, B, and C were measured by hydrolysis of following substrates, respectively: carbobenzoxy-L-glutamyl-L-phenylalanin (Makinodan and Ikeda, 1976), benzoyl-DL-arginine β-naphtylamide (Barrett, 1977b), and glycyl-L-tyrosineamide (de la Haba et al., 1959). Cathepsin D activity was determined by a modification of the method of Anson (1938): the reaction mixture, containing 1.5 ml of formate buffer, pH 3.0, prepared by mixing 1.0M sodium formate with 1.0M formic acid (Barrett, 1977c), 0.5ml of 5% (w/v) acid denatured hemoglobin, pH 3.0, and 0.5ml of enzyme solution, was incubated at 37°C for 1 hour. The reaction was stopped by the addition of 2.5 ml of 5% (w/v) trichloroacetic acid (TCA). After standing for 45 minutes at room temperature, the solution was filtered through Whatman No. 42 paper. A blank was prepared in the same manner except that hemoglobin was incubated separately from the other components of the reaction mixture and then combined after adding TCA. The Cu-Folin value of lml of TCA filtrate was measured by the method of Lowry et al. (1951). Activity was expressed as nanomoles of tyrosine released per ml of enzyme solution per hour and specific activity as nanomoles of tyrosine released per mg protein per hour. Neutral proteinase activity was determined as reported previously (Makinodan et al., 1979). Activity of alkaline proteinase was measured by the modified method of a previous paper (Makinodan and Ikeda, 1969b): the reaction mixture, containing 1.5 ml of phosphate buffer (M/10 KH_2PO_4-M/20 $Na_2B_4O_7$). pH 8.0 0.5 ml of 5% (w/v) casein solution and 0.5 ml of enzyme solution, was incubated at 63°C for 1 hr. After this, the procedure was the same as for cathepsin D.

Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard, or the absorbance at 280 nanometer.

Purification of carp muscle cathepsin D

Unless otherwise indicated, all operations were done at 5°C. Ordinary muscle of about 1 kg was homogenized with two volumes of 0.1M NaCl containing 1mM 2Na-EDTA in a Waring Blendor. After standing for 3 hr, homogenate was centrifuged at 13,000 × *g* for 30 min. The supernatant was dialyzed overnight against 0.1M NaCl containing 1mM 2Na-EDTA and centrifuged at 13,000 × *g* for 10 min (crude enzyme solution). To the crude extract, 1M HCl was added to adjust pH to 4.2 and the suspension was heated at 30°C for 10 min. The precipitate was removed by centrifugation,

and then the pH of the supernatant was readjusted to the original pH with 1M NaOH and the solution was centrifuged again to remove precipitate (acid treated fraction). Powdered ammonium sulfate was added to the supernatant to 60% saturation. After standing for 3 hr, the precipitate was collected by centrifugation at 13,000 x *g* for 30 min and dissolved in cold 0.1M NaCl containing 1mM 2Na-EDTA. This fraction was dialyzed overnight against the same NaCl solution as above and centrifuged to remove precipitate (ammonium sulfate fraction). Cold acetone (−20°C) was added to this fraction to give a 30–60% saturated fraction. After standing for 2 hr at −20°C, the precipitate was collected by centrifugation and dissolved in 5 mM Tris-HCl buffer, pH 8.0, containing 20 mM NaCl, 1 mM 2 Na-EDTA, and 100mM sucrose. The solution was dialyzed overnight against the same buffer and centrifuged to remove precipitate (acetone fraction). The acetone fraction (about 160 mg protein) was applied to a DEAE-Sephadex A-50 column (2.2 x 18 cm) equilibrated with Tris buffer as above and eluted stepwise with 0.02M, 0.12M, 0.22M, and 0.52M NaCl in the same buffer. The flow rate was 40 ml per hour. Each fraction was 5 ml. The fractions eluted with 0.12M NaCl were pooled (DEAE-Sephadex fraction). The DEAE-Sephadex fraction (about 35 ml) was dialyzed overnight against 1% glycine solution, concentrated to 3–4 ml with a Collodion Bag and applied to a preparative electrofocusing. The electrofocusing was performed at 8 watts for 10 hr. Then the enzyme fractions were desorbed from the gel by 50 mM phosphate buffer, pH 7.0, and dialyzed against the same buffer (electrofocusing fraction). The electrofocusing fraction (about 10 ml) was concentrated to 3–4 ml with a Collodion Bag and applied to a Sepharose 6B column (1.5 x 90 cm). The enzyme was eluted with 50 mM phosphate buffer, pH 7.0, containing 20 mM NaCl, 1 mM 2 Na-EDTA, and 100 mM sucrose at a flow rate of 4 ml per hour. Each fraction was 3 ml. Proteolytically active fractions were pooled (Sepharose fraction).

Determination of molecular weight

Molecular weight of the purified cathepsin D was determined by Sephadex G-100 gel filtration (Andrews, 1964). The column (1.5 x 90 cm) was eluted with 20 mM phosphate buffer, pH 7.0. The flow rate was 7 ml/hr with 2.5 ml fraction volume. The molecular weights taken for the reference proteins were: bovine serum albumin, 67,000; ovalbumin, 45,000; chymotrypsinogen, 25,000; cytochrome c, 12,400.

Electrophoresis

Disc electrophoresis of the purified enzyme preparation was carried out according to the method of Davis (1964): the enzyme (40 μg) was loaded on a 7.5% polyacrylamide gel column, and permitted to migrate in the direction of the anode at 4 mA per column using 50 mM Tris-glycine buffer, pH 8.8. The gel was stained with Coomassie Brilliant Blue. Slab SDS-gel electrophoresis of myofibrils treated with cathepsin D was done by the method of Suzuki (1977) with 15% polyacrylamide gel. In the treatment of myofibrils with catehpsin D, the reaction mixture containing 1.3 ml of 0.2M buffer, 0.1 ml of chloramphenicol solution (final concentration, 100 ppm), 0.1 ml of pepstatin (final concentration, 1 μg/ml) or 0.1 ml of buffer, 0.5 ml of myofibrils solution (1 mg protein), and 0.5 ml of enzyme solution was incubated at 37°C for 24 hr. Preparative electrofocusing and the determination of isoelectric point were performed with an apparatus of LKB-Produkter AB, Bromma, Sweden.

RESULTS

Purification of carp muscle cathepsin D

Fig. 1 shows a chromatographic pattern of DEAE-Sephadex A-50 column elution fractions. Cathepsin D was eluted with 0.12M NaCl in 5mM Tris-HCl buffer, pH 8.0. In the fraction eluted with 0.22M NaCl no cathepsin D activity was observed, but it contained cathepsin A activity. The DEAE-Sephadex fraction was separated into many protein fractions by electrofocusing (Fig 2). The highest cathepsin D activity was observed in the pH 5.3–5.4 fractions. The elution pattern of electrofocusing fraction from Sepharose 6B column is shown in Fig. 3. The enzyme was eluted as a single peak which corresponds to one obvious protein peak. Table 1 gives data on a typical purification of carp muscle cathepsin D.

Purity of the enzyme

Purification of the final enzyme preparation (Sepharose fraction) was about 2,000-fold over the crude enzyme solution. As shown in Fig. 4, the purified enzyme preparation gave a single band in disc electrophoretic analysis and the band coincided with the activity peak which was determined from another gel. Neither activities of cathepsins A, B, C, nor neutral or alkaline proteinase was observed in the purified preparation.

Enzymic properties

Effect of pH on activity. Effect of pH on hydrolysis of hemoglobin is shown in Fig. 5 and 6. When 1.0M buffer was used (final concentration, 0.6M), optimum pH was found to be 2.6–2.8 (Fig. 5). At the acidic limit, activity

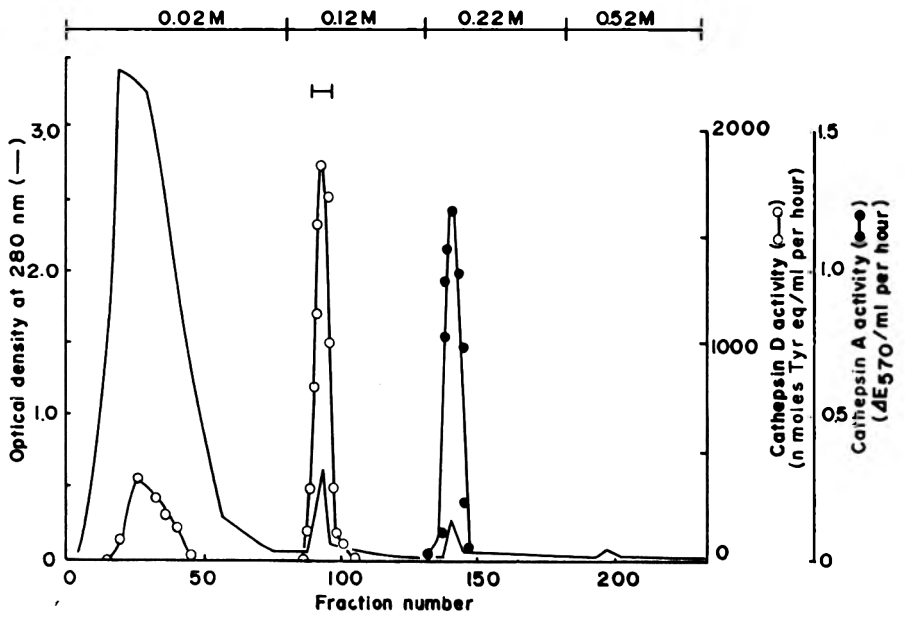


Fig. 1—DEAE-Sephadex A-50 column chromatography of acetone fraction.

fell off rapidly with pH and on the less acidic side, the curve showed a distinct shoulder near pH 4.0. The pH curve obtained with 0.2M buffer (final concentration, 0.12M) showed optimum activity near pH 3.2 and did not form any shoulder (Fig. 6). When the enzyme was preincubated in the absence of substrate at 37°C for 30 min with 50 mM glycine-HCl (pH 1.0–2.7), formate (pH 3.0–5.0), acetate (pH 5.0–6.0), and phosphate (pH 6.0–8.0) buffers, the activity was stable in the range pH 3.0–5.0, fairly stable between pH 5.0–7.0 but at pH 2.0 it was quite lost.

Effect of temperature on activity. The optimum temperature for 1 hr incubation was about 50° C (Fig. 7). When the enzyme was preincubated in the absence of substrate in 1.0M formate buffer, pH 3.2, for 30 min, it was fairly stable at 40°C, retaining about 85% of the activity, but lost the activity about 85% at 50°C.

Effect of inhibitors on activity. The results are shown in Table 2. The enzyme was completely inhibited by the addition of pepstatin (1 μg/ml) and diazoacetylnorleucine methylester (DAN) + Cu^{++} (each 1 mM), both are inhibitors of carboxyl proteinase. Dip-F and soy bean trypsin inhibitor, which inhibit serine proteinase, had no effect. Dithiothreitol (DDT) + EDTA (each 2 mM), which activate thiol proteinase, and 4-chloromercuribenzoate (pCMB, 1mM), which inhibits thiol proteinase, had no effect on the enzyme activity. DTT + EDTA and o-phenanthroline (1 mM) which inhibit metallo proteinase had no effect. Solvents such as ethanol, methanol and iso-propanol as well as 1 mM cysteine and glutathione had no effect. 2-mercaptoethanol increased the activity about 40%.

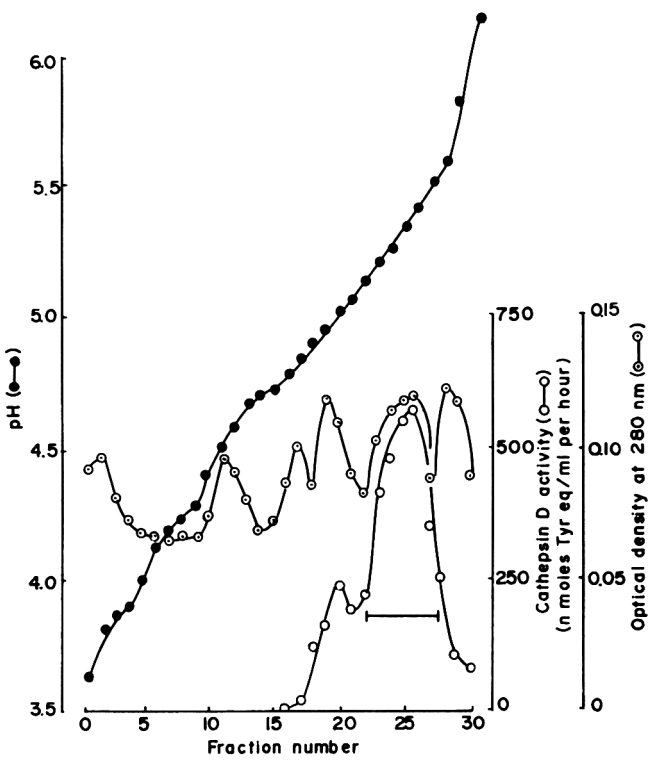


Fig. 2–Preparative electrofocusing of DEAE-Sephadex fraction in an amphoryte pH gradient from pH 4 to 6.

Physicochemical properties

Fig. 8 illustrates the determination of molecular weight by gel filtration. The extrapolated molecular weight was estimated to be 41,000. From the position of the most active fraction in electrofocusing (Fig. 2), the isoelectric point was determined to be pH 5.4.

Action of the enzyme on casein and carp muscle myofibrils

The pH-activity curves of casein and myofibrils are presented in Fig. 9. For casein substrate, maximum activity was shown at pH 5.0–5.5, but hardly any activity was observed at pH 6.5. Optimum pHs for myofibrils in the absence and the presence of 3 M urea were found to be 3.2 and 4.0, respectively. Myofibrils were not hydrolyzed above pH 6.0. The activity in the presence of 3M urea was lower than that in the absence at every pH examined. The auto-

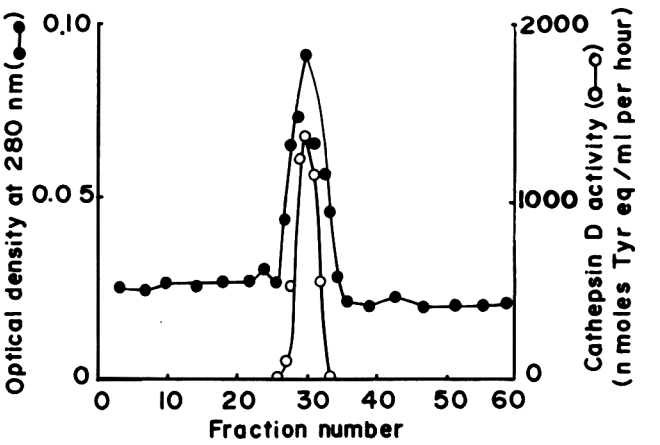


Fig. 3–Elution pattern of electrofocusing fraction on Sepharose 6B.

Table 1–Typical purification chart for carp muscle cathepsin D

Fraction	Volume (ml)	Protein (mg/ml)	Activity[a]	Purification (fold)	Yield (%)
Crude enzyme	2720	6.54	124	1	100
Acid treatment (pH 4.2)	1570	3.80	140	1.9	65
Ammonium sulfate fractionation (0–60%)	85	10.2	1150	5.9	29
Acetone fractionation (30–60%)	50	3.50	1120	17	17
DEAE-Sephadex A-50 chromatography	40	0.200	910	240	11
Electrofocusing	17	0.075	800	560	4.0
Sepharose 6B gel filtration	15	0.022	815	2000	3.6

[a] Nanomoles tyrosine equivalent per ml of enzyme solution per hour

lysis of myofibrils was not observed. Hydrolysis of myofibrils was also examined by SDS-slab gel electrophoresis (Fig. 10). At pH 3.2, the high molecular weight and 45,000 dalton-bands vanished and many distinct bands appeared at dalton lower than 67,000 after 24 hr incubation. This phenomenon was not observed in the presence of pepstatin. At pH 6.5, electrophoresis pattern hardly changed after incubation.

DISCUSSION

PREVIOUSLY, we (Makinodan and Ikeda, 1969c) reported some properties of carp muscle acid proteinase with a partially purified enzyme preparation. The reported results coincide with the present ones. A more purified preparation (Makinodan and Ikeda, 1976) was obtained later, but it still seemed to contain some contaminating proteins. The present cathepsin D preparation, however, was homogeneous in disc electrophoretic analysis.

Optimum pH with 1M buffer (final concentration, 0.6M) was 2.6–2.8, but with 0.2M buffer (final concentration, 0.12M) it was near 3.2. The cause of such difference of optimum pH is not clear. The position of the optimum pH is possibly influenced by an effect of ionic strength on hemoglobin (Cunningham and Tang, 1976). The pH curve with the 1.0M buffer showed a shoulder near pH 4.0. It is well known that cathepsin D shows a shoulder on the less acidic side of the optimum pH (Barrett, 1977c). The optimum pH value, 3.2, was lower in comparison with 4.6 for cod muscle cathepsin against urea denatured hemoglobin (Siebert et al., 1965). About 25% of maximum activity was found near pH 6.5 on cod muscle cathepsin, but no activity was observed at such pH using the carp muscle enzyme. Optimum pH of muscle cathepsin D of *Tilapia mossambica* with 0.04M (final concentration) acetate buffer against acid denatured hemoglobin is 2.8 and the pH curve shows a shoulder near pH 4.0 (Doke et al., 1980). The difference in optimum pH value depends possibly on the different substrate used in addition to species specificity. As for purified muscle cathepsin D of land animals, an optimum pH of 4.0 is reported with 0.05–0.25M (final concentration) acetate buffer against urea denatured hemoglobin (Suzuki and Fujimaki, 1968; Fukushima et al., 1971).

The optimum temperature for 1 hr incubation was about 50°C, but the enzyme was very labile at the same temperature in the absence of the substrate. The protective effect against heating by substrate is generally known (Makinodan

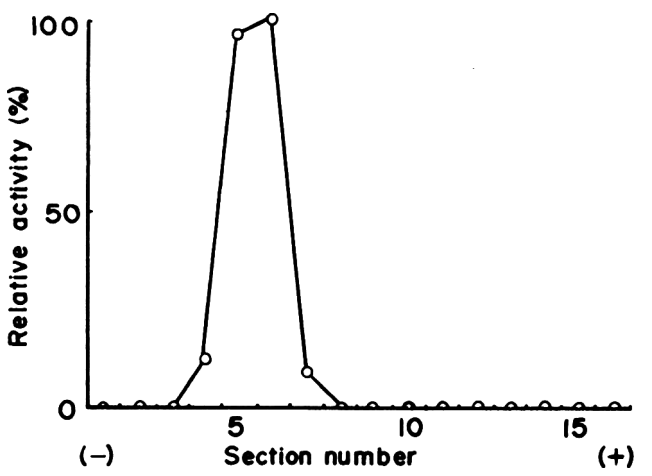


Fig. 4—Disc electrophoresis of the purified enzyme preparation. Graph demonstrates the activity of 3 mm sections of another gel.

Table 2—Effect of inhibitors on activity

Inhibitor	Conc	Relative activity (%)
None		100
Pepstatin	1 μg/ml	0
DTT + EDTA	2 mM (each)	102
Dip-F	1 mM	107
Soy bean trypsin inhibitor	100 μg/ml	112
o-Phenanthroline	1 mM	100
pCMB	1 mM	90
DAN + Cu^{++}	1 mM (each)	0

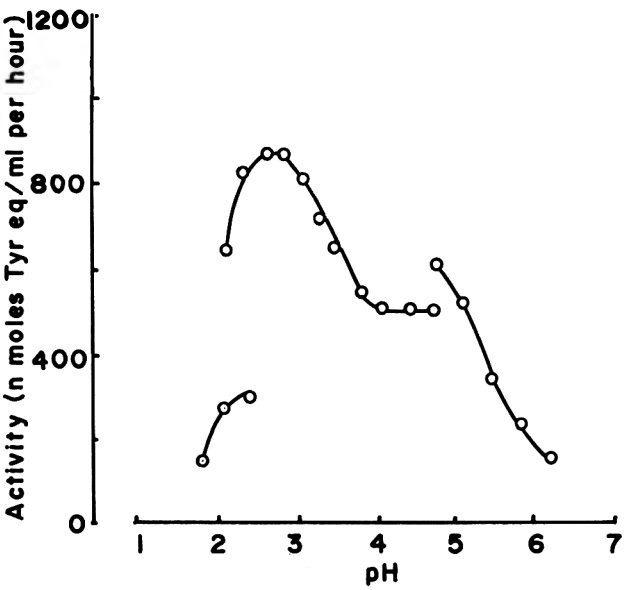


Fig. 5—Effect of pH on hydrolysis of hemoglobin in 0.6M buffer (final concentration). The buffers were glycine-HCl (pH 1.8–2.4), formate (pH 2.1–4.6), and acetate (pH 4.0–6.0).

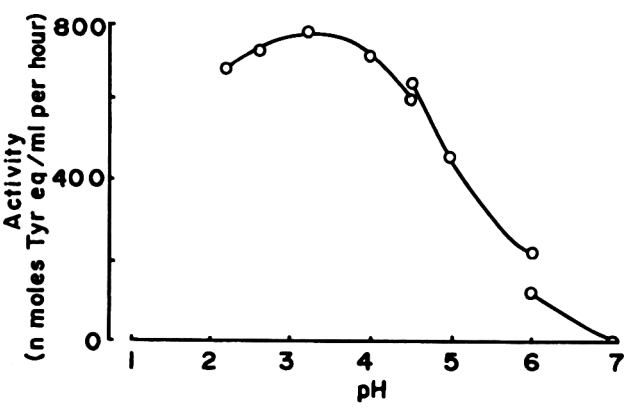


Fig. 6—Effect of pH on hydrolysis of hemoglobin in 0.12M buffer (final conc). The buffers were formate (pH 2.2–4.5), acetate (pH 4.5–6.0), and phosphate (pH 6.0–7.0).

and Ikeda, 1969b). Optimum temperature of chicken muscle cathepsin D is 40°C (Fukushima et al., 1971) and the activity at 50°C is very low. This property is different from that of carp muscle cathepsin.

The activity was inhibited by pepstatin and DAN + Cu^{++}, but hardly affected by DTT + EDTA, Dip-F, Soy bean trypsin inhibitor, o-phenanthroline and pCMB. The molecular weight was 41,000 and isoelectric point was 5.4. From these results the enzyme was identified as cathepsin D under the classification of Barrett (1977a). Carp muscle cathepsin D was not affected by cysteine, a similarity with cathepsin D of chicken muscle (Fukushima et al., 1971) and of *Tilapia mossambica* muscle (Doke et al., 1980). The activity was stimulated about 40% by 2-mercaptoethanol. This result coincides with the previous report (Makinodan and Ikeda, 1969c).

Molecular weights of 32,000, 38,000 and 50,000 are reported for the muscle acid proteinase or cathepsin D of winter flounder (Reddi et al., 1972), *Tilapia mossambica* (Doke et al., 1980) and cod (Siebert et al., 1965), respectively. Siebert (1973) stated that as the molecular weight of cathepsin D shows species and/or tissue specificity, it should not be taken as a strong criterion for cathepsin D. As for muscle cathepsin D of land animals, the molecular weight is 36,000 for chicken (Fukushima et al., 1971) and 42,000–45,000 for rat (Schwart and Bird, 1977).

The acid proteinase activity has been observed in fish muscle for a long time and it was almost the only protein-

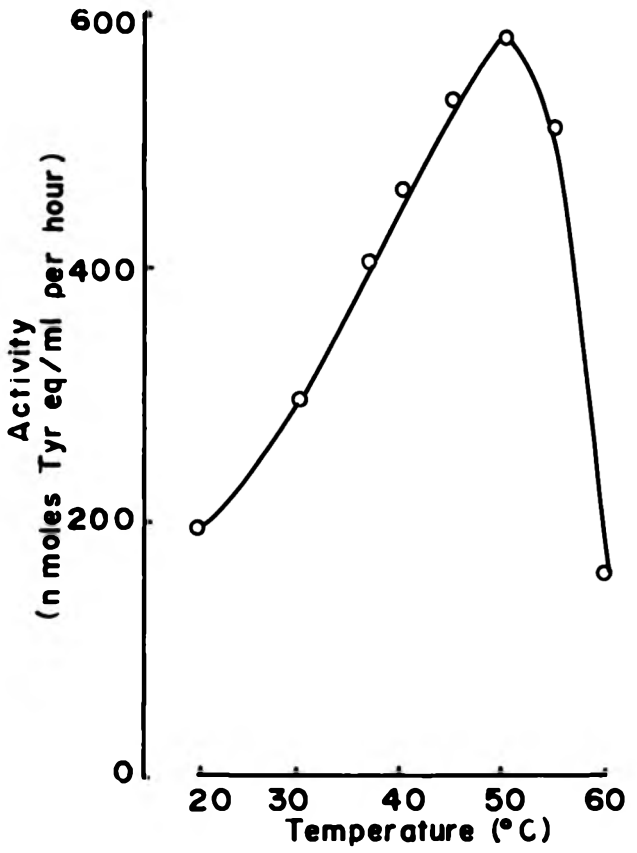


Fig. 7—Temperature-activity curve of the enzyme at pH 3.2 for 1 hr.

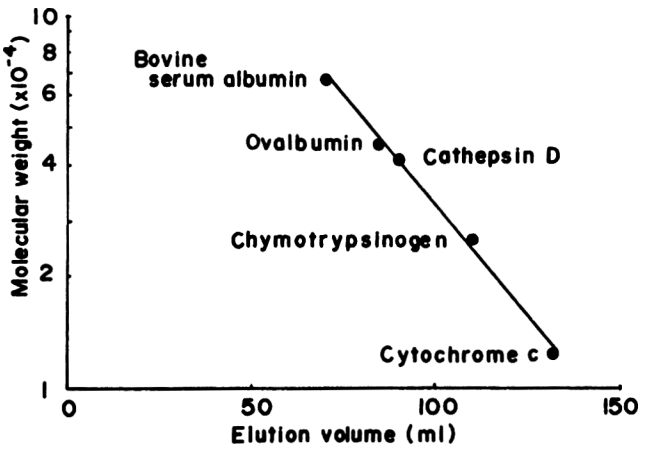


Fig. 8—Determination of molecular weight of carp muscle cathepsin D by Sephadex G-100 gel filtration.

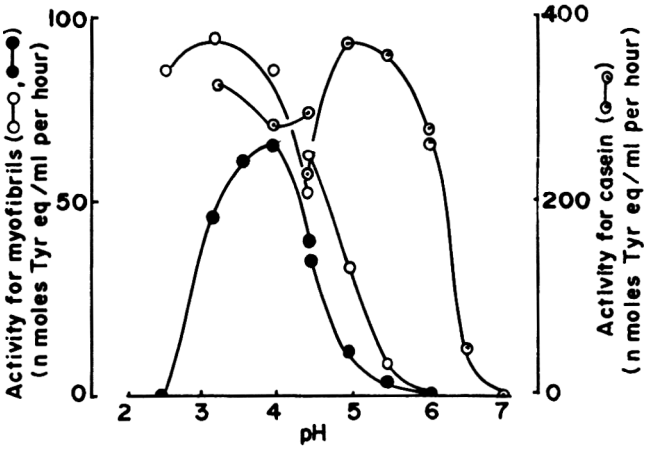


Fig. 9—pH-activity curves for casein and carp muscle myofibrils. The buffers were 0.12 M (final concentration) formate (pH 2.4–4.5), acetate (pH 4.5–6.0), and phosphate (pH 6.0–7.0). o—o: No urea addition; •—•: Urea (3M) addition.

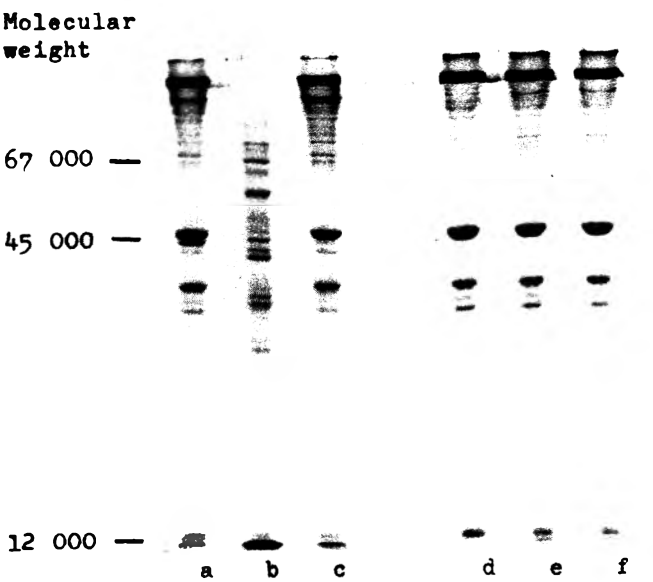


Fig. 10—Slab SDS polyacrylamide gel electrophoretic patterns of myofibrils treated with cathepsin D: (a) Zero hour-incubation at pH 3.2; (b) Without pepstatin at pH 3.2; (c) With pepstatin at pH 3.2; (d) Zero hour-incubation at pH 6.5; (e) Without pepstatin at pH 6.5; (f) With pepstatin at pH 6.5.

ase activity of muscular tissue observed before 1960. The acid proteinase has often been discussed in connection with the autolysis of fish muscle (Oya and Shimada, 1923; Siebert and Schmitt, 1965; Manita et al., 1969). However, the pH of fish muscle does not decrease so much after the death of fish, the ultimate pH for carp ordinary muscle is 6.3–6.6 (Makinodan et al., 1980). This fact makes it difficult to relate the enzyme to the autolysis. On the other hand, cathepsin D is a typical endopeptidase. Carp muscle cathepsin D acts on hemoglobin and releases nona or deca peptides (Makinodan et al., 1979), and the released products from hemoglobin are further hydrolyzed by muscle cathepsin A (Makinodan and Ikeda, 1976). These facts seem to suggest that muscle cathepsin D is possibly involved in the initial stages of meat protein degradation.

We sought the activity near neutrality of the carp muscle cathepsin D. The pH optimum for hemoglobin was near 3.2 with 0.2M formate buffer, but for casein it was 5.0–5.5. The result seemed to suggest that cathepsin D might be active near neutrality against some substrate. Therefore the activity against myofibrils was examined. The optimum pH in the presence of 3M urea in comparison with that in its absence shifted one pH unit to a neutral range. However, considering the decrease in the maximum activity, this shift possibly relates to the unstability of the enzyme under low pH conditions and the presence of urea (Barrett, 1977c; Wojtowicz and Odense, 1970). Myofibrils, however, were not hydrolyzed above pH 6.0. The same result was observed by slab SDS-gel electrophoresis. These facts do not support the possibility of participation in the autolysis of the cathepsin D. Reddi et al. (1972) carried out an interesting study about catheptic activity of winter flounder muscle and recognized that muscle lysosomal fraction hydrolyzed the sarcoplasmic fraction at pH 3–7. But these fractions may contain components other than proteins and proteinases. Accordingly it seems that it is still open to discussion whether the hydrolysis of sarcoplasmic fraction near neutrality reflects the hydrolysis of proteins by a proteinase.

Kazakova and Orekhovich (1975) reported that when rat liver cathepsin D was coupled to activated Sepharose, the preparations were totally inactive in acid media and exhibited maximum activity at pH 7.0 against hemoglobin. If this is true, the relation of lysosome-bound cathepsin D to autolysis may be clarified to some degree. However, the same experiments in other laboratories could detect no change in pH optimum in comparison with the free enzyme (Barrett, 1977c), nor could we for carp muscle cathepsin D.

From the above results, the participation of muscle cathepsin D in autolysis is very doubtful. Geist and Crawford (1974) reported that fish muscle cathepsins did not play a significant role in muscle degradation during cold storage. Cathepsin D, however, exists generally in tissues of creatures, so there is probably a purpose for its existence. Cathepsin D is possibly a physiologically important enzyme which exists in lysosomes in the living body and takes part in metabolism of proteins.

REFERENCES

Andrew, P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. 91: 22.

Anson, M.L. 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J. Gen. Physiol. 22: 79.

Barrett, A.J. 1977a. Introduction to the history and classification of tissue proteinases. In "Proteinases in Mammalian Cells and Tissues," Ed. Barrett, A.J. North-Holland Publishing Co., Amsterdam.

Barrett, A.J. 1977b. Cathepsin B and other proteinases. In "Proteinases in Mammalian Cells and Tissues," Ed. Barrett, A.J. North-Holland Publishing Co., Amsterdam.

Barrett, A.J. 1977c. Cathepsin D and other carboxyl proteinases. In "Proteinases in Mammalian Cells and Tissues," Ed. Barrett, A.J. North-Holland Publishing Co., Amsterdam.

Brid, J.W.C., Berg, T., Milanesi, A. and Stauber, W.T. 1969. Lysosomal enzymes in aquatic species. 1. Distribution and particle properties of muscle lysosomes of the gold fish. Comp. Biochem. Physiol. 30: 457.

Cunningham, M. and Tang, J. 1976. Purification and properties of cathepsin D from porcine spleen. J. Biol. Chem. 251: 4528.

Davis, B.J. 1964. Disk electrophoresis. 2. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404.

de la Haba, G., Cammarata, P.S., and Timasheff, S.N. 1959. The partial purification and some physical properties of cathepsin C from beef spleen. J. Biol. Chem. 234: 316.

Doke, S.N., Ninjoor, V., and Nadkarni, G.B. 1980. Characterization of cathepsin D from the skeletal muscle of fresh water fish, Tilapia mossambica. Agric. Biol. Chem. 44: 1521.

Fukushima, K., Gnoh, G.H. and Shinano, S. 1971. Purification and properties of a proteolytic enzyme, cathepsin D, from chicken muscle. Agric. Biol. Chem. 35: 1495.

Geist, G.M. and Crawford, D.L. 1974. Muscle cathepsins in three species of pacific sole. J. Food Sci. 39: 548.

Kazakova, O.V. and Orekhovich, V.N. 1975. Some properties of cathepsins chemically fixed to carriers. Int. J. Peptide Protein Res. 7: 23.

Lowry, O.H., Rosebrough, N., Farr, A. and Randall, J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265.

Makinodan, Y. 1981. Fish muscle protease. In "Minor Components of Fish and Shell-Fish—Their Biochemistry and Food Chemistry" (Japanese). Ed. Ikeda, S. Koseisha Koseikaku Co., Tokyo.

Makinodan, Y. and Ikeda, S. 1969a. Studies on fish muscle protease. 1. On the existence of two kinds of proteinases active in acid and in slightly alkaline pH range. Bull. Japan. Soc. Sci. Fish. 35: 672.

Makinodan, Y. and Ikeda, S. 1969b. Studies on fish muscle protease. 2. Purification and properties of a proteinase active in slightly alkaline pH range. Bull. Japan. Soc. Sci. Fish. 35: 749.

Makinodan, Y. and Ikeda, S. 1969c. Studies on fish muscle protease. 3. Purification and properties of a proteinase active in acid pH range. Bull. Japan. Soc. Sci. Fish. 35: 758.

Makinodan, Y. and Ikeda, S. 1976. Studies on fish muscle protease. 6. Separation of carp muscle cathepsins A and D, and some properties of carp muscle cathepsin A. Bull. Japan. Soc. Sci. Fish. 42: 239.

Makinodan, Y., Hirotsuka, M., and Ikeda, S. 1979. Neutral proteinase of carp muscle. J. Food Sci. 44: 1110.

Makinodan, Y., Hirotsuka, M., and Ikeda, S. 1980. Autolysis of carp muscle. Bull. Japan. Soc. Sci. Fish. 46: 1507.

Manita, H., Koizumi, C., and Nonaka, J. 1969. Aseptic autolysis of mackerel muscle. Bull. Japan. Soc. Sci. Fish. 35: 1027.

Mush, K. and Siebert, G. 1971. Differenzierung von zwei Kathepsinen aus Dorschmuskel. Hoppe-Seyler's Z. Physiol. Chem. 352: 878.

Oya, T. and Shimada, K. 1923. Gyoniku no jikashoka ni tsukite. Suiko Shiken Hokoku. 19:1.

Reddi, P.K., Constantinides, S.M. and Dymsza, H.A. 1972. Catheptic activity of fish muscle. J. Food Sci. 37: 643.

Saito, K. and Sameshima, M. 1958. Studies on the biochemical change in fish muscle. 8. On the proteolytic activity of fish muscle extracts. Bull. Japan. Soc. Sci. Fish. 24: 201.

Schwart, W.N. and Bird, J.W.C. 1977. Degradation of myofibrillar proteins by cathepsins B and D. Biochem. J. 167: 811.

Siebert, G. 1958. Activität Eiweiss spaltender Enzyme in Fischen. Experientia. 14: 65.

Siebert, G. 1973. Properties of cathepsins from fish muscle. Wiss. Zeits. Martin-Luther-Univ. Halle-Wittenberg. 6. Intracell. Protein Catabolism, Proc. Symp.

Siebert, G. and Schmitt, A. 1965. Fish tissue enzymes and their role in the deteriorative changes in fish. In "The Technology of Fish Utilization" Ed. Kreuzer, R. Fishing News Ltd., London.

Siebert, G., Schmitt, A., and von Malortie, R. 1965. Reinigung und Eigenschaften von Dorschmuskel-Kathepsin. Hoppe-Seyler's Z. Physiol. Chem. 342: 20.

Suzuki, K. 1977. Sulabugata SDS-akuriruamidogeru denkieido ho. Iden. 31: 43.

Suzuki, A. and Fujimaki, M. 1968. Studies on proteolysis in stored muscle. 2. Purification and properties of a proteolytic enzyme, cathepsin D, from rabbit muscle. Agric. Biol. Chem. 32: 975.

Takahashi, T. and Yamasawa, M. 1969. Studies on carp muscle cathepsins. J. Tokyo Univ. Fish. 55: 99.

Ting, C.Y., Montgomery, W. and Anglemier, A.F. 1968. Partial purification of salmon muscle cathepsins. J. Food Sci. 33: 617.

Tokiwa, T. and Matsumiya, H. 1969. Fragmentation of fish myofibril. Effect of storage condition and muscle cathepsin. Bull. Japan. Soc. Sci. Fish. 35: 1099.

Wojtowicz, M.B. and Odense, P.H. 1970. The effect of urea upon the activity measurement of cod muscle cathepsin with hemoglobin substrate. Can. J. Biochem. 48: 1050.

Wojtowicz, M.B. and Odense, P.H. 1972. Comparative study of the muscle catheptic activity of some marine species. J. Fish. Res. Bd. Canada. 29: 85.

Ms received 5/30/81; revised 8/29/81; accepted 9/2/81.

An outline of this report was presented at a spring meeting of the Japanese Society of Scientific Fisheries, Tokyo, Japan, 1980.

This work was supported in part by a research grant from the Ministry of Education of Japan.

Instron Measurements and Sensory Scores for Texture of Poultry Meat and Frankfurters

KENNETH J. PRUSA, JANE A. BOWERS, and EDGAR CHAMBERS IV

ABSTRACT

The relationship of sensory scores and Instron measurements of poultry meat and frankfurters was studied. Chicken samples were separated by tensile grips; turkey samples were sheared with a Warner-Bratzler shear attachment and compressed with a simulated-molar attachment; and poultry frankfurters were tested with the Warner-Bratzler shear, puncture probe, and simulated-teeth attachments. Generally, for chicken higher correlation coefficients between sensory scores and Instron measurements resulted when baseline length and area measurements of curves were made than when peak height was measured. For turkey, Instron measurements with either the Warner-Bratzler shear or the simulated-molar attachment were not related significantly to sensory scores. For frankfurters, Instron measurements usually were significantly correlated with sensory scores for firmness.

INTRODUCTION

THE INSTRON Universal Testing Machine, used widely in food research, has been used to evaluate texture of meat, meat products, and intact poultry muscle.

To establish the relationship between Instron measurements of meat texture and sensory analysis of meat texture, Voisey et al. (1975) used a compression attachment mounted on the Instron; for frankfurters, they found high correlation with sensory scores (firmness r=0.92, and chewiness r=0.89). Quinn et al. (1978) in evaluating frankfurters determined these correlation coefficients between sensory scores and Instron measurements: firmness, 0.94; cohesiveness, 0.95; and resilience, 0.36. Simon et al. (1965), using puncture probes shaped as molars and incisors to evaluate texture of whole frankfurters, found excellent correlation (r=−0.87) between puncture tests and taste panel evaluation of tenderness. Baker et al. (1970), who evaluated textural changes of chicken frankfurters of different pH, found that Instron puncture measures and taste panel scores were related (r=0.948).

Although some information exists on use of the Instron for evaluating poultry products, more work needs to be done. We developed simulated teeth attachments for the Instron and compared them and other Instron attachments with sensory scores for intact poultry muscle and a processed poultry product (frankfurters).

EXPERIMENTAL

Sensory analysis

Chicken (Prusa et al., 1981) and turkey (Chambers et al., 1982) samples (4 x 1½ x ½ cm) from pectoralis major muscles treated with three levels of papain and cooked in microwave and conventional ovens were cut perpendicular to the muscle fibers and served to a 10 or 11-member, semi-trained panel for sensory analysis. Panelists were given a 21 cm structured, linear scale (divided and labeled every 3 cm beginning 1.5 cm from the end) to use in evaluating tenderness and mealiness.

Poultry frankfurters containing two levels of nitrite and potassium sorbate or sorbic acid were prepared according to Chambers et al. (1982) and presented to a 10-member, semi-trained sensory panel, who scored them for firmness on a 21 cm structured, linear scale divided the same as for chicken and turkey.

Authors Prusa, Bowers, and Chambers are affiliated with the Dept. of Foods & Nutrition, Kansas State Univ., Manhattan, KS 66506.

Instron measurements

Duplicate samples (4 x 1 x ½ cm), cut perpendicular to the muscle fibers, were used to measure connective tissue strength between fibers; other samples (4 x ½ x ½ cm) for measuring fiber strength were cut parallel to the muscle fibers. Samples were placed between tensile grips mounted on an Instron Universal Testing Machine, Model 1122 and pulled apart with 2 kg of force (crosshead speed, 20 mm per min; chart speed, 100 mm per min). Three measurements were taken from the curves: (1) area under the curve, (2) peak height, and (3) baseline length from beginning of curve to a line drawn perpendicular from peak height to baseline.

Duplicate turkey samples (4 x 1 x 1 cm, pectoralis major muscle) were compressed by using a simulated molar attachment mounted on the Instron (with a force of 10 kg, crosshead speed of 100 mm per min, and recorder chart speed of 200 mm per min). Additionally, core samples (1.27 cm diam) cut parallel to the fibers were sheared with a Warner-Bratzler shear attachment mounted on the Instron. Peak height and area under the curve were measured.

Instron measurements (shear, compression, and puncture) were made on samples of poultry frankfurters. One sample, a 2.5-cm section, was sheared by using a Warner-Bratzler shear attachment mounted on the Instron. Additionally, a simulated incisor attachment was used to measure texture of a 2.5-cm section (through the skin) and a molar attachment (with the molars biting through the skin) to measure the compression of a 6.25 cm section is shown in Fig. 1. Another measurement was made on 1 cm sections (same style of sample as for taste panel), placed upright and compressed with the molar attachment biting through the interior of the sample without first rupturing the outer skin. Molar (3.8 in. diam) and incisor (½ in. x 1/16 in.) attachments were made of aluminum by the Kansas State University Physics Laboratory. The last measurement was with a 0.317-cm puncture probe mounted on the Instron;

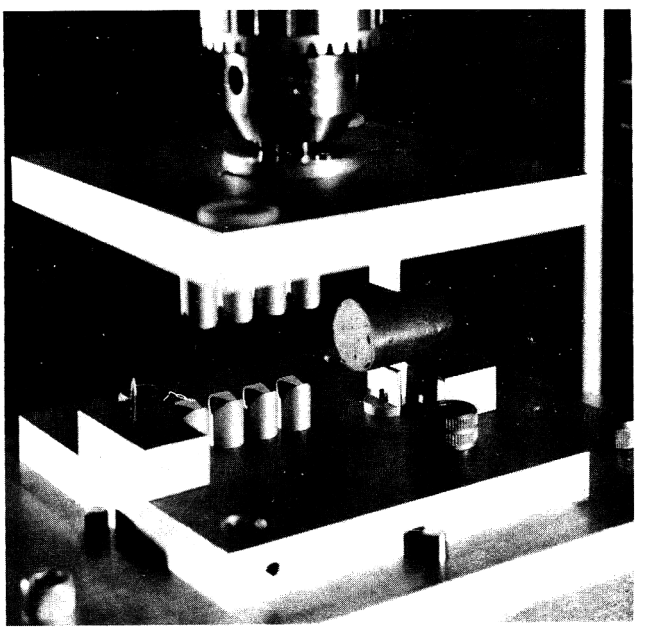


Fig. 1—Simulated incisor attachment for Instron.

the probe moved through samples (2.5 cm), first rupturing the top skin, continuing through the interior and also puncturing the bottom skin. All measurements were taken with 10 kg of force, applied at a crosshead speed of 100 mm per min and graphed at a chart speed of 200 mm per min. Peak height and area under the curve were measured.

Correlation coefficients were determined for Instron measurements and sensory scores for firmness (frankfurters) and tenderness and mealiness (chicken and turkey) by using both the means of six treatments (n=6) and for the 58 or 60 individual observations (n=58 or 60).

RESULTS & DISCUSSION

CORRELATION COEFFICIENTS based on both treatment means and individual observations are presented in Table 1. Correlations were higher when treatment means were used than when individual observations were used because averaging 10 observations for each treatment reduced variation. For chicken and turkey, the correlation coefficients would be expected to be neagtive because as tenderness and mealiness increase, Instron measurements should decrease. For frankfurters, coefficients would be expected to be positive because as firmness scores increase, Instron values should increase.

Table 1—Correlation coefficients for Instron measurements and sensory attributes of intact chicken and turkey muscle and frankfurters

	Tenderness		Mealiness	
	n=6[a]	n=60[b]	n=6[a]	n=60[b]
Chicken				
Fiber[c]				
Peak height	−0.20 ns	−0.01 ns	−0.19 ns	−0.13 ns
Baseline length	−0.58 ns	−0.19 ns	−0.33 ns	−0.22 ns
Area under curve	−0.90 *	−0.08 ns	−0.75 ns	−0.13 ns
Connective tissue[d]				
Peak height	−0.70 ns	−0.12 ns	−0.78 ns	−0.05 ns
Baseline length	−0.70 ns	−0.24 ns	−0.90 *	−0.38 **
Area under curve	−0.66 ns	−0.02 ns	−0.78 ns	−0.25 ns
Turkey				
Warner-Bratzler				
Area under curve	0.13 ns	−0.32 *	0.50 ns	−0.06 ns
Peak height	0.47 ns	0.30 *	0.75 ns	0.06 ns
Simulated molar				
Area under curve	−0.49 ns	−0.20 ns	−0.70 ns	−0.21 ns
Peak height	−0.53 ns	−0.23 ns	−0.77 ns	−0.20 ns

	Firmness	
Frankfurter	n=6[a]	n=60[b]
Warner-Bratzler		
Area under curve	0.88 *	0.47 ***
Peak height	0.92 **	0.59 ***
Puncture probe		
Area under curve	0.99 ***	0.25 ns
Peak height	0.98 ***	0.68 ***
Simulated incisor		
Area under curve	0.96 **	0.59 ***
Peak height	0.98 ***	0.59 ***
Simulated molars I		
Area under curve	0.95 **	0.55 ***
Peak height	0.57 ns	0.11 ns
Simulated molars II		
Area under curve	0.74 ns	0.39 **
Peak height	0.53 ns	0.19 ns

[a] Treatment means (treatment described in Chambers and Bowers (1981), Chambers et al. (1982) and Prusa et al. (1981)
[b] Individual observations, only 58 for chicken
[c] Sample cut parallel to muscle fiber
[d] Sample cut perpendicualr to muscle fiber
***, significance at 0.1% level
**, significance at 1% level
*, significance at 5% level
ns, not significant

Chicken

For relating Instron measurements of fiber strength to both tenderness and mealiness of chicken (Table 1), correlation coefficients were higher (using treatment means) for measurements of area under the curve or of baseline length than for those of peak height. Generally, peak height measurements from the Instron curves produced low and nonsignificant correlation coefficients when related to tenderness and mealiness scores.

Instron measurements (peak height, baseline length, area under curve) of connective tissue strength between fibers were related moderately (r=−0.66 to −0.90) to mealiness and tenderness scores when treatment means were used. For treatment means and individual observation calculations, baseline length measurements of Instron curves had higher correlation coefficients with sensory scores than the other two measurements.

In general, more correlation coefficients were significant between sensory scores and Instron measurements when baseline length and area measurements of curves were made than when peak height was measured.

Turkey

Correlation coefficients from the turkey data, presented in Table 1, were negative when they indicated good agreement between sensory scores and Instron measurements. Generally, measurements with the Warner-Bratzler shear attachment did not relate to tenderness or mealiness of turkey, as indicated by the low and in some cases positive correlation coefficients. Measurements using the simulated molar attachment had higher correlation coefficients with sensory scores (usually negative as expected) than did those using the Warner-Bratzler shear; but even so, none was significant.

Frankfurters

For frankfurters, positive correlation coefficients between firmness scores and Instron measurements were expected (Table 1). In most cases all correlation coefficients were moderate or high. Measurements using the Warner-Bratzler shear, puncture probe, and simulated incisor attachment were more highly related to firmness scores than were measurements with the simulated molar attachment.

The results of our work indicate that by using the Instron Universal Testing Machine one can obtain a good measure of firmness of frankfurters, but attachments for measure of the texture of intact muscle should be further refined.

REFERENCES

Baker, R.C., Darfler, J., and Vadehran, D.V. 1970. Effect of pH on the quality of chicken frankfurters. J. Food Sci. 35: 693.
Chambers, E. IV, Bowers, J.A., Prusa, K., and Craig, J. 1982. Sensory attributes, Instron measurements, and residual nitrite of reduced-nitrite poultry frankfurters with sorbic acid or potassium-sorbate. J. Food Sci.
Chambers, E. IV, and Bowers, J.A. 1981. Sensory characteristics of postmortem papain injected turkey cooked conventionally or by microwaves. J. Food Sci. 46(5): 1627.
Prusa, K.J., Chambers, E. IV., Bowers, J.A., Cunningham, F., and Dayton, A.D. 1981. Thiamin content, texture and sensory evaluation of postmortem papain-injected chicken. J. Food Sci. 46(6): 1684.
Quinn, J.R., Raymond, D.P., and Larmond, E. 1978. Instrumental measurement of wiener texture. Canadian Institute of Food Sci. & Technol. J. 12(3): 154.
Simon, S., Field, J.C., Karmlich, W.E., and Tauber, F.W. 1965. Factors affecting frankfurter texture and a method of measurement. Food Technol. 19: 410.
Voisey, P.W., Randall, C.J., and Larmond, E. 1975. Selection of an objective test of wiener texture by sensory analysis. Canadian Institute of Food Sci. & Technol. J. 8: 23.
Ms received 4/20/81; revised 10/14/81; accepted 10/16/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 6-10, 1981.
Contribution No. 81-376-j, Dept. of Foods & Nutrition, Kansas Agricultural Experiement Station, Manhattan, KS 66506.

Protein Efficiency Ratio and Amounts of Selected Nutrients in Mechanically Deboned Spent Layer Meat

E. L. MOTT, J. H. MacNEIL, M. G. MAST, and R. M. LEACH

ABSTRACT

Three types of mechanically deboned spent layer meat (MDSL) were found to have adjusted protein efficiency ratios (PER) equivalent or superior to a standard (casein) diet. These same materials proved to be good sources of calcium, iron and zinc. Percentage protein ranged from 15.39 for MDSL from whole spent layers to 16.55 for MDSL from spent layer frames with wings. Fat levels were highest for whole birds (20.41%) and lowest for frames without skin (16.84%). High levels of unsaturated fatty acids were found to be associated with these products.

INTRODUCTION

MECHANICALLY DEBONED poultry meat (MDPM) from spent layers is currently being used in a variety of food products—specifically in sausage-type foods such as frankfurters and bologna. These products have met with consumer approval and expanded usage in other food items is underway.

Chemical and physical characteristics of MDPM were first identified by Grunden et al. (1972). Further studies by Cunningham and Mugler (1974), Dhillon and Maurer (1975) and Mast and MacNeil (1976) provided information on the functional properties of MDPM. Microbial qualities of MDPM were identified by Ostovar et al. (1971), Maxcy et al. (1973) and Mulder and Dorresteijn (1975).

Several studies on the nutritional characteristics of MDPM have been conducted. Essary and Ritchey (1968) determined the amino acid profile of mechanically deboned turkey meat. MacNeil et al. (1978) reported similar amino acid profiles for mechanically deboned broiler meat and ground beef. Biological evaluation of protein quality, as expressed by Protein Efficiency Ratio (PER), for raw mechanically deboned broiler meat was found to be comparable to a standard casein-lactalbumin diet (Brinkman and MacNeil, 1976) and a standard casein diet (MacNeil et al., 1978). Mechanically deboned turkey meat (MDTM) was also found to have a significantly higher PER value when compared to a casein diet (MacNeil et al., 1979).

Little comprehensive information concerning mechanically deboned spent layer meat (MDSL) is available in the literature. It is the intent of this paper to provide this information.

EXPERIMENTAL

THREE TYPES OF MDSL were obtained from a commercial poultry processing plant in New Jersey. Samples consisted of deboned meat from raw whole spent layers, spent layer frames with wings (MDSL with skin) and spent layer frames (MDSL without skin). Poultry frames include backs, breast cage and ribs, and pelvic girdle areas. Four 10 kg boxes of each type of meat were obtained. One box from each type was packed in insulated containers and immediately transported unfrozen to the university for initial analysis. The remaining boxes representing samples of each group were placed in the plant's freezer along with the regular production run of mechanically deboned poultry meat. This material was removed from the freezer and transported to the university after 1 wk. The study was replicated three times with all chemical analyses conducted in duplicate.

Official methods of the AOAC (1975) were used to determine fat (petroleum ether extractables), moisture, ash, fatty acids (preparation according to Metcalfe et al., 1966) and protein by Kjeldahl nitrogen determination (N x 6.25 for meat protein). Iron and zinc were measured by atomic absorption spectrophotometry (Perkin-Elmer Co., Norwalk, CT), calcium content by a Corning calcium analyzer (Corning Scientific Inst., Medford, MA), calorie content by a Parr Automatic Adiabatic Calorimeter, Parr Instrument Co., Moine, IL), amino acids with a Beckman Model 121 amino acid analyzer (Beckman Instruments, Palo Alto, CA), using procedures outlined in Beckman Instruction Manual A-IM3 fluoride content using a specific ion electrode (Dolan et al. 1977) and oxidative stability changes by the 2-thiobarbituric acid (TBA) test (Tarladgis et al., 1960).

Amino acid analysis and the PER study were conducted on pooled samples of each type of meat from the three replicates. Frozen blocks of meat were cut into small cubes using a meat band saw, and freeze-dried. The material was then ground in a Hobart meat grinder, placed in sealed containers and held at 21°C until incorporated into the experimental diets. Proximate analysis, amino acids and fatty acids were determined on freeze-dried material as well as fresh MDSL to detect possible changes during freeze-drying.

AOAC (1975) procedures for PER were followed except that fat contents of the diets were adjusted to complement the higher fat content of the test material. For example, inclusion of the test material at the recommended level to provide the required 9% protein resulted in an 18% dietary fat content. Thus a standard casein diet with an 18% fat level was included as well as a 9% fat level casein diet. This procedure is similar to that suggested by Hurt et al. (1974). It has been shown in two previous studies, MacNeil et al. (1978) and MacNeil et al. (1979), that there were no significant differences between the PER values obtained with the high and low fat casein diets. Subsequent testing used the conventional 9% fat level diets for adjusting the PER values. Each test diet was fed to 10 male weanling rats (Wistar strain, Charles River Breeding Labs, Wilmington, MA) which were individually housed in stainless steel cages with raised wire bottoms. Food and water were supplied *ad libitum*. PER values were computed for each rat as grams of weight per gram of protein consumed. PER values obtained were adjusted with the reference casein diet being given the value of 2.5.

Data were analyzed by analysis of variance to determine effects of treatment and replication on the characteristics of MDPM. When significant differences were found by the analysis of variance, Duncans New Multiple Range Test (1955) was applied.

RESULTS & DISCUSSION

MDSL produced from whole spent layers (Table 1) contained 15.39% protein, 20.41% fat, and 62.47% moisture. The proteinand moisture levels were significantly lower and fat content was significantly higher for this type of meat than those levels observed in previous studies (MacNeil et al., 1978, 1979). This reflects the composition of the sample prior to deboning: MDSL from whole spent layers has a high meat-to-bone ratio and includes the heavy carcass fat deposits and skin of the bird. Thus, the increased fat concentration reflects the presence of carcass fat as well as

Authors MacNeil and Mast are with the Dept. of Food Science, The Pennsylvania State Univ., University Park, PA 16802. Author Leach is with the Dept. of Poultry Science, The Pennsylvania State Univ., University Park, PA 16802. Author Mott, formerly with The Pennsylvania State Univ., is now with the School of Public Health, Univ. of Michigan, Ann Arbor, MI 48109.

the fact that fat from skin is expressed with the meat. Reduced protein concentration is thought to be due to a dilution effect and also due to the fact that skin protein (collagen) is discarded with the bone during the deboning process. Work on the effect of skin on the resultant composition of the MDSL product was first done by Froning et al. (1973) and later confirmed by MacNeil et al. (1977). Whole MDSL and MDSL with skin had a significantly lower ash concentration as compared to MDSL without skin. This is thought to be due to the dilution effect of fat from skin on the samples.

Table 1—Proximate analysis[a] and selected nutrient content of mechanically deboned spent layer meat (wet wt)

Characteristic	Whole	W/Skin	W/O Skin
Protein (%)	15.39b	16.19a	16.55a
Fat (%)	20.41a	16.91b	16.84b
Moisture (%)	62.47b	66.35a	66.63a
Ash (%)	1.19b	1.28b	1.39a
Calcium (%)	0.19a	0.20a	0.23a
Iron (mg/100g)	1.20b	4.25a	4.05a
Zinc (mg/100g)	1.87a	2.48a	2.40a
Fluoride (ppm)	—	12.27b	14.58a
Kilocalories/100g	271.4a	234.4b	23.13b

[a] Means followed by the same letter are not significantly different ($p < 0.01$) from each other. Each mean represents six determinations.

Table 2—Amino acid analysis of freeze dried deboned spent layers meat (grams of amino acid residue per 100 g of total amino acid residues)

Amino acid	Whole	W/Skin	W/O Skin
		Essential Amino Acids	
Histidine	3.47b	5.00a	5.07a
Lysine	9.59a	11.19a	9.17a
Threonine	4.81a	4.84a	4.42a
½ Cystine	0.42a	0.41a	0.65a
Valine	4.01b	4.42ab	4.74a
Methionine	2.95a	2.52b	2.66b
Isoleucine	3.95b	5.61a	5.62a
Leucine	8.46a	7.87b	7.91b
Phenylalanine	4.02b	4.09ba	4.32a
Tryptophan	ND	ND	ND
Total	41.68	45.95	44.56
		Nonessential Amino Acids	
Arginine	6.60a	6.25a	4.34b
Aspartic	10.73a	8.74b	9.08b
Serine	4.39a	4.21b	4.29b
Glutamic	16.95a	15.91a	17.11a
Proline	4.26a	4.11a	5.01a
Glycine	5.12b	4.45a	5.51a
Alanine	6.28a	5.71ab	5.51b
Tyrosine	3.62a	3.62a	3.67a
Total	58.45	53.95	54.52

[a] Each value represents the mean of four determinations on the pooled sample.
[b] ND — not determined
[c] Means followed by the same letter are not significantly different ($p > 0.01$) from each other.

Table 1 (Selected nutrient content of the three types of MDSL) shows that MDSL is a good source of calcium, iron and zinc. Iron concentration is significantly lower in whole MDSL due to the presence of additional meat on the carcass prior to deboning. High fluoride concentrations, due to the age of the bird and the brittleness of the bones, in MDSL with skin and MSDL without skin could be reduced by making the appropriate adjustment to the deboner screen. Kilocalories, as expected, reflect the fat concentrations of the individual samples.

Amino acid profiles of the three MDSL types are shown in Table 2 and are grouped into essential and nonessential amino acids. This study did not include a determination of tryptophan since it is readily destroyed by the acid hydrolysis procedure used. Histidine is included as an essential amino acid because it is essential in the diets of infants and rats. Cystine is included since it may replace part of methionine. There was little variation in the amino acid profile of each MDSL type. Analysis of variance on the essential amino acid grouping showed that whole MDSL was significantly different from MDSL with skin and without skin for histidine, methionine, isoleucine, and leucine. Studies on commercial lean ground beef performed in this laboratory revealed a total of 40g essential amino acids per 100g of total amino acids as compared to 41.68g, 45.98g, and 44.56g essential amino acids per 100g of total amino acids for whole MDSL, MDSL with skin, and MDSL without skin.

The results of the PER test are shown in Table 3. In test 1 when whole fowl was evaluated the PER value for this material, while higher, was not significantly better than the high-fat standard casein diet but was higher than the low-fat diet. In test 2 when two types of MDSL was compared to the conventional 9% fat casein diet both were found to be significantly higher. It is evident then that the whole fowl or MDSL obtained from fowl frames, with or without skin, is a good source of high quality protein.

Fatty acid composition (Table 4) shows little difference between two types of MDSL tested. It should be noted that MDSL contains the expected high level of unsaturated fatty acids normally associated with poultry products.

—Continued on page 663

Table 3—Growth and protein efficiency ratios (PER) for rats fed mechanically deboned spent layer meat

Diet	Total wt gained (g)	Total feed consumed (g)	Total protein consumed (g)	Adjusted PER
Test I				
Casein (8% fat)	111.69b	401.88b	36.17b	2.36b
Casein (18% fat)	111.88b	381.91b	34.37b	2.50ab
Whole fowl	142.97a	461.97a	41.58a	2.65a
Test II				
Casein (9% fat)	106.69b	435.67b	39.21b	2.50b
Fowl w/skin	146.57a	505.50a	45.49a	2.94a
Fowl w/o skin	139.88a	503.91a	43.35a	2.73a

[a] Means within columns followed by the same letter are not significantly different ($p < 0.01$) from each other.

Table 4—Fatty acid composition in mechanically deboned spent layer meat

Treatment	Lauric	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
	12:0	14:0	16:0	16:1	18:1	18:1	18:2	18:3
Whole		0.94	21.18	5.36	4.10	45.50	22.11	0.79
w/skin		1.13	26.26	7.06	4.43	41.82	19.30	trace

[a] Each value represents the mean of 4 determinations on the pooled sample

Bovine Bone Marrow Lipids

G. J. MILLER, M. R. FREY, J. E. KUNSMAN and R. A. FIELD

ABSTRACT

Characteristics of marrow lipids in bones from three different anatomical locations (cervical, lumbar, femur) in steers and cows on low energy (range) or high energy (feedlot) diets are reported. Cervical marrow contained the least and femur marrow the most total lipid and triglycerides (TG) were the major type of lipid present in all marrows. Phosphatidyl choline was the major PL present in all marrows. The predominant fatty acids were 16:0, 18:0 and *cis* 18:1. There were no consistent effects due to anatomical location of bone, diet or sex upon any of the acids measured. TG structures were similar in all marrows and the fatty acid composition of TG from steer marrow resembled that of steer intramuscular TG.

INTRODUCTION

AT PRESENT, bovine bone marrow is a part of the American diet via soups, sauces and mechanically deboned red meat (MDM) in food products. There is a possibility that the latter source of marrow may increase and the USDA Select Panel report on MDM (Kolbye and Nelson, 1977) noted a need for additional information concerning specific lipid patterns of bovine bone marrow from bones suitable for mechanical deboning. Mello et al. (1976) reported the types of lipids present in whole bones from six different anatomical locations of calves, heifers and cows and the major portion of that lipid represented marrow lipid. Christie (1978), in a recent review of lipids in ruminant tissues, reported some values for bovine bone marrow lipids. This report concerns lipids present in bone marrow from three different bones of steers and cows on low energy (range) and high energy (feedlot) diets.

METHODOLOGY

FIVE STANDARD GRADE STEERS and five utility grade cows, referred to as range steers and range cows, were grazed on native range at least 4 months prior to slaughter and five choice grade steers and five commercial grade cows, referred to as feedlot steers and feedlot cows, received a fattening ration for at least 4 months prior to slaughter. The fattening ration consisted of about 9 kg of a grain mix (corn, barley, oats, beet pulp; 4:2:2:1 by weight) and about 4.5 kg hay per day. Between 15–20g marrow from cervical, lumbar and femur bones of the animals were obtained 24 hr postmortem. Marrow was removed from cervical and lumbar bones by centrifugation (Sanchez et al., 1978) and from split femur bones. The marrows were stored at 4°C prior to lipid extraction (not more than 48 hr). The marrows were homogenized and 10.0g were extracted (Kunsman and Field, 1976) and the extracts washed as described by Folch et al., (1957). The chloroform was removed by rotary evaporation, lipids redissolved in hexane, and the solution passed through 1 micron pore size Teflon membrane filters (Miller et al., 1979). Aliquots were evaporated for gravimetric analysis of total lipids and the remainder stored in hexane at −20°C until analyzed (maximum of 6 months).

Cholesterol was determined by the method of Kovacs et al. (1979) and results have been incorporated into a previous report (Kunsman et al., 1981). Lipid phosphorus in the cervical and lumbar marrow was determined by the procedure of Dodge and Phillips (1967). Lipid from femur marrow contained low levels of phosphorus and it was necessary to dry ash (550°C) relatively large amounts of lipid prior to colorimetric analysis for phosphorus. Samples of the total lipids from the sixty marrows based on equal weights of lipid were composited by bone, diet and sex prior to the analysis of triglycerides (TG), TG structures and individual phospholipids (PL).

TG and PL were isolated by column chromatography using a 1.5 cm (i.d.) column containing 5g silicic acid (Biosil A, Bio-Rad Laboratories, Richmond, CA). The column was loaded with 200 mg lipid in hexane and washed with 12 ml hexane. TG was eluted with 60 ml hexane:ethyl ether (95:5). eighty ml chloroform were then passed through the column to remove neutral lipids and 20 ml chloroform:acetone (1:1) to remove glycolipids [glycolipids were not detected under these conditions using the colorimetric procedures of Radin et al. (1955)]. PL were eluted by increasing the amount of methanol in chloroform (50 ml chloroform:methanol, 18:32, 60 ml 3:57 and finally 20 ml methanol). Thin layer chromatography was used to check contents of each column fraction.

TG were determined gravimetrically after removal of solvent. Enzymatic hydrolysis of TG for determination of fatty acids in the *sn*-2 position was performed according to Breckenridge (1978) as modified by Miller et al. (1981). Preparation of diglycerides, synthesis of phosphatidyl phenols and enzymatic hydrolysis of the phenols for determination of fatty acids in the *sn*-1 position were performed according to Brockerhoff (1967). *Ophiophagus hannah* venom was used as the source of phospholipase A_2 activity. Fatty acids in the *sn*-3 position were obtained by difference.

After solvent removal from the PL fraction, the PL were dissolved in chloroform and subjected to high performance thin-layer chromatography (HPK plates, Whatman Inc., Clifton, NJ) using solvent system F of Touchstone et al. (1980). Areas defined by charring after sulfuric acid spray were scraped into tubes and phosphorus determined (Dodge and Phillips, 1967). Individual PL were identified by comparison to standards (Supelco, Inc., Bellefonte, PA).

Fatty acid methyl esters were prepared as described for butter oil by Macgee and Allen (1977) using carbon tetrachloride as extracting solvent. Packed columns of SP 2330 (10% on 100/120 mesh Chromosorb W/AW, Supelco, Bellefonte, PA) were used for analysis of long chain fatty acids (LCFA) and OV 101 (10% on 100/120 mesh Gas Chrom Q, Applied Sciences, State College, PA) for medium chain fatty acids (MCFA; Miller et al., 1980). Identification was by both comparing to known gas chromatograph/mass spectrometer standards (Miller et al., 1980) and by rechromatographing hydrogenated and argentation thin-layer chromatograph separated samples. For quantification heneicosanoic acid (21:0) served as internal standard for LCFA and tetradecanoic acid (14:0), analyzed using 21:0 as internal standard, then served as internal standard for MCFA (Miller et al., 1981). *Cis* and *trans*-octadecenoic acids (18:1) were determined by capillary gas chromatography using a wall-coated (polycyanopropylsiloxane–Alltech Associates, Arlington Heights, IL) 50 m x .5 mm glass column. Injector temperature was 140°C, detector temperature 200°C and the column was programmed from 140–200°C at 1°C/min. Helium at 3 ml/min served as carrier gas, nitrogen was used as make-up for the flame ionization detector and the instrument was run in the split mode (20:1). Mixtures of the methyl esters of *cis* 6-, 9-, 11-, 18:1 and *trans* 6-, 9-, 11-, 18:1 were chromatographed and it was found that the system would not separate the individual acids but would separate *trans* 18:1 from *cis* 18:1. Therefore, standard mixtures of *trans* 11- 18:1 (18:1ω7t) and *cis* 9- 18:1 (18:1ω9c) in ratios of .01:1, .05:1, .07:1 were prepared and chromatographed. Peak height ratios of *trans* 18:1 and *cis* 18:1 were determined from recorder trace and compared to the known weight ratios. There was an agreement of 95:1 ± 7.9% (average ± standard deviation). Using these ratios and total 18:1

Authors Miller, Frey, Kunsman, and Field are with the Div. of Animal Science, Univ. of Wyoming, Laramie, WY 82971.

of the mixtures as determined by packed column chromatography to calcualte the amount of *trans* 18:1 in the standard mixtures gave an accuracy of 95.6 ± 6.8 when compared to actual amounts.

RESULTS & DISCUSSION

ALL LIPID VALUES are reported as quantity of lipid per 100g fresh marrow. While many of the values reported are single values from composited samples, they can be useful to processors and nutritionists in estimating the amount of each lipid provided by bovine bone marrow in a food product.

Lipid values for marrows are shown in Table 1. Cervical marrow contained the least and femur the most total lipid. The reason for this is probably that cervical marrow is more involved in hematopoiesis and is not heavily infiltrated with adipocytes as is "yellow" bone marrow (Christie, 1978). There did not appear to be consistent effects of diet or sex upon levels of total lipid in marrows. Cervical marrow of steers appeared to contain more lipid than that of cows, whereas lumbar marrow of cows contained more than that of steers. In all cases, femur marrow contained very high levels of lipid. There were considerable variations within groups in total lipid, cholesterol and PL.

Foods may contain any or all of the various classes of lipids but those of greatest concern are TG, PL and cholesterol. As shown in Table 1, the predominant lipid present in all marrows was TG and in this respect marrow resembles bovine adipose tissue (Christie, 1978). Therefore, one of the major food values of marrow lipid will reside in food energy derived from fatty acids and/or the presence of dietary essential fatty acids. Marrows contained lower levels of cholesterol and PL than TG and these values declined from cervical marrow to femur marrow, indicating that the higher values of total lipid in femur marrow represented an increase in TG. The average ratio of cholesterol:PL in marrow and lipid is about 1:4 in cervical, 1:3 in lumbar and 1:1 in femur bones. Values calculated from data in other reports (Christie, 1978; Miller et al., 1979, 1981) show that such ratios vary from about 1:1–1:3 in adipose tissue lipid, 1:12–1:14 in skeletal muscle lipid and 1:12–1:18 in liver lipids of ruminants. Therefore, marrow resembles adipose tissue rather than muscle or liver tissue with respect to these ratios.

Individual PL, expressed as percent of total measured, are shown in Table 1. Phosphatidylcholine (PC) was the predominant PL in all marrows. Femur marrow PL did not contain any measurable lysoPC, lysophosphatidylethanolamine and very little if any phosphatidylserine. In addition, femur marrow PL contained relatively more PC and less sphingomyelin (SPH) than did PL of the other two marrows. A decrease in SPH was also noted in the lumbar and femur marrow PL of cows when compared to those of steers.

As mentioned, one of the major contributions of bone marrow lipid to food is fatty acids, since a majority of the lipids are TG. Table 2 shows the amounts of fatty acids in total lipids from 100g marrow. Femur marrow provides more of all fatty acids than cervical marrow because the highest levels of lipid are found in femur marrow. Lumbar marrow is intermediate with respect to levels of fatty acids provided. As with most ruminant tissue, the predominant acids present in bone marrows were 16:0, 18:0 and 18:1. The branched chain acids present, Br 13:0 and Br 15:0, were mixtures of *iso* and *anteiso* isomers. Assuming 18:2 was essentially all linoleic acid, then 100g bovine marrow could provide about 800 mg (range cow, cervical) to 1700 mg (feedlot steer, femur) of dietary essential fatty acids (linoleic, linolenic-18:3, arachidonic-20:4 acids). Table 3 lists the amounts of acids provided by triglycerides in 100g bone marrow. As expected, the amounts of fatty acids in total lipids (Table 2) and triglycerides are similar. There were no consistent effects of bone, diet or sex upon any of the acids measured in total lipids or triglycerides when expressed as weight/100g marrow (Tables 2 and 3).

Extensive dietary trials have suggested the TG structure of a fat is involved in its atherogenic potential (Myher et al., 1977). For example, it is proposed that the higher atherogenicity of native peanut oil in comparison to randomized peanut oil may arise from a relative metabolic unavailability of linoleic acid in the *sn*-2 position of native peanut oil TG due in part to the presence of long chain saturated acids (20:0–24:0) in the other positions, particularly the *sn*-3 position (Myher et al., 1977). It has also been suggested that increases in serum cholesterol are not merely dependent upon levels of 14:0 in butterfat TG but are probably related to its distribution among the various types of TG and the position it occupies in them (Mukherjee and Sengupta, 1981). Positional distribution of the fatty acids in marrow TG were determined and it was found that diet, sex and anatomical location of bone did not have any consistent effects upon distributions of any of the acids measured. Therefore, values obtained from all marrows in the study were composited and appear in Table 4. With respect to even-numbered saturated acids in marrow TG, 10:0 predominated in positions *sn*-1 and 3; 12:0 and 14:0 in *sn*-1 and 2; 16:0 in *sn*-1; 18:0 and 20:0, the major long chain saturated acids of marrow TG, in *sn*-1 and 3. In the case of odd-numbered saturated acids, 13:0 resembled 12:0 and predominated in positions *sn*-1 and 2; 15:0 was like 16:0 and predominated in position *sn*-1; 17:0 was similar to 18:0 and 20:0, with preference for *sn*-1 and 3. In regards to branched saturated acids, Br 13:0 showed little prefer-

Table 1—Lipid compositions of cervical, lumbar and femur marrows from range and feedlot steers and cows

	Cervical				Lumbar				Femur			
Lipid	RS[a]	FS[a]	RC[a]	FC[a]	RS	FS	RC	FC	RS	FS	RC	FC
Total lipid[b]	26.6±3.1	27.8±3.8	22.7±5.8	23.9±5.6	43.3±9.8	46.6±15.2	62.7±18.2	73.3±9.5	82.6±1.1	84.3±3.2	87.4±0.5	87.0±5.3
Triglyceride[b]	24.9	25.2	21.2	22.2	40.9	43.9	60.3	71.8	80.1	82.6	85.7	85.6
Cholesterol[c]	151±32	223±22	182±32	201±24	106±14	155±31	115±23	127±9	77±16	95±11	92±12	99±9
Phospholipid[c]	603±96	791±97	736±129	882±71	336±71	502±150	378±120	329±116	97±17	101±10	81±14	78±9
LPC[d]	5.5	6.1	6.0	7.0	4.4	6.1	4.3	4.1	0	0	0	0
SPH[d]	18.8	19.0	17.6	17.3	16.3	17.4	10.3	9.4	7.0	10.1	5.2	5.1
PC[d]	40.8	39.6	37.8	38.6	40.2	38.5	41.6	46.9	71.4	60.8	61.6	62.2
PS&LPE[d]	8.0	9.0	7.6	7.8	7.4	7.6	6.6	5.8	0	0	0	0
PI[d]	6.4	7.6	6.8	7.1	6.3	7.8	7.1	8.6	5.2[e]	3.6[e]	13.7[e]	12.3[e]
PA[d]	6.4	4.6	7.5	6.2	7.8	8.1	10.1	8.6	8.0	10.2	6.3	9.8
PE[d]	6.9	8.4	10.6	10.5	10.0	10.4	12.8	11.0	6.4	10.6	7.6	5.5
PG&DPG[d]	7.1	5.6	6.1	5.5	7.6	4.2	7.1	5.6	2.0	4.8	5.6	5.1

[a] RS-range steer; FS—feedlot steer; RC—range cow; FC—feedlot cow.
[b] Expressed as g/100g fresh marrow ± standard deviation where appropriate.
[c] Expressed as mg/100g fresh marrow ± standard deviation.
[d] LPC—lysophosphatidyl choline; SPH—sphingomyelin; PC—phosphatidyl choline; PS—phosphatidyl serine; LPE—lysophosphatidyl ethanolamine; PI—phosphatidyl inositol; PA—phosphatidic acid; PE—phosphatidyl ethanolamine; PG—Phosphatidyl glycerol; DPG—diphosphatidyl glycerol: all expressed as percent of total.
[e] May be contaminated with PS. Good separations were not obtained with femur samples.

ence for any one position and Br 15:0 predominated in the *sn*-2 position. The monoenoic acids 14:1, 16:1 and *cis* 18:1 predominated in the *sn*-2 position, whereas *trans* 18:1 was similar to long chain saturated acids in preference for *sn*-1 and 3. The longest chain monoenoic acid, 20:1, predominated in position *sn*-3. With respect to polyunsaturated acids 18:2, 18:3 and 20:4, all predominated in positions 2 and 3 with preference for the *sn*-2 position. These results are similar to those reported for beef adipose TG (Christie, 1978) and beef intramuscular TG (Miller et al., 1981).

Bone marrow TG contained relatively low levels of 14:0 and this acid predominated in positions 1 and 2 (Tables 3 and 4). An increase in serum cholesterol produced by dietary butterfat has been reported to be related to its content of 14:0 and the researchers showed that the 14:0 in butterfat was present exclusively in the 2 position of trisaturated TG (Mukherjee and Sengupta, 1981). The 18:2 of bone marrow TG was found mainly in the 2 and 3 positions with a preference for the 2 position (Table 4) whereas native peanut oil TG contained 18:2 mainly in 1 and 2 positions with most in 2 position (Myher et al., 1977). Marrow TG also differed from native peanut oil TG in that little or no 22:0 or 24:0 was present.

The fatty acid compositions of intramuscular TG from range and feedlot steers are available from another report (Miller et al., 1981) and they are compared to fatty acid compositions of feedlot and range steer bone marrow TG in Table 5. In this table, fatty acids are expressed as percent of total fatty acids to allow meaningful comparisons Although there was considerable similarity among marrow and intramuscular TG within dietary groups, there were

Table 2—Fatty acids from total lipids of cervical, lumbar and femur marrows from range and feedlot steers and cows

		Cervical				Lumbar				Femur			
Fatty acid[a]		RS[b]	FS[b]	RC[b]	FC[b]	RS	FS	RC	FC	RS	FS	RC	FC
	10:0	10.2	7.7	9.1	11.3	16.5	17.4	42.0	38.3	57.7	40.5	63.3	50.8
	12:0	12.6	9.9	18.5	19.4	26.8	23.0	60.4	52.3	119	75.6	67.3	64.2
Br	13:0	3.1	1.8	5.0	4.7	5.7	4.8	11.9	10.3	10.4	7.6	6.8	8.4
	13:0	2.3	1.4	3.3	3.6	6.2	5.7	12.1	13.2	20.1	11.9	9.9	11.0
	14:1	28.9	23.7	31.1	29.5	68.2	70.1	141	160	242	310	171	150
	20:0	16.3	12.5	13.8	13.1	20.2	13.6	34.1	46.6	66.9	40.4	66.6	48.8
	20:4	25.7	25.3	20.2	20.9	23.4	25.6	24.0	24.3	45.4	42.8	33.4	31.5
	14:0	0.42	0.32	0.49	0.38	0.59	0.58	1.24	1.33	2.64	2.55	2.26	2.20
Br	15:0	0.09	0.06	0.10	0.08	0.17	0.15	0.30	0.40	0.52	0.46	0.42	0.47
	15:0	0.12	0.08	0.13	0.11	0.22	0.19	0.46	0.61	0.76	0.70	0.59	0.76
	16:0	5.96	5.48	5.36	5.29	9.03	9.29	15.6	17.7	21.1	22.0	24.8	24.3
	16:1	0.52	0.48	0.46	0.51	1.00	0.88	1.64	2.06	2.01	1.99	1.93	1.84
	17:0	0.32	0.29	0.25	0.28	0.44	0.42	0.61	0.75	1.10	1.08	0.95	1.00
	18:0	5.73	5.21	4.54	4.60	7.92	7.66	10.5	11.5	16.3	15.5	14.7	15.5
Cis	18:1	8.26	9.99	6.94	7.89	15.8	18.5	23.5	28.7	23.8	26.0	27.8	28.5
Trans	18:1	0.44	0.41	0.35	0.27	0.90	0.80	1.10	0.90	2.00	1.50	2.00	1.20
	18:2	0.62	0.80	0.62	0.75	1.00	1.09	1.20	1.14	1.04	1.22	0.82	0.83
	18:3	0.24	0.08	0.19	0.18	0.40	0.19	0.43	0.36	0.53	0.48	0.32	0.33
	20:1	0.13	0.10	0.10	0.11	0.28	0.21	0.35	0.42	0.54	0.54	0.44	0.40

[a] Fatty acids 10:0 thru 20:4 are expressed as mg/100g marrow and 14:0 thru 20:1 as g/100g marrow; Fatty acids are from pooled samples. Br refers to mixtures of iso and anteiso branched chain acids.
[b] RS—range steer; FS—feedlot steer; RC—range cow; FC—feedlot cow.

Table 3—Fatty acids from triglycerides of cervical, lumbar and femur marrows from range and feedlot steers and cows

		Cervical				Lumbar				Femur			
Fatty acid[a]		RS[b]	FS[b]	RC[b]	FC[b]	RS	FS	RC	FC	RS	FS	RC	FC
	10:0	13.1	5.5	7.3	7.7	17.5	17.8	38.2	41.0	38.0	45.0	58.0	52.6
	12:0	19.1	6.1	12.5	11.9	24.5	24.0	48.6	56.2	101	77.8	80.3	75.2
Br	13:0	3.2	2.9	4.5	3.6	4.7	3.0	8.9	10.1	11.1	7.9	10.8	9.6
	13:0	4.8	1.2	2.8	2.6	5,4	5.0	11.0	13.6	18.5	12.9	11.4	13.7
	14:1	24.7	19.6	26.0	28.6	69.5	70.7	152	165	278	290	150	168
	20:0	12.8	10.9	11.3	10.4	15.1	10.6	27.2	35.3	56.0	32.2	55.8	41.7
	20:4	16.0	19.7	12.5	16.8	27.2	23.9	23.6	30.9	38.4	43.4	25.8	24.0
	14:0	0.42	0.26	0.36	0.36	0.58	0.56	1.23	1.32	2.56	2.42	2.17	2.02
Br	15:0	0.09	0.07	0.09	0.07	0.17	0.15	0.32	0.40	0.53	0.45	0.43	0.53
	15:0	0.12	0.07	0.11	0.10	0.20	0.19	0.46	0.59	0.75	0.70	0.74	0.77
	16:0	5.47	4.84	4.68	4.97	8.54	8.97	15.0	17.1	19.9	21.1	23.7	23.3
	16:1	0.44	0.36	0.46	0.48	0.99	0.90	1.50	1.97	1.89	2.16	1.86	1.85
	17:0	0.29	0.25	0.25	0.21	0.37	0.33	0.61	0.65	0.84	0.84	0.81	0.82
	18:0	5.26	5.20	4.32	4.25	6.91	7.01	9.94	10.2	15.6	12.8	14.1	15.2
Cis	18:1	7.82	9.40	6.61	7.16	14.7	17.3	22.5	26.5	22.5	26.5	26.5	26.3
Trans	18:1	0.34	0.30	0.33	0.36	0.84	0.71	1.06	0.86	2.13	1.28	1.68	1.21
	18:2	0.59	0.72	0.53	0.68	0.88	0.95	1.01	1.07	1.01	1.30	0.80	0.75
	18:3	0.24	0.12	0.20	0.13	0.36	0.15	0.34	0.31	0.48	0.20	0.42	0.27
	20:1	0.12	0.10	0.10	0.10	0.27	0.21	0.33	0.39	0.61	0.70	0.46	0.39

[a] Fatty acids 10:0 thru 20:4 are expressed as mg/100g marrow and 14:0 thru 20:1 as g/100g marrow; Fatty acids are from pooled samples. Br refers to mixtures of iso and anteiso branched chain acids.

some specific differences. Intramuscular TG contained less 18:0 than did the bone marrow TG. Intramuscular TG contained considerably less 18:2 and 18:3 than cervical and lumbar marrow TG. Higher levels of polyunsaturated fatty acids in bovine bone marrow as compared to adipose tissue has been noted previously (Christie, 1978). Fatty acid composition of intramuscular TG more closely resembled that of femur TG than did the other two bone marrow TG, particularly with respect to 14:0, 18:2 and 18:3.

It appears that lipid of bovine bone marrows resembles that of bovine adipose tissue with respect to levels of TG and PL with somewhat more cholesterol present in marrows (Table 1; Christie, 1978). In addition, bovine bone marrow TG, the major lipid present, resembles bovine *longissimus* intramuscular TG with respect to fatty acid composition (Table 5). Therefore, the incorporation of bovine bone marrow into the American diet provides lipid that, in terms of composition, has been a part of that diet in the past and does not represent the addition of any new or strange dietary lipid components. While *trans* monounsaturated 18 carbon acids are present, they are present at low levels (Table 2). It has been estimated that the average American diet contains about 8% *trans* fatty acids due mainly to the use of hydrogenated vegetable oils as a major source of dietary fat (Emken et al., 1979). Increased use of bovine bone marrow would not add a significant amount of *trans* 18:1 to that total.

Table 4—Positional distribution of fatty acids in bovine bone marrow triglycerides

	sn-1	sn-2	sn-3
		mole percent[a]	
10:0	0.08 ± 0.03	0.04 ± 0.03	0.18 ± 0.05
12:0	0.21 ± 0.05	0.13 ± 0.04	0.05 ± 0.04
Br 13:0	0.02 ± 0.003	0.02 ± 0.003	0.02 ± 0.01
13:0	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
14:0	3.42 ± 0.47	5.36 ± 0.59	0.29 ± 0.38
14:1	0.02 ± 0.05	0.89 ± 0.07	0.05 ± 0.06
Br 15:0	0.24 ± 0.13	1.64 ± 0.16	0.15 ± 0.16
15:0	1.35 ± 0.18	0.50 ± 0.10	0.40 ± 0.18
16:0	63.7 ± 3.6	13.1 ± 2.7	10.6 ± 2.4
16:1	1.78 ± 0.52	4.66 ± 0.28	2.22 ± 0.70
17:0	1.65 ± 0.23	0.52 ± 0.20	1.19 ± 0.35
18:0	18.7 ± 0.3	8.48 ± 1.44	30.0 ± 3.7
Cis 18:1	7.32 ± 1.18	58.5 ± 2.1	47.2 ± 1.9
Trans 18:1	0.84 ± 0.29	0	5.06 ± 0.32
18:2	0.28 ± 0.08	3.89 ± 0.36	1.30 ± 0.40
18:3	0.15 ± 0.04	1.33 ± 0.14	0.23 ± 0.16
20:0	0.07 ± 0.02	0	0.08 ± 0.03
20:1	0.12 ± 0.04	0.74 ± 0.09	0.95 ± 0.06
20.4	0.02 ± 0.004	0.07 ± 0.01	0.06 ± 0.004

[a] Means ± standard deviations of values obtained from cervical, lumbar and femur marrows of range and feedlot steers and cows. Br refers to mixtures of iso and anteiso branched chain acids.

REFERENCES

Breckenridge, W.C. 1978. "Handbook of Lipid Research 1. Fatty Acids and Glycerides," Ed. A. Kuksis, p. 197. Plenum Press, New York.

Brockerhoff, H. 1967. Stereospecific analysis of triglyceride: an alternative method. J. Lipid Res. 8: 167.

Christie, W.W. 1978. The composition, structure and function of lipids in the tissues of ruminant animals. Prog. Lipid Res. 17: 111.

Dodge, J.T. and Phillips, G.B. 1967. Composition of phospholipids and phospholipid fatty acids and aldehydes in human red cells. J. Lipid Res. 8: 667.

Emken, E.A., Rohwedder, W.K., Dutton, H.J., DeJarlais, W.J., Adlof, R., Dougherty, R.M., and Iacono, J.M. 1979. Incorporation of deuterium-labeled cis- and trans-9-octadecenoic acids in humans. Lipids, 14: 547.

Folch, J., Lees, M., and Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 266: 497.

Kolbye, A. and Nelson, M.A. 1977. Health and Safety Aspect of the Use of Mechanically Deboned Meat. Final Report and Recommendations. Select Panel Meat and Poultry Inspection Program. USDA, Washington, DC.

Kovacs, M.I.P., Anderson, W.E., and Ackman, R.G. 1979. A simple method for the determination of cholesterol and some plant sterols in fishery-based food products. J. Food Sci. 44: 1299.

Kunsman, J.E., Collins, M.A., Field, R.A., and Miller, G.J. 1981. The cholesterol content of beef bone marrow and mechanically deboned meat. J. Food Sci. 46(6): 1785.

Kunsman, J.E. and Field, R.A. 1976. The lipid content of mechanically deboned red meats. J. Food Sci. 41: 1439.

MacGee, J. and Allen, K.G. 1977. Recovery of short, medium and long chain fatty acid methyl esters using wet halogenated solvents. J. Am. Oil Chem. Soc. 54: 375.

Mello, F.C., Field, R.A., Forenza, S., and Kunsman, J.E. 1976. Lipid characterization of bovine bone marrow. J. Food Sci. 41: 226.

Miller, G.J., Kunsman, J.E., and Field, R.A. 1980. Characteristics of soft subcutaneous fat in ram lambs fed corn and corn-silage diets. J. Food Sci. 45: 259.

Miller, G.J., Masor, M.L., and Riley, M.L. 1981. Intramuscular lipids and triglyceride structures in range and feedlot steers. J. Food Sci. 46(5): 1333.

—Continued on page 665

Table 5—Fatty acid compositions of triglycerides from range and feedlot steer bone marrows and longissimus muscles

	Range steer				Feedlot steer			
	Bone marrow			Longissimus muscle[b]	Bone marrow			Longissimus muscle[b]
Fatty acid[a]	Cervical	Lumbar	Femur		Cervical	Lumbar	Femur	
10:0	0.06	0.05	0.05	0.08	0.03	0.05	0.06	0.06
12:0	0.09	0.07	0.15	0.10	0.03	0.06	0.11	0.08
Br 13:0	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.004
13:0	0.02	0.02	0.03	0.01	0.01	0.01	0.02	0.01
14:0	1.97	1.66	3.69	3.46	1.19	1.49	3.41	3.37
14:1	0.12	0.20	0.40	0.43	0.09	0.19	0.41	0.46
Br 15:0	0.42	0.49	0.76	0.56	0.34	0.39	0.63	0.20
15:0	0.56	0.57	1.08	0.56	0.33	0.50	0.99	0.51
16:0	25.7	24.4	28.7	30.6	22.2	23.8	29.7	29.2
16:1	2.07	2.83	2.73	4.19	1.65	2.39	3.04	4.35
17:0	1.36	1.06	1.21	–	1.13	0.88	1.18	–
18:0	24.7	19.8	22.5	16.2	23.9	18.6	18.1	13.0
Cis 18:1	36.7	42.0	32.4	38.7	43.2	46.1	37.3	44.6
Trans 18:1	1.60	2.40	3.07	2.54	1.38	1.90	1.80	1.37
18:2	2.77	2.52	1.46	1.39	3.31	2.52	1.83	1.79
18:3	1.13	1.03	0.69	0.65	0.55	0.40	0.28	0.21
20:0	0.06	0.04	0.08	0.16	0.05	0.03	0.05	0.09
20:1	0.56	0.77	0.88	0.33	0.46	0.56	0.99	0.60
20:4	0.08	0.08	0.06	–	0.09	0.06	0.06	–

[a] Expressed as percent fatty acid in total fatty acids measured. Marrow fatty acids are from pooled samples. Muscle triglycerides are intramuscular. Br refers to mixtures of iso and anteiso branched chain acids.
[b] Data calculated from Miller et al. (1981).

A Research Note

Water Activity of Fresh Foods

JORGE CHIRIFE and CONSTANTINO FERRO FONTAN

ABSTRACT

This work presents a compilation of water activity (a_w) values for fresh foods namely fruits, vegetables and meats. Water activities were calculated from reported data on the cryoscopic temperature (or initial freezing point) of fruits, vegetables and meats, amounting to eighty six different items. In practically all cases studied the water activity is in the range 0.970–0.996.

INTRODUCTION

IT IS NOW generally accepted that water activity (a_w) is more closely related to the physical, chemical and biological properties of foods than is the total moisture content (Troller and Christian, 1978). Several traditional as well as recent methods of food preservation (drying, curing, salting, freezing, intermediate moisture foods) are at least partially based on the reduction of water activity (Leistner and Rödel, 1975; Troller, 1979; Plitman et al., 1975, Vigo et al., 1981). By this reason various methods have been developed for measuring the a_w of foods and extensive information is available on a_w values of raw/processed low moisture and intermediate moisture foods (Troller and Christian 1978; Vigo et al., 1981, Chirife, 1978; Stoloff, 1978; Labuza et al., 1976). Little information is available, however, on the a_w values of raw foods such as fresh fruits, vegetables and meats. Besides the theoretical interst, this type of information may be useful for a more optimal design of food preservation methods based on a_w lowering (Favetto et al., 1981).

The purpose of the present work is to calculate the a_w of a diversity of fresh foods, namely fruits, vegetables and meats.

RESULTS & DISCUSSION

WATER is the major component of fresh fruit, vegetables and meats comprising about 75–95% of the food weight. The water activity of these foods is solely determined by the nature and concentration of the dissolved chemical species naturally occurring in it, such as sugars, organic acids, inorganic salts, and other soluble substances. Insoluble constituents are not expected to make any significant contribution to a_w lowering due to the high moisture contents considered (Chirife, 1978). As the concentration of solutes (nonionic and/or ionizable) naturally present in the water phase of fresh foods is relatively small, water activity is expected to be close, but different from one.

It is well known that direct measurement of a_w at very high water activities (i.e. > 0.98) presents several difficulties when using the a_w-measurement devices most used in the food area (Prior et al., 1977). By this reason it was decided to derive the water activity of fresh foods from reported values of its cryoscopic temperature (or initial freezing point), since the incipient ice formation in fruits, vegetables and meats is well documented in the literature. Recently, Miracco et al. (1981), Chirife et al. (1981), Alzamora et al. (1981) and Rey and Labuza (1981) concluded that cryoscopic measurements constitute an accurate and convenient measure of high water activity solute systems.

The relationship between water activity and freezing point depression (θ_F) may be accurately expressed by the following equation (Ferro Fontan and Chirife, 1981)

$$-\ln a_w = 9.6934 \cdot 10^{-3}\,\theta_F + 4.761 \cdot 10^{-6} \cdot \theta_F^2 \quad (1)$$

Equation (1) was applied to a diversity of literature data on freezing points of fruits, vegetables, meats and other fresh foods, and the results are shown in Table 1. Strictly speaking Eq. (1) provides the water activity at the freezing temperature and not, let us say at "room" temperature. However for high water activities, such as those considered here, a_w may be safely assumed to be independent of temperature (Ferro Fontán and Chirife, 1981).

In a recent work on the determination of the cryoscopic temperature of fruits and vegetables, Guegov (1980) demonstrated, as it was to be expected, that the cryoscopic temperature (and hence the a_w) was correlated with the concentration of soluble solids in the food. Thus, ranges of a_w values reported in Table 1 correspond to variations found in a same food having different concentrations of soluble solids (effect of variety, degree of ripeness).

CONCLUSIONS

THERE are some observations which can be made about the results shown on Table 1. It is noteworthy that the a_w values derived from freezing point data reported by different authors agree reasonably well. The a_w of the large majority of fresh fruits, vegetables and meats is in the range 0.970–0.996. In the average, fruits have somewhat lower a_w than vegetables; the average a_w for 42 fruits is 0.983 while for 39 vegetables it is 0.990. The different meats (fish, pork, lamb, beef) have much the same $a_w = 0.990$. This result is in agreement with results of Leistner and Rödel (1975) who reported that different porcine and bovine muscles have practically the same a_w.

Figure 1 shows a histogram for the a_w (average) distribution in vegetables and fruits; a gaussian fit to the histogram is also shown for illustrative purposes. However, it should be noted that the histograms show considerable skewness.

Mellor (1981) has suggested that the freezing point of some intact biological tissue may be misleading as it may be lower than the value for the soluble liquid fraction. Guegov (1981), however, reported that the cryoscopic temperature of fruit and vegetables depends mainly on the soluble solids content and structure plays only a minor role. Similarly, Briozzo (1981) reported freezing point data for the soluble liquid fraction of beef and pork and the values were in very good agreement with other literature data obtained from meat tissues. Dickerson (1968) also

Author Chirife is with the Departamento de Industrias Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, (1428) Buenos Aires, Republica Argentina. Author Ferro Fontan is with the Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and is a member of Consejo Nacional de Investigaciones Científicas y Tecnicas de la República Argentina.

Table 1—Water activity of fresh foods

Fruits	
Apples	0.980[a]
	0.988–0.975[c]
Apple juice	0.986[b]
Apricots	0.987[a]
	0.985–0.977[c]
Bananas	0.987[a]
	0.971–0.964[c]
Bilberries	0.989[b]
Blackberries	0.989[b]
Blackberries	0.986[a]
Blueberries	0.982[a]
Cherries	0.977[a]
	0.986–0.959[c]
Cherries	0.977[a]
	0.986–0.959[c]
Cherries juice	0.986[b]
Cranberries	0.989[a]
Currants	0.990[a]
Dates	0.974[a]
Dewberries	0.985[a]
Figs	0.974[a]
Grapefruit	0.982[a]
	0.985–0.980[c]
Gooseberries	0.989[a]
Grapes	0.986–0.974[a]
	0.982–0.963[c]
Grape juice	0.983[b]
Lemons	0.984[a]
	0.989–0.982[c]
Limes	0.980[a]
Mangoes	0.986[a]
Melons	0.989–0.988[a]
	0.991–0.970[c]
Nectarines	0.984[a]
Oranges	0.979[a]
	0.987–0.979[c]
Orange juice	0.988[b]
Papaya	0.990[a]
Peaches	0.987[a]
	0.985[b]
	0.989–0.979[c]
Pears	0.985[b]
	0.989–0.969[c]
Persimmons	0.976[a]
Pineapple	0.988–0.985[a]
Plums	0.982[a]
	0.978[b]
	0.980–0.969[c]
Quinces	0.979[a]
	0.981–0.972[c]
Raspberries	0.991–0.986[a]
	0.988[b]
	0.994–0.984[c]
Raspberry juice	0.988[b]
Sour cherries	0.983–0.971[c]
Strawberries	0.990[a]
	0.991[b]
	0.997–0.986[c]
Strawberry juice	0.991[b]
Sweet cherries	0.975[b]
Tangerines	0.987[a]
Water melon	0.992[a]
Vegetables	
Artichokes	0.987–0.976[a]
Asparagus	0.992[a]
	0.994[b]
Avocado	0.989[a]
Beans, green	0.990[a]
	0.996–0.994[c]
Beans, lima	0.994[a]
Beets	0.985[a]
	0.988–0.979[c]
Broccoli, sprouting	0.991[a]
Brussels sprouts	0.990[a]
Cabbage	0.992[a]
	0.992–0.990[c]
Carrots	0.983[a]
	0.989[b]
	0.993–0.988[c]
Cauliflower	0.990[a]
	0.987–0.984[c]
Celeriac	0.990[a]
Celery	0.994[a]
	0.992–0.987[c]
Celery leaves	0.997–0.992[c]
Corn sweet	0.994[a]
Cucumbers	0.992[a]
	0.998–0.995[c]
Eggplant	0.991[a]
	0.993–0.987[c]
Endive	0.995[a]
Green onion	0.996–0.992[c]
Leeks	0.991[a]
	0.989–0.976[c]
Lettuce	0.996[a]
Mushrooms	0.989[a]
	0.995–0.990[c]
Onions	0.990[a]
	0.986[b]
	0.986–0.974[c]
Parsnips	0.988[a]
Peas, green	0.990[a]
	0.982[b]
	0.983–0.980[c]
Peppers	0.992[a]
	0.997–0.992[c]
Potatoes	0.988[a]
	0.997–0.992[c]
Potatoes, sweet	0.985[a]
Pumpkins	0.989[a]
	0.992–0.984[c]
Radishes	0.990[a]
	0.990–0.980[c]
Rhubarb	0.989[a]
Rutabagas	0.988[a]
Salsify	0.987[b]
Small radishes	0.996–0.994[c]
Spinach	0.996[a]
	0.996–0.992[c]
	0.991–0.988[a]
Squash	0.998–0.994[c]
Tomatoes	0.991[a]
	0.998–0.994[c]
Tomato pulp	0.993[b]
Turnips	0.988[a]
Meats	
	0.992–0.989[d]
Beef	0.990–0.980[e]
	0.990[f]
Fish, cod	0.990[g]
cod	0.994–0.990[g]
various species (sea water)	0.989[h]
Lamb carcasses	0.990[d]
Pork	0.990[e]
Milk products	
Cream, 40% fat	0.979[a]
Milk, whole	0.995[a]
	0.995–0.994[j]
1.5% fat	0.995[i]

[a–j] Water activity values calculated from freezing point data reported by:

[a] Rha, (1975)
[b] Dickerson, Jr. (1968).
[c] Guegov (1980).
[d] Briozzo (1981).
[e] Leistner and Rodel (1975).
[f] Burgess et al. (1965).
[g] Planck, (1963).
[h] Earle and Fleming (1967).
[i] Alzamora et al. (1981).
[j] Zarb and Hourigan (1979).

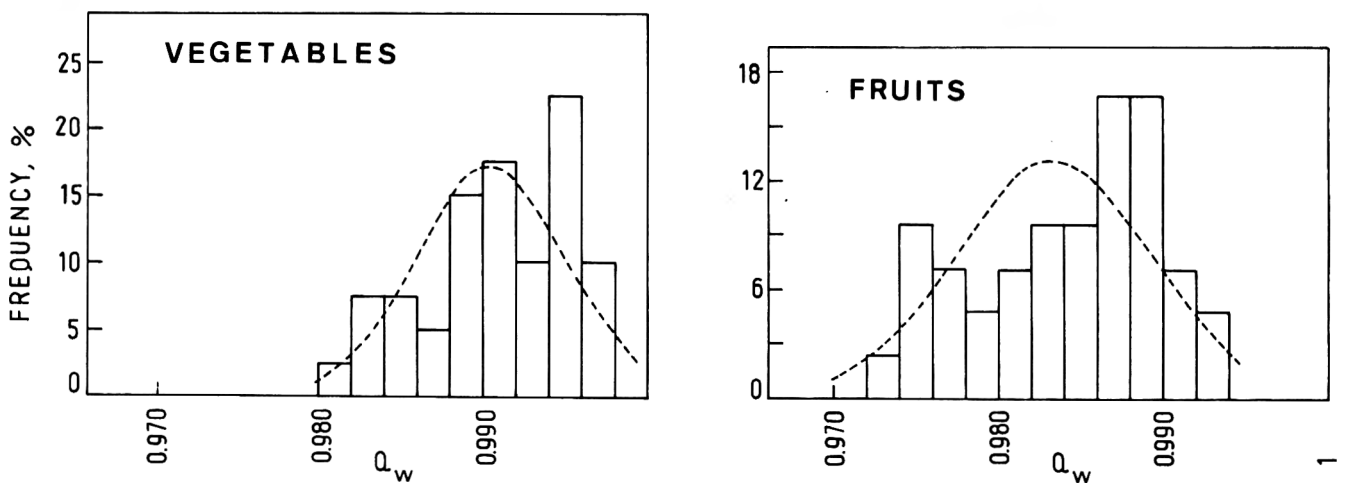


Fig. 1—Histogram for water activity (average) distribution in vegetables and fruits.

quoted freezing point data for various berries and their juices and the values were identical. It is noteworthy that Guegov (1980) – whose freezing point data constitute a large percentage of those used in this work – compared the analytical dependency of the cryoscopic temperature with soluble solids of various fruits and vegetables with other literature data and found a good agreement. We have also found through a statistical analysis (not reported here) a reasonable degree of correlation between Guegov's data and those quoted by Rha (1975).

REFERENCES

Alzamora, S.M., Chirife, J. and Ferro Fontán, C. 1981. A note on the effect of surface active agents on water activity of IM food solutions. J. Food Science, in press.

Briozzo, J. 1981. Unpublished results. Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

Burgess, G.H.O., Cutting, C.L., Lovern, A. and Waterman, J.J. 1965. "Fish Handling and Processing", Her Majestig's Stationary Office, Edinburg, 1965.

Chirife, J. 1978. Prediction of water activity in intermediate moisture foods. J. Food Technol., 13: 417.

Chirife, J., Ferro Fontan, C. and Vigo, S. 1981. A study of water activity prediction in molasses solutions. J. Agric. Food Chem., 29: 1085.

Dickerson, Jr., R.W. 1968. Thermal properties of foods, in: "The Freezing Preservation of Foods", fourth edition, vol. 2, AVI Publishing Co., Westport, Conn. U.S.A.

Earle, R.L. and Fleming, A.K. 1967. Cooling and freezing of lamb and mutton carcasses. I. Cooling and freezing rates in legs. Food Technol., 21: 79.

Favetto, G., Chirife, J. and Bartholomai, G. 1981. A study of water activity lowering in meat during immersion-cooking in sodium chloride-glycerol solutions. 2. Kinetics of a_w lowering and effect of some process variables. J. Food Technol., in press.

Ferro Fontán, C. and Chirife, J. 1981. The evaluation of water activity in aqueous solutions from freezing point depression. J. Food Technol., 16: 21.

Guegov, J. 1980. General correlation between the cryoscopic temperature and the soluble solids content of fruit and vegetables. J. Food Technol., 15: 607.

Labuza, T.P., Acott, K.A., Tatini, S.R., Lee, R., Flink, J. and McCall, W. 1976. Water activity determination: A comparative study of different methods, J. Food Sci., 41: 910.

Leistner, L. and Rodel, W. 1975. The significance of water activity for micro-organisms in meats, in: "Water Relations of Foods", edited by R.B. Duckworth, Academic Press, London, New York.

Mellor, J.D. 1981. Critical evaluation of thermophysical properties of foodstuffs and outline of future developments. COST 90 Final Seminar, Catholic University of Leuven, Belgium, Sept. 9-11.

Miracco, J.L., Alzamora, S.M., Chirife, J. and Ferro Fontan, C. 1981. On the water activity of lactose solutions. J. Food Sci. 46: 1612.

Planck, R. 1963. "El Empleo del Frío en la Industria de la Alimentación", ed. Reverté, España, 1963.

Plitman, M., Park, Y., Gómez, R. and Sinskey, A.J. 1973. Viability of Staphylococcus aureus in intermediate moisture meats. J. Food Sci., 38: 1004.

Prior, B.A., Casaleggio, C. and Van Vuuren, H.J.J. 1977. Psychrometric determination of water activity in the high a_w range. J. Food Protection, 40: 537.

Rha, C. 1975. Thermal properties of food materials, in: "Theory, Determination and Control of Physical Properties of Food Materials", edited by C. Rha, D. Riedel Publishing Company, Dordrecht, Holland.

Rey, D.K. and Labuza, T.P. 1981. Characterization of the effect of solutes on the water-binding and gel strength properties of carrageenan. J. Food Sci., 46: 786.

Stoloff, L. 1978. Calibration of water activity measuring instruments and devices: Collaborative study. J. Assoc., Off., Anal., Chem., 61: 1166.

Troller, J.A. and Christian, J.H.B. 1978. "Water Activity and Food", Academic Press, New York.

Troller, J.A. 1979. Food spoilage by microorganisms tolerating low a_w environments. Food Technol., 39: 72.

Vigo, M.S., Chirife, J., Scorza, O.C., Cataneo, P., Bertoni, M.H. and Sarrailh, p. 1981. Estudio sobre alimentos tradicionales de humedad intermedia elaborados en la Argentina. I. Determinacion de actividad acuosa (a_w), pH, humedad y solidos solubles. Rev. Agroq. y Tecnol. de Alimentos (Spain), 21: 91.

Zarb, J.M. and Hourigan, J.A. 1979. An enzimic, cryoscopic method for the estimation of lactose in milk products. The Australian J. of Dairy Technol., December, p. 184.

Ms received 8/31/81; accepted 10/9/81.

The authors acknowledge the financial support from Consejo Nacional de Investigaciones Cientificas y Técnicas de la República Argentina (Legajo No. 9373/80).

PER & NUTRIENTS IN MD SPENT LAYER MEAT . . . From page 656

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemist, Washington, DC.

Brinkman, G.L. and MacNeil, J.H. 1976. Protein quality of mechanically deboned poultry meat as measured by rat PER Nutrition Reports International 14: 365.

Cunningham, F.E. and Mugler, D.S. 1974. Deboned fowl meat offers opportunities. Poultry Meat 25: 46.

Dhillon, A.S. and Maurer, A.J. 1975. Stability study of comminuted poultry meats in frozen storage. Poultry Sci. 54: 1407.

Dolan, T., Legetter, L., McNeal, J., and Malanoski, A. 1977. Determination of fluoride in deboned meat. Scientific Services, FSQS, USDA, Washington, DC.

Duncan, D.B. 1955. Multiple range and multiple F-tests. Biometrics 11:1.

Essary, E.O. and Ritchey, S.J. 1968. Amino acid composition of meat removed from boned turkey carcasses by use of a commercial boning machine. Poultry Sci. 47: 1953.

Froning, G.W., Satterlee, L.D., and Johnson, F. 1973. Effect of skin content prior to deboning on emulsifying and color characteristics of mechanically deboned chicken back meat. Poultry Sci. 52: 923.

Grunden, L.P., MacNeil, J.H., and Dimick, P.S. 1972. Poultry product quality: Chemical and physical characteristics of mechanically deboned poultry meat. J. Food Sci. 37: 274.

Hurt, H.D., Forsythe, R.H., and Krieger, C.H. 1974. Factors which influence the biological evaluation of protein quality by the protein efficiency ratio method. Am. Chem. Soc. "Symposium on Chem. and Bio. Methods for Protein Quality Evaluation." Marcel Dekker, Inc., New York.

Mast, M.G. and MacNeil, J.H. 1976. Physical and functional properties of heat pasteurized mechanically deboned poultry meat. Poultry Sci. 55: 1207.

MacNeil, J.H., Graves, K.L., and Leach, R.M. Jr. 1977. Comparison of hand and mechanically deboned broiler necks. Unpublished manuscript.

MacNeil, J.H., Mast, M.G., and Leach,R.M. 1978. Protein efficiency ratio and levels of selected nutrients in mechanically deboned Poultry Meat. J. Food Sci. 43: 864.

MacNeil, J.H., Mast, M.G., and Leach, R.M. 1979. Protein deficiency ratio and amounts of selected nutrients in mechanically deboned turkey meat. J. Food Sci. 44: 1293.

Maxcy, R.B., Froning, G.W., and Hartung, T.E. 1973. Microbial quality of ground poultry meat. Poultry Sci. 52: 2061.

Metcalfe, L.D., Schmitz, A.A., and Pelka, J.R. 1966. Analytical Chem. 38: 514.

Mulder, R.W., Mulder, A.W. and Dorresteijn, L.W.J. 1975. Microbiological quality of mechanically deboned poultry meat. Spelderhold Mededeling 243.

Ostovar, K., MacNeil, J.H., and O'Donnell, K. 1971. Poultry product quality 5% microbiological evaluation of deboned poultry meat. J. Food Sci. 36: 1005.

Tarladgis, B.G., Watts, B.M., and Younathan, M. 1960. Distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Amer. Oil Chem. Soc. 37: 44.

USDA. 1975. Mechanically deboned poultry and poultry meat. Animal Health Inspection Serivce, MPI Bull. 75-30.

Ms received 3/7/81; revised 10/20/81; accepted 10/21/81.

Paper No. 6185 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Published as part of research conducted under NE-73 Regional Research and partially supported by the American Egg Board.

A Research Note

Comparison of Stability of Thiamin Salts at High Temperature and Water Activity

T. P. LABUZA and J. F. KAMMAN

ABSTRACT

Current literature indicates conflicting results as to the relative stability of thiamin mononitrate versus thiamin hydrochloride. These salts were tested for stability at 75–95°C and at a_w's 0.58 and 0.86. The observed difference in stability of the two salts can be explained by the higher activation energy (26.3 vs 22.4) for the mononitrate which results in a crossover point at about 95°C in an Arrhenius plot. Below 95°C the mononitrate was more stable while above 95°C the hydrochloride is more stable. In addition, stability of both forms was slightly less at the higher a_w. The loss of thiamin mononitrate in a fluctuating square wave temperature could be accurately predicted using the results of the Arrhenius plot.

INTRODUCTION

THIAMIN has long been considered to be the most thermally unstable of the vitamins used in the enrichment of cereal products. Commercially, the vitamin is available in either the hydrochloride or mononitrate salt form. As seen in Table 1, one of the major differences between the two forms is its solubility in water, with the hydrochloride being much more soluble and thus generally preferred for liquid application or liquid coating systems. The mononitrate, although less soluble, is generally preferred for enrichment fo dry mixes because of its greater stability. For example, Hollenbeck and Obermeyer (1952) found that the mononitrate form was much more stable than the hydrochloride form in enriched flour stored at 24–38°C. However, later work by Farrer (1953) showed the hydrochloride form to be the more stable when heat processed in buffer solution at 100°C. Thus, the literature presents conflicting results as to relative stability. The objective of this research was to compare stability of the two salt forms at several temperatures and two water activities (a_w) to determine why the difference in stability exists. In addition, this research included a square wave fluctuating temperature study of thiamin mononitrate at high temperatures to see if the conclusions of Kamman and Labuza (1981), based on Arrhenius kinetics (Labuza, 1979), would also apply.

MATERIALS & METHODS

Semolina dough

Unenriched semolina (International Multifoods, Minneapolis, MN) was fortified with thiamin to about 100 mg per 300g by blending in a twin shell blender for 30 min. Separate batches were made for the mononitrate and hydrochloride salts (Hoffmann LaRoche). One hundred grams were then mixed with an appropriate amount of distilled water to achieve the desired a_w in a small Brabender Farinograph (Brabender Instruments) mixing bowl attached to a Brabender Farinograph (Brabender Instruments) mixing bowl attached to a Brabender model D-3002 prep center. Water activities of 0.58 and 0.86 were used (moisture contents of 14g H_2O/100g and 22g H_2O/100g respectively, Kamman and Labuza, 1981). The mixing was done at high speed (200 rpm) for 10 min until a uniform and homogeneous system resulted. Approximately 6g portions of the dough were then vacuum sealed into 6 x 9 cm retort-type pouches for the study.

Test conditions

The pouches containing the semolina dough was immediately subjected to the test conditions by immersion into a circulating oil bath held at either 75, 85 or 95°C or a square wave of 75/95°C with 125 min at each temperature. The temperature of a sample pouch was followed by equipping it internally with a thermocouple. Once equilibration had occurred (about 60 sec), the initial sample was taken in quadruplicate and designated as the time zero samples. Triplicate samples were then removed at appropriate time intervals for a total time to reach at least 75% destruction. All samples removed from the oil bath were immediately immersed in liquid nitrogen for 5 sec and then stored at 4°C until analyzed. The square wave study was done only with thiamin mononitrate and at a_w of 0.58.

Thiamin analysis

The HPLC technique of Kamman et al. (1980) was used.

RESULTS

THE RATE CONSTANTS and half lives for loss of thiamin for each salt type are shown in Table 1. A first order reaction was followed very closely for both salts as noted by the high correlations determined by the least squares method. It is also obvious that the hydrochloride form is less stable at the lower temperatures; however, its stability comes close to that for the mononitrate at 95°C. Examination of the difference in activation energies explains this phenomenon. It is well known in thermal processing in

Table 1–Kinetic constants for thiamin loss in a semolina dough system subjected to high temperatures

a_w	(°C)	$k(min^{-1})^a$ ± 95% Confidence interval	r^2	Half Life $\theta_{1/2}$ (min)	E_A kcal/mole
		Hydrochloride			
0.58	75	3.72 ± 0.01	0.986	1,863	
	85	11.41 ± 3.64	0.928	607	22.8
	95	22.45 ± 2.57	0.994	309	
0.86	75	5.35 ± 2.57	0.888	1,295	
	85	12.20 ± 4.45	0.913	568	22.0
	95	30.45 ± 8.91	0.941	228	
		Mononitrate			
0.58	75	2.88 ± 0.01	0.960	2,406	
	85	7.91 ± 0.01	0.993	876	26.1
	95	22.69 ± 2.57	0.990	305	
0.86	75	2.94 ± 0.01	0.993	2,357	
	85	8.31 ± 0.01	0.997	834	26.5
	95	23.89 ± 0.01	0.999	290	
0.58	75/95°C				
	Actual	11.71 ± 0.01	0.990	592	
	Predicted	11.87 ± 0.02		584	

[a] x 10^4

Author Labuza is with the Dept. of Food Science & Nutrition, Univ. of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

examining the rates of two reactions (e.g., microbial death versus vitamin stability; see Lund, 1975), that if they have different activation energies, there will be a crossover temperature below which one reaction is faster and above which the other reaction is faster. This is the basis of HTST processing in which for high temperature processing the rate of destruction of spores is much faster than the rate of quality loss while at normal retort processing the quality loss rate becomes significant with respect to the death rate of spores.

Fig. 1 shows this also applies to the differences in the stability of the two salts, probably because of electrophilic transfer mechanisms due to the salt. At around 95°C the lines begin to cross; thus, somewhere above 95/110°C the hydrochloride salt should be more stable while at 25–45°C the hydrochloride should be much less stable. Previous inconsistencies in the literature may thus be explained solely by an Arrhenius relationship. At temperatures of less than 95°C, the mononitrate form of thiamin should be the preferred from for enrichment of cereals. Above 110°C the hydrochloride form is more stable. Since processed foods generally will not be stored above 100°C, the mononitrate, although less soluble and less stable to thermal processing, would be the preferred form to use if the product is to be fortified. It should also be noted in Table 1 that both salts are less stable at the higher a_w, as would generally be expected (Kirk, 1981). In addition, in examining the data in Table 1, the crossover point at a_w 0.86 is about 110–115°C suggesting again that electron transport mechanisms for degradation of the vitamin in the aqueous environment are probably the cause of the difference in E_A's.

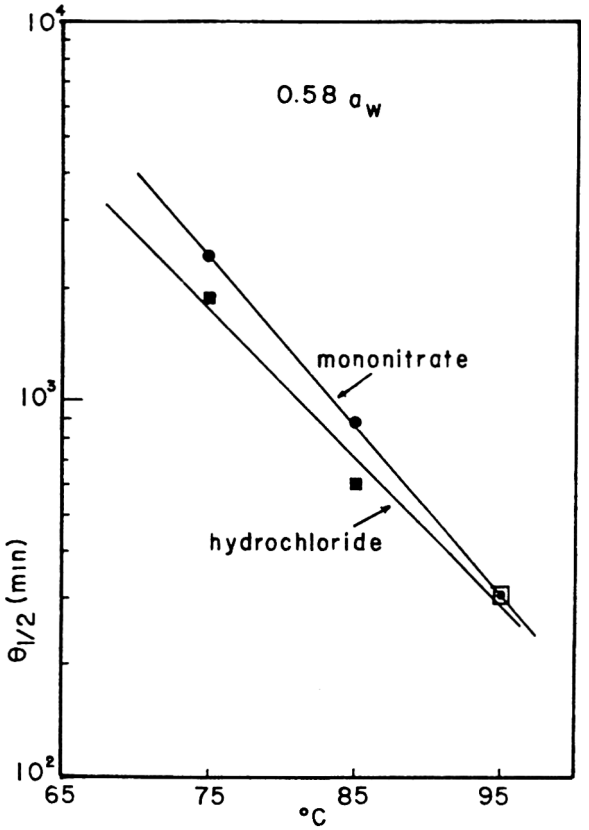


Fig. 1—Comparison of the relative stability (half life in minutes) of thiamin hydrochloride and thiamin mononitrate as a function of temperature.

Table 1 also presents the actual and predicted rate constants and half life for the fluctuating study using thiamin mononitrate. Kamman and Labuza (1981) showed that simple Arrhenius methods could be used to predict the loss of thiamin in pasta held under fluctuating conditions of 25/45°C. This present work was extended to a higher temperature range. As seen, the actual rate constant as determined by least squares analysis of the data comes very close to that predicted from the Arrhenius approach developed by Labuza (1979). The effective temperature was 89°C which is 4°C above the mean temperature of the fluctuations (85°C). These results thus further support the usefulness of applying kinetics to food stability problems.

REFERENCES

Farrer, K.T.H. 1953. The thermal destruction of vitamin B_1. Austr. J. Exp. Biol. 31: 247.

Hollenbeck, C.M. and Obermeyer, H.G. 1952. Relative stability of thiamin mononitrate and thiamin hydrochloride in enriched flour. Cereal Chem. 29: 82.

Kamman, J.F., Labuza, T.P., and Warthesen, J.J. 1980. HPLC techniques for measuring thiamin anf riboflavin in fortified foods. J. Food Sci. 45: 1497.

Kamman, J.F. and Labuza, T.P. 1981. Kinetics of thiamin and riboflavin loss in pasta as a function of constant and variable storage conditions. J. Food Sci. 46: 1467.

Kirk, J.D. 1981. The effect of water activity on stability of vitamins. In "Water Activity: Relation To Food Quality," Ed. L.B. Rockland and G.F. Stewart. Academic Press, New York.

Labuza, T.P. 1979. A theoretical comparison of losses in foods under fluctuating temperature sequences. J. Food Sci. 44: 1162.

Lund, D. 1975. Heat processing, Ch. 3. In "Physical Principles of Food Preservation," Ed. O.R. Fennema. Marcel Dekker, New York.

Ms received 8/31/81; accepted 10/20/81.

This paper is scientific journal series No. 11869 from the Univ. of Minnesota Agric. Exp. Station. This project was supported in part by the Univ. of Minnesota Agricultural Experiment Station Project 18-78 and Grant No. PFR-79-10370 of the National Science Foundation.

BOVINE BONE MARROW LIPIDS . . . From page 660

Miller, G.J., Riley, M.L., and Field, R.A. 1979. A convenient microfiltration procedure for purification of bovine and ovine subcutaneous tissue lipid extracts. J. Agri. Food Chem. 27: 206.

Mukherjee, S. and Sengupta, S. 1981. Studies on lipid responses to interesterified soya-oil-butterfat mixture in hypercholesteremic rats and human subjects. J. Am. Oil Chem. Soc. 58: 287.

Myher, J.J., Marai, L., Kuksis, A., and Kritschevsky, D. 1977. Acylglycerol structure of peanut oils of different atherogenic potential. Lipids 12: 775.

Radin, N.S., Larin, F.B., and Brown, J.R. 1955. Determination of cerebrosides. J. Biol. Chem. 217: 789.

Sanchez, L., Kunsman, J.E., Field, R.A., and Kruggel, W.G. 1978. Determination of red marrow in muscle/marrow mixtures. Proc. West. Sect. Am. Soc. Anim. Sci. 29: 139.

Touchstone, J.C., Chen, J.C., and Beaver, K.M. 1980. Improved separation of phospholipids in thin layer chromatography. Lipids 15: 61.

Ms received 6/15/81; revised 9/14/81; accepted 9/17/81.

Support by USDA Competitive Grants Office under Grant 43-8584 and the Univ. of Wyoming Agricultural Experiment Station is gratefully acknowledged. Wyoming Experiment Station Journal Article No. 1142.

A Research Note

The Nutrient Composition of Fresh Fiddlehead Greens

A. A. BUSHWAY, A. M. WILSON, D. F. McGANN, and R. J. BUSHWAY

ABSTRACT

Fiddlehead greens of the ostrich fern, *Matteuccia struthiopteris*, var. *penn.*, from two separate locations were analyzed for their nutrient composition. Greens contained (in %) 87 water, 1.12 fiber, 4.23 crude protein, 4.02 ash, 3.06 carbohydrate and 0.5 fat. The fatty acid composition consisted mostly of palmitate, linoleate and linolenate. Unsaturated fatty acids account for approximately 65% of the fatty acids associated with fiddlehead lipids. Niacin, riboflavin, thiamin, vitamin C, and vitamin A were present at concentration ranges (based on wet weight) of 4.07–5.57, 0.15–0.24, 0.018–0.024, 19.00–32.69 mg/100g greens and 2175–2709 I.U./100g, respectively. Spectrochemical analysis identified 17 minerals with potassium, phosphorus, magnesium and calcium being the most prominent. The low sodium content (0.5 mg/100g greens) should be noted. The high potassium to sodium ratio could make fiddlehead greens an integral part of the diet of persons requiring low intakes of sodium.

INTRODUCTION

FIDDLEHEAD GREENS of the ostrich fern, previously called *Pteretia noduosa* (Michx.) Nieuwl, now named *Matteuccia struthiopteris* var. *pensylvanica* (Willd.) Morton, are harvested in the northeastern states as well as in Canada. The fiddlehead is sold fresh in local markets, canned and frozen commercially, and home processed by canning, freezing, or salting (Blake, 1942, 1955). Gibbons (1979) reports that the fiddlehead grows from Newfoundland to Alaska and from the Dakotas to Missouri and West Virginia.

No nutritional data on the ostrich fern exist in the literature although several sources list the fiddlehead as an edible plant (Blake 1942, 1955; Briwa, 1956; Gibbons, 1979). Since fiddlehead greens are consumed by the population of the northeastern United States and eastern Canada, the nutritional composition of fresh greens from two locations was investigated.

EXPERIMENTAL

FIDDLEHEADS of the ostrich fern, *Matteuccia struthiopteris* var. *pensylvanica*, were harvested from the banks of the Union and Piscataquis Rivers in Central Maine in May, 1981. Fiddleheads were stored at 4–5°C and all samples were analyzed within 3 days of harvest.

Proximate composition, vitamin, and mineral analyses

Crude protein was determined by the macro-Kjeldahl method using 6.25 as the multiplication factor while crude fiber was determined by the method of the Association of Official Analytical Chemists (AOAC, 1980). The spectrophotometric, fluorometric, 2,6-dichloroindophenol and fluorometric methods were used to analyze for niacin, riboflavin, vitamin C and thiamin respectively (AOAC, 1980). Carbohydrate was determined by subtracting the total percent composition from 100. Vitamin A was determined by a newly developed high-performance liquid chromatographic (HPLC) method (Bushway and Wilson, 1981).

Samples for mineral analysis were dry ashed, dissolved in dilute HCl and diluted with deionized water. All minerals were analyzed by Inductively Coupled Plasma (ICP) spectroscopy using a Jarrell-Ash Plasma Comp 975 except sodium which was analyzed by atomic absorption spectroscopy (Akpapunam and Markakis, 1981; Bushway et al. 1981). Procedures used were those found in Jarrell-Ash bulletin 96-975.

Amino acid analysis was performed by hydrolysis in 5.7N HCl at 110°C for 24 hr. Analyses was performed according to the method of Rushoff et al., (1980) using a Beckman 119 amino acid analyzer equipped for automatic sample injection.

Lipid extraction and fatty acid analysis

Ten grams of freeze-dried and ground fiddleheads were extracted for 15 hr in chloroform:methanol (2:1. v/v) in a Soxhlet extractor. After extraction, solvent was removed using a rotary evaporator and the lipid was kept under nitrogen until fatty acid analysis was performed.

Twenty mg samples of fiddlehead lipid were saponified in 0.2N methanolic NaOH. Fatty acid methyl esters were prepared by hydrolysis with 14% boron trifluoride in methanol for 10 min at 100°C (Morrison and Smith, 1964) and were separated on a 0.3 × 180 cm glass column packed with 10% SP 2340 on 100/120 mesh Chromosorb W with temperature programming from 150 to 220°C at 4°C/min. A Sigma 2 gas chromatograph equipped with a flame ionization detector was used. Helium was employed as carrier gas with a flow rate of 30 ml/min. Quantitation of individual fatty acids was performed with peak areas determined with a Hewlett Packard integrator Model 3390A.

RESULTS & DISCUSSION

THE PROXIMATE ANALYSIS of fiddleheads is shown in Table 1. The percentage of protein is about twice that found in beet greens, swiss chard and spinach (Watt and Merrill, 1980). Preliminary amino acid analysis indicated the fiddleheads were low in methionine and high in aspartic acid and leucine. The fat, crude fiber and moisture content of fiddleheads compares favorably with that of beet greens, broccoli, spinach, swiss chard and collards, but the ash content is two to three times as high (Watt and Merrill, 1980). With the increased interest in the role played by fiber in health and disease (Spiller and Amen, 1976; Roth and Mehlman, 1978), fiddleheads, could make a significant contribution to daily fiber intake.

Ranges for vitamin data are given in Table 1 since values varied between sampling location. Comparisons of the vita-

Table 1—Proximate analyses and vitamin composition of fresh fiddleheads

Assay	%[a]	Vitamin[b]	Vitamin content[c]
Moisture	87.10	Niacin	4.07 –5.57
Fat	0.49	Riboflavin	0.15 –0.24
Crude Fiber	1.12	Thiamin	0.018–0.024
Crude Protein	4.23	Vitamin C	19.00 –32.69
Total Carbohydrate	3.06	Vitamin A	2175 –2709

[a] Each value represents the mean of two samples from two different locations.
[b] Values are presented as ranges since fiddleheads were harvested from two different locations. Four to five samples from each location were analyzed.
[c] Values for niacin, riboflavin and thiamin are in mg/100g wet weight. For vitamin A, values are in I.U./100g wet weight.

All authors are affiliated with the Dept. of Food Science, Holmes Hall, Univ. of Maine, Orono, ME 04469.

min content of fiddlehead greens (Table 1) with that of beet greens, dandelion greens, swiss chard and spinach (Watt and Merrill, 1980) showed that fiddleheads were high in niacin and low in vitamin A. The thiamin, riboflavin and vitamin C contents were similar to those for other greens. The lower vitamin A content may reflect differences between methods. The HPLC method eliminates the measurement of carotenoids that do not have vitamin A activity, and thus would give a more accurate value for vitamin A than previous methods.

As with other vegetable lipids, the fatty acid composition of fiddleheads consists of a high percentage of unsaturated acids. Palmitic (25.7%), linoleic (18.4%) and linolenic (26.7%) were found in the highest concentrations followed by stearic (4.2%), oleic (8.7%) and 13-docosenoic acid (8.1%). No fatty acids of chain length greater than C:24 were detected under the condition used.

The mineral composition of fiddlehead greens (Table 2) shows levels of potassium, magnesium and phosphorus as expected for greens. The sodium content of fiddlehead greens is low and is similar to that of asparagus (Watt and Merrill, 1980). Trace amounts of boron, copper, iron, manganese and zinc were found.

Table 2—Elemental analysis of fresh fiddleheads on a wet weight basis

Element	Sample[a] ppm	Sample[a] mg/100g	% RDA of 100g sample
Macro-elements			
Ca	326	32.6	4
K	3605	361.0	–
Mg	339	33.9	10
P	1050	105.0	13
Na	5	0.5	–
Micro-elements			
Al	0.3	0.03	–
B	4.7	0.47	–
Ba	–	0.01	–
Cd	0.1	0.13	–
Cr	–	0.01	–
Cu	3.4	0.34	–
Fe	11.2	1.12	11
Mn	4.2	0.42	–
Ni	1.0	0.10	–
Si	4.5	0.45	–
V	–	0.01	–
Zn	5.7	0.57	4

[a] Each value represents the mean of two samples from two different locations.

A 100g portion of wet fiddlehead greens would furnish approximately 25, 13, 2, 46 and 71% of the Recommended Dietary Allowance (RDA) for niacin, riboflavin, thiamin, vitamin C and vitamin A, respectively. The percent RDA of the mineral elements supplied by a 100g serving of fiddleheads is shown in Table 2. A 100g portion of fiddlehead greens would supply 10% or more of the RDA's for magnesium, phosphorus and iron. No RDA has been established for many of the micro-elements although minimum daily intakes have been recognized (Anon, 1980). Based on these data, a 100g serving of fiddlehead greens would furnish 25% of the chromium, 11% of the copper, and 10% of the manganese required on a daily basis.

From a nutritional standpoint, fiddleheads are a good source of vitamins A and C, niacin and riboflavin. Although vitamin content varied depending on harvest location, the differences were not significant. The vitamin content is similar to the values for potatoes which are recognized as an excellent source of vitamin C (Watt and Merrill, 1980). The fiber content of fiddleheads represents a substantial proportion of the solids content and could contribute to dietary fiber intakes. The low sodium content would make fiddleheads a valuable part of the diet of persons requiring low sodium intake. Fiddlehead greens then are comparable in nutritional quality to many common green vegetables.

REFERENCES

Akpapunam, M.A. and Markakis, P. 1981. Physiochemical and nutritional aspects of cowpea flour. J. Food Sci. 46: 972.

Anonymous. 1980. "Recommended Dietary Allowances," 9th ed. National Research Council, Washington, D.C.

AOAC. 1980. "Official Methods of Analysis," 13th ed., p. 123, 129, 771, 774. Association of Official Analytical Chemists, Washington, DC.

Blake, S.F. 1942. The ostrich fern as an edible plant. Amer. Fern J. 32: 61.

Blake, S.F. 1955. The ostrich fern as an edible plant; A second note. Amer. Fern J. 45: 135.

Briwa, K.E. 1956. Spring greens for the picking. Circular 296. Maine Cooperative Extension Service, Orono, ME.

Bushway, A.A., Belyea, P.R., and Bushway, R.J. 1981. Chia seed as a source of oil, polysaccharide and protein, J. Food Sci. 46: 1349.

Bushway, R.J. and Wilson, A.M. 1981. Determination of α- and β-chromatography. Canadian Institute of Food Sci. & Technol. In press.

Gibbons, E. 1979. "Handbook of Edible Wild Plants." Donning, Va. Beach, Norfolk, VA.

Morrison, W.R. and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boronfluoride-methanol. J. Lipid Res. 5: 600.

Roth, H.P. and Mehlman, M.A. 1978. "Symposium on the Role of Dietary Fiber in Health." Am. J. Clin. Nutr. (31).

Rushoff, L.L., Blakeney, E.W. Jr., and Culley, D.D. Jr. 1980. Duckweeds (Lemnaceae family): A potential source of protein and amino acids. J. Agric. Food Chem. 28: 848.

Spiller, G.A. and Amen, R.J. 1976. "Fiber in Human Nutrition." Plenum Publ. Co., New York.

Watt, B.K. and Merrill, A.L. 1980. "Composition of Foods." USDA Agriculture Handbook No. 8. U.S. Govt. Prtg. Off., Washington, D.C.

Ms received 7/1/81; revised 10/20/81; accepted 10/20/81.

A Research Note

Rehydration of Freeze-Dried Cultures of Lactic Streptococci

R. N. SINHA, A. K. SHUKLA, MADAN LAL, and B. RANGANATHAN

ABSTRACT

Cell suspensions (0.2 ml each) of three lactic streptococci were freeze-dried from distilled water. In order to determine the optimum conditions of rehydration the effect of different factors such as composition, temperature, and pH of rehydrating media on viability of the freeze-dried cells of the lactic streptococci has been studied. Rehydration of freeze-dried cells with 10% solution of dextrose, sucrose or reconstituted skim milk at pH 6.5 and 22°C can be recommended as optimum conditions for maximum recovery of viable cells.

INTRODUCTION

DURING THE USE of freeze-dried organism, rehydration has been considered as an important step. An organism which survives the various steps such as freezing, drying and storage, may lose its viability during rehydration (Fry, 1966). Poor recovery of cells following freeze-drying may be attributed to inadequacies in rehydration procedure (Leach and Scott, 1959). The present study pertains to study of factors such as composition, temperature, and pH of rehydrating media on the viability of freeze-dried lactic streptococci.

MATERIALS & METHODS

CELLS of *Streptococcus lactis* C_{10}, *S. cremoris* C_1, and *S. lactis* subsp. *diacetylactis* DRC_1 grown in 30 ml yeast dextrose broth were centrifuged. The washed cells were resuspended in 3 ml of glass distilled water. 0.2 ml each of the cell suspension was transferred to glass ampoule (100 x 6/7 mm) and freeze-dried (Sinha et al., 1972, 1974). Ampoules containing freeze-dried cells were opened immediately after freeze-drying and 0.2 ml of the rehydrating medium was added. Viable cell counts were determined by the standard plate method using yeast dextrose agar. The petriplates were incubated at 30°C for 48 hr.

RESULTS & DISCUSSION

COMPARATIVE DATA on the influence of eleven different rehydrating media on the viability of freeze-dried lactic streptococci are presented in Table 1. Maximum viability of cells was obtained with all three cultures in the rehydrating medium containing sucrose, followed by reconstituted skim milk and dextrose. When distilled water was used as rehydrating medium, the minimum number of viable cells were recovered with *S. lactis* C_{10}, *S. cremoris* C_1 and *S. lactis* subsp. *diacetylactis* DRC_1, the values being 173×10^5, 310×10^5, and 412×10^5 per ampoule, respectively. Other rehydrating media such as yeast extract, peptone, sodium glutamate and horse serum gave intermediate values in regard to number of viable cells (Table 1).

Analysis of variance of the data indicated significant ($P < 0.05$) differences in the viable cell counts due to different rehydrating media. Analysis of the data for the critical difference indicated similar effects in the cases of surcrose, reconstituted skim milk and dextrose, although the influence of the above three rehydrating media on the viability of cells significantly differed from other rehydrating media listed in Table 1. Distilled water, normal saline, phosphate buffer and lactose did not differ significantly from one another as they gave very low viable cell counts.

The data on distilled water as rehydrting medium yielding minimum cell counts are in conformity with the results of earlier workers (Wickerham and Flickinger, 1946; Wasserman and Hopkins, 1957; Leach and Scott, 1959; Pedersen, 1965). The poor protection afforded by distilled water to the freeze-dried cells of lactic streptococci may be similar to the disturbance in osmotic balance caused by exposure of cells to hypotonic solutions and consequent leakage of UV-absorbing substances from such cells (Leach and Scott, 1959; Meryman, 1966). From the results of the present investigation, it appears that in addition to temporary osmotic imbalance, some other phenomenon may also be involved, since it has been observed that rehydration of freeze-dried cells of lactic streptococci with normal saline and phosphate buffer (which are known to maintain osmotic equilibrium) yielded very low viable cell counts. Iandolo and Ordal (1966) ascribed sub-lethal thermal injury to cells of *Staphylococcus aureus* to their increased sensitivity to salt. It is therefore likely that low viable cell counts obtained with freeze-dried lactic streptococci after rehydration with normal saline and phosphate buffer may have to be explained on the above basis.

Compared to recovery in lactose, higher viable cell counts were obtained with sucrose and dextrose (Table 1). Almost similar results have been reported by Iljin (1953) in plant tissues and by Record et al. (1962) in *Escherichia coli* and other gram negative organisms. Such a phenomenon may be due to differences in control of water flux into cells by rehydrating media (Leach and Scott, 1959).

Table 1—Effect of different rehydrating media on the viability of freeze-dried cells of lactic streptococci[a]

Rehydrating medium	*S. lactis* C_{10}	*S. cremoris* C_1	*S. lactis* subsp. *diacetylactis* DRC_1
	Viable cell count ($\times 10^5$/ampoule)		
Distilled water	173	310	412
Normal saline	275	362	487
Sucrose (10% w/v)	543	684	865
Dextrose (10% w/v)	486	603	748
Lactose (10% w/v)	190	354	418
Yeast extract (5% w/v)	346	493	698
Peptone (10% w/v)	345	601	679
Sodium glutamate (5% w/v)	379	528	726
Horse serum	324	499	654
Reconstituted skim milk (10% w/v)	478	672	861
Phosphate buffer (pH 7.0)	315	424	488

[a] Rehydration was carried at ambient temperature

Authors Sinha and Lal are with the Div. of Dairy Bacteriology, National Dairy Research Institute, Karnal-132001 (Haryana), India. Author Shukla is with Pumjab Dairy Development Corp., Bhatinda-151001, India. Author Ranganathan is affilaited with the Southern Regional Station, National Dairy Research Institute, Bangalore-560 030, India.

In freeze-drying, sodium glutamate has been considered superior to reconstituted skim milk as suspending medium (Morichi et al., 1963; Vanderzant and Hyder, 1969; Sinha et al., 1970; 1972). But a reverse trend has been observed when these two have been used as rehydrating media. Ray et al (1971) reported faster repair of cell injury during rehydration with skim milk. Hence the present observations confirm that injury and repair of cells during rehydration are different as compared to other stages of freeze-drying such as freezing, drying, and storage (Meryman, 1966).

Rehydration of freeze-dried cells of *Streptococcus lactis* C_{10}, *S. cremoris* C_1 and *S. lactis* subsp. *diacetylactis* DRC_1 at 5, 22 and 32°C showed that maximum viable cells were obtained at 22°C (Table 2). In general, rehydration at 5°C gave better results than 37°C with all rehydrating media except distilled water and normal saline.

It has been shown that rehydration at refrigeration temperature may cause leakage of intracellular substances from the cells, thereby resulting in low viability (Leach and Scott, 1959; Fung and Vanden Bosch, 1975). Ray et al. (1971) showed that the rate of repair of the injured freeze-dried cells of *Salmonella anatum* decreased from 35°C when temperature of rehydration was lowered to 10 and 1°C.

Table 2—Effect of temperature of rehydration on the viability of freeze-dried cells of lactic streptococci

Rehydrating medium	Temp of rehydration	*S. lactis* C_{10}	*S. cremoris* C_1	*S. lactis* subsp. *diacetylactis* DRC_1
		Viable cell count ($\times 10^5$/ampoule)		
Distilled water	5	115	423	137
	22	405	432	359
	37	291	238	282
Normal saline	5	283	241	289
	32	638	333	411
	37	541	274	190
Sucrose (10% w/v)	5	785	772	758
	22	1450	919	813
	37	803	535	641
Peptone (10% w/v)	5	484	272	604
	22	815	613	737
	37	549	308	558
Reconstituted skim milk (10% w/v)	5	578	1220	718
	22	1030	1370	765
	37	499	788	565
Sodium glutamate (10% w/v)	5	637	358	588
	22	893	471	676
	37	383	217	565

Only one rehydrating medium, namely, sodium glutamate was used at six different levels of pH such as 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 as rehydrating medium for the recovery of viable cells of freeze-dried *S. cremoris* C_1. Maximum viable cell counts (596×10^5 per ampoule) were obtained at pH 6.5.

Analysis of variance of the data on viable cell counts indicated significant differences ($P < 0.05$) among temperature and pH of rehydrating media.

On the basis of the above findings, rehydration of the freeze-dried lactic streptococci with a 10% solution of dextrose, sucrose or reconstituted skim milk at pH 6.5 and at 22°C, can be recommended as optimum for obtaining maximum viable cell counts. Since the cells were freeze-dried after suspending in distilled water, the present recommendation needs to be further confirmed on rehydration of cells freeze-dried from other suspending media also.

REFERENCES

Fry, R.M. 1966. In "Cryobiology," p. 665, Ed. H.T. Meryman, Academic Press, New York and London.

Fung, D.Y.C. and Vanden Bosch, L.L. 1975. Repair, growth and enterotoxigenesis of Staphylococcus aureus S-6 injured by freeze-drying. J. Milk Fd. Technol. 38: 212.

Iandolo, J.J. and Ordal, Z.J. 1966. Repair of thermal injury of Staphylococcus aurus. J. Bacteriol. 91: 134.

Iljin, W.S. 1953. Cause of death of plants as a consequence of loss of water, conservation of life in desiccated tissue. Bull. Torrey Bot. Cl. 80: 166 (cf). [Cited by Leach and Scott (1959)].

Leach, R.H. and Scott, W.J. 1959. The influence of rehydration on the viability of dried microorganisms. J. Gen. Microbiol. 21: 295.

Meryman, H.T. 1966. Freeze drying. In "Cryobiology," p. 665. Academic Press New York and London.

Morichi, T., Irie, R., Yano, N., and Kembo, H. 1963. Protective effect of glutamic acid and related compounds on bacterial cells subjected to freeze drying. J. Gen. Microbiol. 9: 149.

Pederson, T.A. 1965. Factors affecting viable cell counts of freeze-dried Cryptococcus terricolus cells. Antonie van Leeuwenhoeck 31: 232.

Ray, B., Jezeski, J.J., and Busta, F.F. 1971. Repair of injury in freeze-dried Salmonella anatum. Appl. Microbiol. 22: 401.

Record, B.R., Taylor, R., and Miller, D.S. 1962. The survival of Escherichia coli on drying and rehydration. J. Gen. Microbiol. 28: 585.

Sinha, R.N., Dudani, A.T., and Ranganathan, B. 1974. Protective effect of fortified skim milk as suspending medium for freeze-drying of different lactic acid bacteria. J. Food Sci. 39: 641.

Sinha, R.N., Nambudripad, V.K.N., Dudani, A.T., and Laxminarayana, H. 1970. Studies of some factors affecting the viability of freeze-dried starter cultures during storage. 18th Int. Dairy Congr. IE: 128: Brief Communication.

Sinha, R.N., Nambudripad, V.K.N., Dudani, A.T., and Laxminarayana, H. 1972. Effect of suspending media on the viability of freeze-dried culture of Streptococcus lactis, J. Food Sci. Technol. 9: 85.

Vanderzant, C. and Hyder, K. 1969. Influence of condition of rehydration on the enumeration of bacteria from freeze dehydrated model food systems. J. Milk Food Technol. 32: 390.

Wasserman, A.E. and Hopkins, W.J. 1957. Effect of freeze-drying on some enzyme system of Serratia marcescens. Appl. Microbiol. 6: 49.

Wickerham, I.K. and Flickinger, M.H. 1946. Viability of yeasts preserved two years by the Lyophil process. Brewers Dig. 21: 55.

Ms received 5/2/81; revised 8/31/81; accepted 9/9/81.

A Research Note

Protein Size and Meltability in Enzyme-Treated, Direct-Acidified Cheese Products

R. R. MAHONEY, H. N. LAZARIDIS, and J. R. ROSENAU

ABSTRACT

SDS-electrophoresis was used to follow changes in protein size in a direct-acidified cheese product treated with protease. Increased meltability was related to decreased protein size. Excessive proteolysis preferentially eliminated most of the intermediate-sized proteins producing a short, grainy texture and a bitter flavor.

INTRODUCTION

DIRECT-ACIDIFICATION METHODS have many advantages over conventional processes for cheese manufacture (Lazaridis and Rosenau, 1980). Cheeses prepared in this fashion, however, often exhibit poor meltability which limit their use. Lazaridis et al. (1981) developed an enzymatic method of enhancing meltability in these cheese products. They reported a strong correlation between meltability and proteolysis expressed in terms of nonprotein nitrogen. However, at advanced levels of proteolysis, textural defects and bitterness were noted. They therefore postulated that meltability was related to protein size.

The objective of this work was to study changes in the proteins of direct-acidified cheese products produced by enzymatic treatment and relate them to meltability.

EXPERIMENTAL

Materials

Chymotrypsingen A, ovalbumin and ribonuclease A were from Pharmacia Fine Chemicals, Piscataway, NJ. Trypsin inhibitor, insulin and sodium dodecyl sulfate (SDS) were from Sigma Chemical Company, St. Louis, MO. All other chemicals were reagent grade or better.

Cheese products

Three experimental products of different meltability were prepared as described by Lazaridis et al. (1981). The product preparation differed only in the degree of enzymatic hydrolysis of the cheese protein. The first sample (control) was not exposed to enzymatic action and had poor meltability. The second sample (optimum) was treated with 0.03% enzyme (curd weight basis)–Embiozyme FP 600, Midwest Biochemical Corporation, Milwaukee, WI–for 10 min at 50°C and pH 7.0. These conditions describe a near optimum enzymatic treatment in terms of rheological and organoleptic characteristics for the product (Lazaridis, 1980), and result in a cheese with good meltability. The third sample (excessive) was treated with 0.037% enzyme for 20 min at 50°C and pH 7.0. These conditions correspond to an excessive treatment, resulting in a product of high meltability but with a short texture and noticeable bitterness. Commercial mozzarella cheese with meltability slightly greater than that of the optimum sample was obtained from a local supermarket. Casein was prepared from milk by acid precipitation (Lazaridis, 1980).

Analysis

The proteins in the cheeses were solubilized as described by Lazaridis et al. (1981) and stirred overnight. Soluble protein was determined by the Biuret method (Bruening et al., 1970) and accounted for 89–93% of the protein nitrogen in the original sample (Lazaridis, 1980).

SDS-electrophoresis in tubes was performed as described by Weber and Osborn (1975) with modifications: the samples were denatured by heating at 90°C for 15 min; the gels contained 13% acrylamide and 0.5% bisacrylamide and were loaded with either 50 μg cheese protein or 10 μg calibration protein. Meltability was determined by the Schreiber test (Kosikowski, 1977).

RESULTS

THE MOLECULAR WEIGHTS of the proteins in the cheese samples were obtained by reference to a calibration curve based on the migration of standards (Fig. 1). There is a pronounced inflection in the curve in the region below 10,000 daltons, Nonlinearity in this molecular weight range has also been observed by other groups (Dunker and Rueckert, 1969; Swank and Munkres, 1971). A possible reason for the deviation from linearity is that the intrinsic charge becomes relatively more important at lower molecular weights (Swank and Munkres, 1971). Alternatively, the deviation might be due to intrinsic differences in the tertiary structure of the proteins/peptides. Molecular weight values in this region are therefore only approximate. A schematic diagram of the electrophoretic patterns of the samples is shown in Fig. 2. Increased proteolysis caused a shift towards lower molecular weights which correlated with increased meltability. The control sample (A) showed no changes as compared to casein (E). The optimum sample (B), which exhibited acceptable meltability and good organoleptic characteristics, showed bands corresponding to two groups of large polypeptides derived from the caseins. Both this sample and the mozzarella (D) contained polypeptides in the range 10–25,000 daltons. These polypeptides are evidently capable of supporting a closed-textured but meltable structure.

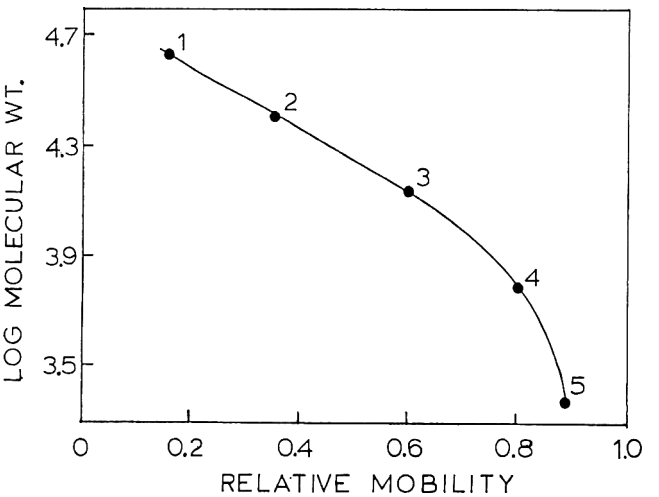


Fig. 1–Calibration curve for molecular weights of proteins using SDS electrophoresis: 1. ovalbumin; 2. chymotrypsingen A; 3. ribonuclease A; 4. bovine trypsin inhibitor; 5. bovine insulin.

Author Mahoney is with the Dept. of Food Science & Nutrition, and Authors Lazaridis and Rosenau are with the Dept. of Food Engineering, Univ. of Massachusetts, Amherst, MA 01003.

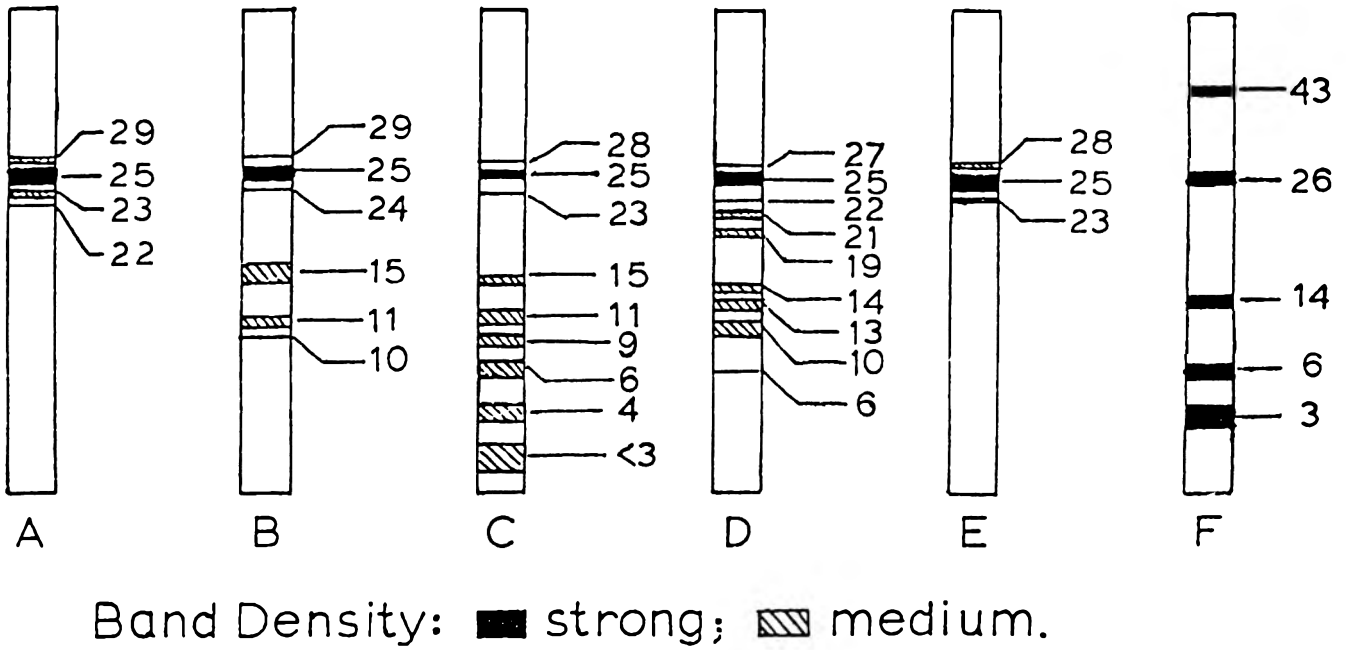


Fig. 2—Schematic diagram of SDS-electrophoretic patterns of cheese proteins. The number assigned to each bond is the molecular weight in thousands of daltons: A—control; B—optimum enzyme treatment; C—excessive enzyme treatment; D—mozzarella; E—casein; F—calibration proteins.

In the sample which received excessive enzyme treatment (C) both the caseins and the intermediate polypeptides in (B) above were broken down to give a series of smaller polypeptides whose molecular weight was mostly below 11,000. Evidently, these small polypeptides cannot support a closed structure since the cheese samples had a short, grainy unacceptable texture. These samples also had a bitter taste, which may be due to small peptides with a high percentage of hydrophobic amino acids (Matobe and Hata, 1972).

The electrophoretic pattern for casein (E) and the control sample (A) is similar to that of α_s casein (Mullin and Wolfe, 1974). However, the peptides found in the excessively treated sample (C) were much smaller than those found when casein was treated with rennin or trypsin (Mullin and Wolfe, 1974) presumably because the enzyme used in this study is much less specific.

It is of interest to note that advanced proteolysis effectively eliminated most of the intermediate-sized proteins. This may be due to the better availability of peptide bonds in partially hydrolyzed proteins as compared to the higher molecular weight caseins. This pattern of protein breakdown helps explain the fact that meltability is largely but not solely dependent on the degree of proteolysis (Lazaridis, 1980) since a cheese sample may still contain enough large molecular weight proteins or aggregates to support a fairly rigid structure, even though many peptide bonds have been broken.

REFERENCES

Bruening, G., Criddle, R., Preiss, J., and Rudert, F. 1970. Photometric methods for protein determination. In "Biochemical Experiments." Wiley—Interscience, Wiley & Sons, Inc., NY.

Dunker, A.K. and Rueckert, R.R. 1969. Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. 244: 5074.

Kosikowski, F.V. 1977. "Cheese and Fermented Milk Foods," 2nd ed. Edwards Brothers, Inc., Ann Arbor, MI.

Lazaridis, H.N. 1980. Enzymatic control of rheological properties in direct-acidified cheese products. Ph.D. thesis, Univ. of Massachusetts, Amherst, MA.

Lazaridis, H.N. and Rosenau, J.R. 1980. Effects of emulsifying salts and carrageenan on rheological properties of cheese-like products prepared by direct acidification. J. Food Sci. 45: 595.

Lazaridis, H.N., Rosenau, J.R., and Mahoney, R.R. 1981. Enzymatic control of meltability in direct-acidified cheese products. J. Food Sci. 46: 332.

Matoba, T. and Hata, T. 1972. Relationship between bitterness of peptides and their chemical structure. Agric. Biol. Chem. 36: 1423.

Mullin, W.J. and Wolfe, F.H. 1974. Disc gel electrophoresis of caseins treated with proteolytic and glycolytic enzymes. J. Dairy Sci. 57(1): 9.

Swank, R.T. and Munkres, K.D. 1971. Molecular weight analysis of oligopeptides by electrophoresis in polyacrylomode gel with sodium dodecyl sulfate. Analytical Biochem. 39: 462.

Weber, K. and Osborn, M. 1975. Proteins and sodium dodecyl sulfate: Molecular weight determination on polyacrylamide gels and related procedures. In "The Proteins," 3rd ed., Vol. 1., Ed Neurath, H. and Hill, R., p. 179. Academic Press, New York.

Ms received 9/8/81; accepted 10/15/81.

This research was supported (in part) by the Massachusetts Agricultural Experiment Station, Univ. of Massachusetts at Amherst.

A Research Note

Evaluation of Potassium Chloride as a Salt Substitute in Bread

C. JANE WYATT and K. RONAN

ABSTRACT

Breads made with white flour and a mixture of wheat and white flours were commercially prepared using three different levels of NaCl and a 1:1 mixture of NaCl/KCl. The Na and K content was determined by flame photometry on the dry ash. The 0.75% NaCl and 1.0% KCl/NaCl treatments for both white and wheat breads were judged equally desirable. The Na:K ratios of the 0.75% NaCl and 1% NaCl/KCl white breads are 7.00 and 1.20 respectively. The Na:K ratios for the 0.75% NaCl and 1% NaCl/KCl wheat breads are 3.29 and 0.91 respectively. Breads containing 1.00% of a 1:1 mixture of NaCl and KCl are not significantly different in flavor than breads containing 0.75% NaCl but the KCl containing product has a much lower Na:K ratio.

INTRODUCTION

MIXTURES OF SODIUM and potassium chlorides have been studied as possible means of reducing dietary sodium without deprivation of a palatable salty seasoning. These mixtures have the appeal of concurrently reducing dietary sodium and augmenting potentially beneficial dietary potassium (Frank and Mickelsen, 1969). Several investigators have studied the use of sodium and potassium chloride in different foods.

Mickelsen et al. (1977) conducted a study with six subjects to compare the acceptability of a 1:1 mixture of sodium and potassium chlorides to pure sodium chloride. The subjects did not detect a significant difference in potato chips or roasted peanuts seasoned with either pure sodium chloride or the salt mixture.

Work was done by Seman et al. in 1980 to determine the effects of partial replacement of sodium chloride on bologna characteristics and acceptability. Nine different combinations of sodium chloride, potassium chloride and magnesium chloride at high and low ionic strengths (0.42 and 0.21) and low ionic strengths of 0.13% tripotassium phosphate were used. A consumer panel tested the flavor acceptability of the treatments and rated the high ionic strength sodium chloride and high ionic strength sodium chloride-potassium chloride in equal strength mixtures, to be similar in acceptability. It was concluded that partial replacement of sodium chloride with potassium chloride would produce an acceptable commercial bologna product.

Wyatt in 1981 investigated the possibility of replacing sodium chloride with a 1:1 mixture of sodium and potassium chlorides in canned corn and green beans. The concentrations of the mixture used were 1.7% and 2.3%. These concentrations were compared to a reference of 1.5% pure sodium chloride for their overall desirability. The 1.5% sodium chloride reference and the 1.7% salt mixture samples were rated equally desirable when tested in green beans. The 2.3% salt mixture was rated less desirable as it was found to be too salty. Both salt mixture samples were rated less desirable than the pure sodium chloride reference in the corn treatments. The sodium-potassium ratio in processed green beans with no added salt was 0.16. The ratio found for the salt blend which was rated as desirable as the pure sodium chloride reference was 0.65. It was concluded that the 1:1 sodium-potassium chlorides salt mixtures in canned vegetables resulted in a lower sodium-potassium ratio than was found in vegetables processed with pure sodium chloride.

The purpose of this study was to evaluate a 1:1 mixture of sodium/potassium chloride as a salt substitute in white and wheat bread.

Authors Wyatt and Ronan are affiliated with the Dept. of Food Science & Technology, Oregon State Univ., Corvallis, OR 97331.

EXPERIMENTAL

Bread preparation

Wheat and white bread samples were made by a local bakery in Corvallis, OR following a standard homestyle recipe for white and wheat bread. These samples contained 0.5%, 0.75% and 1.0% concentrations of both 100% sodium chloride and a 1:1 mixture of sodium and potassium chloride. The dough was mixed and baked according to commercial baking procedures used by the bakery. All samples were placed in plastic freezer bags and held at 0°F until evaluated. All breads were tested within one week of baking.

Two different brands of commercial white and wheat breads were obtained at a local supermarket for analytical purposes only.

Mineral analyses

Bread samples were torn into small pieces using plastic gloves and mixed in a plastic bag to yield a representative mixture for assay. Samples were dry ashed in a muffle furnace at 550°C. Sodium and potassium concentrations were determined using a Coleman flame photometer model number 21 and appropriate element filters. Deionized water was used as a diluent. All analyses were done in triplicate.

Flavor panels

Bread samples were evaluated by an untrained panel (40 judges) selected only on the basis of availability. The samples were served on coded paper trays to the judges seated in individual testing booths. Each judge was asked to score the samples for overall desirability on a nine point hedonic scale ranging from 1 "extremely undesirable" to 9 "extremely desirable." Four different groups of samples were evaluated: 0.5, 0.75, 1.00% NaCl white and wheat breads and 0.5, 0.75, 1.00% NaCl/KCl white and wheat breads. Mean scores were determined for all samples.

A triangle test was done by comparing the sodium chloride sample with the highest mean score to the salt mixture sample which most closely matched it in degree of saltiness. The data were analyzed and the significance of differences were determined at the 5% and 1% level (ASTM, 1968).

RESULTS & DISCUSSION

THE SODIUM and potassium content and the overall desirability of the experimental breads are presented in Table 1. The experimental sodium chloride white bread samples contained an average of 61.5 mg/100g potassium. The sodium content of these samples were: 578.4 mg/100g 1.0% treatment, 415.0 mg/100g 0.75% treatment, and 283.3 mg/100g 0.5% treatment. Commercial white bread products contained an average of 62.0 mg/100g potassium and 630.0 mg/100g sodium.

The experimental sodium chloride wheat bread samples contained an average of 132.2 mg/100g potassium. The sodium content of these samples were: 590.8 mg/100g 1.0% treatment, 422.5 mg/100g 0.75% treatment, and

250.0 mg/100g 0.5% treatment. Commercial wheat breads contained an average of 213.2 mg/100g potassium and 617.9 mg/100g sodium. Commercial breads contained more potassium than the experimental breads because of a higher proportion of wheat flour which contains more potassium than white flour. The 1.0% sodium chloride treatment approximates the sodium level of commercial wheat bread.

The sodium-potassium ratios of the experimental and commercial white and wheat breads are also presented in Table 1.

The sodium and potassium ratios of the 0.75% NaCl white bread is 7.0 and 1.2 for the 1.0% NaCl/KCl bread. For wheat bread the ratios are 3.29 and 0.91. Judges were not able to distinguish between these treatments. The 0.75% sodium chloride white bread and wheat bread received the highest mean score. This score was significantly higher than the 1.0% but not the 0.5% sodium chloride breads. The judges found the 0.75% bread more desirable than the 1.0%, but the 0.75% and 0.5% sodium chloride breads were equally desirable and not significantly different. The 0.75% sodium-potassium chlorides mixture white bread had the highest mean score. This score was significantly higher than the 0.5% score. No significant differences were seen between the 0.75% and the 1.0% salt mixture bread mean scores. The mean scores of the 1.0%, 0.75% and 0.5% sodium-potassium chlorides mixture wheat breads were not significantly different. The judges apparently did not notice any objectionable flavor from the potassium chloride as the mean scores were comparable with the scores for the sodium chloride breads. Sodium chloride apparently masked the bitter flavor of potassium chloride in both white and wheat salt mixture breads.

Table 1—Sodium, potassium content and overall desirability of white and wheat breads

Treatments (%)	Sodium (mg/100g wet wt)	Potassium (mg/100g wet wt)	Sodium: Potassium	Mean score[a]
White bread				
1.0 NaCl	578.40	60.41	9.78	5.48a
0.75 NaCl	145.00	59.32	7.00	6.25b
0.50 NaCl	283.31	64.70	4.38	5.93a,b
Commercial A	644.70	63.91	10.09	
Commercial B	615.31	60.22	10.22	
Wheat bread				
1.0 NaCl	590.81	124.00	4.76	5.87a
0.75 NaCl	422.50	128.51	3.29	6.85b
0.50 NaCl	250.00	144.10	1.73	6.48a,b
Commercial A	585.65	220.42	2.66	
Commercial B	650.31	205.91	3.16	
Salt mixture white bread				
1.0 NaCl/KCl[b]	240.91	200.02	1.20	5.86a,b
0.75 NaCl/KCl	199.50	180.74	1.10	6.33a
0.50 NaCl/KCl	129.22	157.30	0.82	5.43b
Salt mixture wheat bread				
1.0 NaCl/KCl	263.61	290.80	0.91	6.38a
0.75 NaCl/KCl	250.00	270.00	0.92	6.85a
0.50 NaCl/KCl	185.23	220.74	0.84	6.43a

[a] Scores ranged from 1 "extremely undesirable" to 9 "extremely desirable." Mean scores that have the same letter (a,b) among the same test are not significantly different.
[b] NaCl/KCl = a 1:1 mixture

The 0.75% sodium chloride and 1.0% sodium-potassium chlorides mixture breads were tested by the triangle test to determine if a panel of untrained judges could distinguish between the two products. Seventeen judges responded correctly in the white bread test and eighteen in the wheat bread test. To establish significance at the 0.05 level with the triangle test, 19 out of 40 judges had to respond correctly (Roessler et al., 1978). Therefore the judges did not significantly distinguish between the 0.75% sodium chloride and the 1.0% sodium-potassium chlorides mixture breads. The use of a 1:1 sodium-potassium chloride mixture in both white and wheat breads is a successful means of lowering sodium-potassium ratios in bread while producing an acceptable product.

REFERENCES

ASTM. 1968. "Manual on Sensory Testing Methods." Tech. Pub. 434.

Frank, R. and Mickelsen, O. 1969. Sodium-potassium chloride mixtures as table salt. Am. J. Clin. Nut. 22(4): 464.

Mickelsen, O., Makdani, D., Gill, J., and Frank, R. 1977. Sodium and potassium intakes and excretions of normal men consuming sodium chloride or a 1:1 mixture of sodium and potassium chlorides. Am. J. Clin. Nut. 30: 2133.

Roessler, E., Pangborn, R., Sidel, J., and Stone, H. 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference-duo-trio and triangle tests. J. Food Sci. 43(3): 940.

Seman, D., Olson, D., and Mandigo, R. 1980. Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. J. Food Sci. 45(5): 1116.

Wyatt, C.J. 1981. Comparison of sodium and sodium-potassium salt mixtures in processed vegetables. J. Food Sci. 46: 302.

Ms received 7/21/81; revised 9/21/81; accepted 9/24/81.

Technical Paper No. 5922 from the Oregon Agricultural Experiment Station.

This work was partially supported by NSF grant No. PFR-7919119. The authors thank Professor L.A. McGill and her staff for their assistance in conducting the flavor panels.

A Research Note

Effect of Baking on Amino Acids in Pizza Crust

C. C. TSEN, L. S. BATES, L. L. WALL, SR. and C. W. GEHRKE

ABSTRACT

Pizza crusts baked at 316°C for 4.5 min did not change their protein contents. But the high-temperature and short-time baking did reduce the lysine and to a lesser extent, tyrosine, cystine, and threonine in pizza crusts. The loss in total lysine content ranged from 7.1% for whole wheat pizza to 19.4% for a commercial pizza crust. The difference between the total and available lysine for each pizza crust was small, indicating that the nutritive loss of pizza crust should be attributed to the destruction of a portion of lysine in pizza crust.

INTRODUCTION

BROWNING REACTIONS induced by baking or toasting can reduce the nutritive value of bread protein, as measured by protein efficiency ratio (PER) with rat-feeding tests (Tsen et al. 1977; Tsen and Reddy, 1977; Palamidis and Markakis, 1980). Lysine is involved in the browning reaction. Recent work on effects of baking temperature on the nutritive value of balady bread further substantiates that bread-protein quality deteriorates significantly when baking temperature is raised (El-Samahy and Tsen, 1981). Such deterioration suggests that when baking or toasting cereal foods it is important to control temperature and time to preserve their nutritive values.

Bread and pizza crust both are prepared from fermented dough. But pizza is commercially baked at a substantially high temperature (316–344°C) for a short period (4–10 min); whereas most breads are normally baked at 218–232°C for 20–25 min. In view of the popularity of pizza in this country, it would seem to be in the public's interest, as well as of scientific importance, to examine the possible effect of the high-temperature and short-time baking on the destruction of essential amino acids of pizza crusts. The results of such a study are reported here.

MATERIALS & METHODS

COMMERCIAL WHITE FLOUR (bread) and whole wheat flour milled from hard red winter wheat were used. Pizza crust mix (La Crosta) and frozen pizza (Totino's sausage) were the commercial products, from International Multifoods and the Pillsbury Co., Minneapolis, MN, respectively.

The formula for making pizza crust, on a flour basis, called for 1.1% yeast, 2.1% salt, 8% vegetable oil, and 67% water. Flour was mixed with the other ingredients in a standard vertical Hobart A-200 mixer at second speed (medium) for 8 min (5 min for whole wheat pizza crust). The mixer was equipped with a MacDuffee-type bowl and fork, and a water jacket to regulate dough temperature. The mxed-dough, at a temperature 30–32°C, was placed in a glass container and fermented 75 min at 30°C and 85–90% relative humidity. After it had fermented, the dough was divided into 450g pieces, molded, and sheeted by an automatic sheeter (Moline Company, Duluth, MN) to a thickness of approximately 4.0–6.6 mm. The dough sheet was cut into circles 30 cm in diameter. Baking was at 316°C for 4.5 min. The average baking loss was 22%.

To prepare pizza dough from the pizza crust mix, we mixed 368g of crust mix with 180 ml of water (49°C) for 1/4 min at low speed and 1/2 min at medium speed. The mixed dough was fermented for 5 min, then sheeted, cut, and baked into pizza crust. The topping of the frozen pizza was removed, and the remaining crust was baked at 316°C for 4.5 min, as were the other crusts.

For all analyses, we broke the crusts, dried them in a heated oven (air-blowing type) at 37°C for 24 hr, and ground them to a uniform particle size (to pass through a 20 mesh).

We used AACC Methods (1962) for moisture, ash, and protein determinations. Fat was determined by AOCS Method Aa4-38 (1971) with petroleum ether as extracting solvent.

Amino acids were analyzed by ion-exchange chromatograph (DIONEX-D300 analyzer) on p-toluensulfonic acid hydrolyzed samples, as reported by Lorenz et al., 1980, at the Dept. of Grain Science & Industry, Kansas State Univ.

Total and available lysien contents were determined as described by AOAC Method (1980) at the Experiment Station Chemical Laboratory, University of Missouri.

RESULTS & DISCUSSION

Components of pizza crusts

No considerable changes in total protein and ash contents of crusts were observed with baking (Table 1). The fat content, however, was reduced with baking, particularly for crusts prepared from white and whole wheat flours. Some fat, presumed to bound to pizza crust constituents during baking, becomes inextractable by petroleum ether.

Changes in amino acids

Comparing the amino acid contents (listed in Table 2) revealed that arginine, lysine, histidine, tyrosine, glycine, and threonine increased, but that leucine, isoleucine, valine, cystine, proline and aspartic acid were reduced when white or whole wheat flour was processed into pizza crust (unbaked). Those changes obviously resulted from preparing

Table 1—Protein, fat, and ash contents (on a dry basis) of wheat flours and pizza crusts, unbaked and baked

	Protein (N x 5.7) (%)	Fat (%)	Ash (%)
White flour (WF)	13.6	1.0	0.6
Whole wheat flour (WWF)	14.0	1.5	2.0
Pizza crust, white flour			
Unbaked (PCW)	11.6	7.7	2.3
Baked (PCWB)	11.6	6.2	2.4
Pizza crust, whole wheat flour			
Unbaked (PCWW)	12.9	8.8	3.4
Baked (PCWWB)	12.8	6.8	3.4
Pizza crust, commercial			
Unbaked (PCC)	11.7	9.9	2.7
Baked (PCCB)	11.7	9.4	2.8
Pizza crust, commercial mix			
Unbaked (PCCM)	11.8	4.1	3.3
Baked (PCCMB)	11.8	3.8	3.3

Authors Tsen and Bates are with the Dept. of Grain Science & Industry, Kansas State Univ., Shellenberger Hall, Manhattan, KS 66506. Authors Wall and Gehrke are affiliated with the Experiment Station Chemical Laboratory, Univ., of Missouri, Columbia, MO 65201.

Table 2—Amino acids contents in wheat flours and pizza crusts

Amino acid	Amino acid g per 100g protein (corrected to 100% recovery basis)									
	WF[a]	WWF	PCW	PCWB	PCWW	PCWWB	PCC	PCCB	PCCM	PCCMB
Aspartic acid	4.30	5.39	4.19	4.12	5.33	5.36	4.72	4.49	4.79	4.69
Threonine	2.56	2.89	2.66	2.65	2.98	2.91	2.75	2.74	2.94	2.83
Serine	4.93	5.06	4.96	4.88	4.97	5.09	4.83	4.79	4.93	4.94
Glutamic acid	34.44	31.28	34.44	34.46	30.25	30.50	33.35	33.83	33.04	33.32
Proline	10.20	9.55	9.53	9.90	9.05	9.10	9.16	9.23	8.86	9.08
Glycine	3.48	4.09	3.51	3.57	4.27	4.17	3.58	3.53	3.49	3.48
Alanine	3.23	4.04	3.25	3.28	3.50	3.48	3.12	3.15	4.13	4.09
Half cystine	1.72	1.66	1.62	1.56	1.64	1.56	1.55	1.49	1.71	1.61
Valine	3.12	3.21	2.82	2.86	3.12	3.10	2.90	2.70	2.89	2.99
Methionine	1.51	1.50	1.47	1.52	1.50	1.50	1.50	1.41	1.56	1.56
Isoleucine	2.53	2.42	2.31	2.36	2.40	2.39	2.33	2.32	2.40	2.45
Leucine	6.31	6.40	6.10	6.19	6.25	6.30	6.16	6.12	6.29	6.29
Tyrosine	3.01	3.12	3.08	3.00	3.25	3.13	3.18	3.16	3.12	3.05
Phenylalanine	4.38	4.54	4.63	4.66	4.52	4.57	4.73	4.85	4.70	4.69
Histidine	3.19	3.20	3.35	3.51	3.50	3.47	3.48	3.64	3.44	3.41
Arginine	3.50	4.39	4.31	4.12	5.11	5.07	4.50	4.62	4.30	4.17
Lysine	2.51	2.92	3.00	2.76	3.54	3.41	3.20	2.83	3.35	3.05
	Amino acid (g/100g sample)									
Lysine total	–	–	0.31	0.26	0.42	0.39	0.31	0.25	0.35	0.31
Lysine available	–	–	0.29	0.24	0.39	0.37	0.29	0.23	0.33	0.29

[a] WF, WWF, PCW, PCWB, PCWW, PCWWB, PCC, PCCB, PCCM, and PCCMB indicate white flour, whole wheat flour, pizza crusts prepared from white flour, unbaked and baked; from whole wheat flour, unbaked and baked; from commercial frozen pizza, unbaked and baked; and from commercial mix, unbaked and baked; respectively.

pizza crust from fermented dough, which contains yeast and other ingredients in addition to flour.

As compared with the unbaked pizza crusts, the corresponding baked ones showed reduced lysine, tyrosine, cystine, and threonine in their proteins. More lysine than any other amino acid was lost during baking.

In view of the importance of lysine in determining the nutritive value of cereal foods, we also determined the total and available lysine in pizza crusts, baked and unbaked. As shown in the bottom section of Table 2, loss during baking in total lysine content ranged from 7.1% for whole wheat pizza crust to 19.4% for the commercial pizza crust. The difference between total and available lysine for each pizza crust was small (within a range 0.02–0.03%), indicating that the nutritive loss of pizza crusts by baking should be attributed to the destruction, rather than to the reduction in availability, of a portion of lysine in pizza crusts.

REFERENCES

AACC. 1962. "Approved Methods," 7th ed. American Association of Cereal Chemists, St. Paul, MN.

AOAC. 1980. "Official Methods of Analyses," 13th ed., Association of Official Analytical Chemists, Washington, DC.

AOCS. 1971. "Official and Tentative Methods," 3rd ed. American Oil Chemists' Society, Champaign, IL.

El-Samahy, S.K. and Tsen, C.C. 1981. Effects of varying baking temperature and time on the quality and nutritive value of balady bread. Cereal Chem. 58: 546.

Lorenz, K., Dilsaver, W., and Bates, L. 1980. Proso millets. Milling characteristics, proximate composition, and nutritive value of flour. Cereal Chem. 57: 16.

Palamidis, N. and Markakis, P. 1980. Effect of baking and toasting on the protein quality and lysine availability of bread. J. Food Processing & preservation 4: 199.

Tsen, C.C. and Reddy, P.R.K. 1977. Effect of toasting on the nutritive value of bread. J. Food Sci. 42: 1370.

Tsen, C.C., Reddy, P.R.K., and Gehrke, C.W. 1977. Effects of conventional baking, microwave baking, and steaming on the nutritive value of regular and fortified breads. J. Food Sci. 42: 402.

Ms received 10/14/81; accepted 11/7/81.

Contribution No. 82-123-j, Dept. of Grain Science & Industry, Kansas Agricultural Experiment Station, Manhattan, KS 66506.

The financial support of the NC-132 project is gratefully acknowledged.

A Research Note

Protein Quality Characteristics of Iranian Flat Breads

H. A. FARIDI, G. S. RANHOTRA, P. L. FINNEY, and G. L. RUBENTHALER

ABSTRACT

Protein quality of five different types of Iranian flat breads (barbari, lavash, taftoon, sangak and village) and their corresponding unfermented and fermented doughs was assessed by the Protein Efficiency Ratio (PER) assay method. With one exception, the diet intakes of rats increased steadily with the extraction rate of flour used to make breads or doughs. Intakes were significantly ($P < 0.05$) higher on sangak and village breads (and doughs) as compared to the other three types of breads (and doughs). Results showed that PER of breads and doughs gradually increased with the extraction rate of the flour used. The process of baking (breads compared with doughs) lowered the PER value of all breads. The effect of fermentation on PER values was not significant.

INTRODUCTION

CEREALS PROVIDE most of the daily caloric and protein needs in rural as well as urban population of Iran. Protein malnutrition is a serious problem facing people whose diet consists mainly of cereals or starchy products. Reports from countries where rice, maize or wheat are the staple food consistently show a high prevalence of protein-energy malnutrition syndromes among infants and preschool children. Surveys in Iran (Hedayat and Sen-Gupta, 1967) have shown protein intakes to be only marginally adequate on the average and probably to be inadequate for a good number of the population. Inadequacy of the diet in rural Iran are evidenced by the medians for height and weight for age in preschool and school children which are lower than the tenth or even third percentile for well nourished populations (Hedayat et al., 1973). Ronaghy et al. (1968) reported mild hypoproteinemia and anemia exist in a majority of Iranian village boys and girls 12 to 17 years of age.

In recent years, considerable attention has been paid to the effect of processing on the nutritional quality of foods. For example, moderate heat treatment may improve while excessive heat may decrease the nutritive value. The most important factors determining the loss in nutritive value of foods are the duration and temperature level during processing such as baking and the levels of moisture and reducing substances. Eggum and Duggal (1977) reported a minor effect on the Net Protein Utilization (NPU) of Indian chapati, paratha, puri and tandorri during preparation. Shyamala and Kennedy (1962) found the Protein Efficiency Ratio (PER) of Indian chapati to be considerably higher than that of unprocessed wheat. Khan and Eggum (1978) concluded that processing of different cereals into Pakistani bread (unleavened) affects the nutritive quality only to a minor extent.

There is no published work on the effect of baking on the protein quality of Iranian breads. This work is a study of the effects of flour extraction rate, fermentation, and oven baking on the protein quality of five popular Iranian breads—barbari, lavash, taftoon, sangak and village.

Authors Faridi, Finney, and Rubenthaler are with the Western Wheat Quality Laboratory, SEA-USDA-ARS, and Dept. of Food Science & Technology, Washington State Univ., Pullman, WA 99164. Author Ranhotra is with the American Institute of Baking, Manhattan, KS.

MATERIALS & METHODS

THE FIVE TEST BREADS and corresponding doughs (fermented and unfermented) were prepared according to the formulas and procedures shown in Table 1 and detailed elsewhere (Faridi et al., 1982). Breads were oven dried at 50°C overnight and doughs were freeze-dried, finely ground and stored in airtight plastic bags under refrigerated conditions.

Protein quality was assessed by the Standard AOAC method (1975) for measuring the Protein Efficiency Ratios (PER) with some modification. This method measures the relative ability of protein sources to meet the essential amino acid requirement of rats during growth.

Individually housed (under controlled environment) male weanling (Sprague-Dawley) rats were fed test diets for 4 wk and PER values calculated based on the weight increases of the rats in 4 wk (g)/total protein (g) consumed. Determinations were corrected to a value of 2.5 for the reference (Casein-based) diet. Protein sources (breads or doughs) were included in the diet at a 7.5% protein level instead of the AOAC specified 10% level. The reference (casein) diet also contained 7.5% protein. The level of fiber was adjusted to a higher (1.5%) level than the specified 1% level.

The significance of differences between means was determined by the methods described by Steel and Torrie (1980).

RESULTS & DISCUSSION

THE PROTEIN CONTENT of breads/doughs barely exceeded 10% on a dry basis. The flours used were low in protein. With other required (AOAC 1975) ingredients included in the test diets, this permitted no more than 7.5% protein (from breads and doughs) in the diet against the specified 10% level. The compositional data also necessitated the adjustment of fiber in the diet since the village bread contributed substantially more than the specified 1% level. No adjustment was needed in the mineral, fat and moisture content of the test diets.

The data on diet intake, weight gain, and PER values are presented in Table 2. With one exception, the diet intake of rats increased steadily with extraction rate of the flour used to make breads/doughs; intakes were significantly ($P < 0.05$) higher on the sangak and village breads (and doughs) as compared to the other three types of breads (and doughs). The cumulative weight gains at the end of each week were also mostly significantly ($P < 0.05$) higher on the sangak and village breads/doughs. This apparently resulted from higher diet intakes coupled with a higher quality amino acid mixture of diets (Faridi et al., 1982). The effect of favorable amino acid mixture is most striking when the village bread diet is compared with the reference (casein) diet. The intakes on these two diets did not differ significantly but the growth response did ($P < 0.05$).

Like growth response, the PER values of breads gradually increased with the extraction rate of the flour used; this was significantly ($P < 0.05$) so on the breads taftoon, sangak and village. It is noteworthy that PER values of the barbari bread (made with low extraction flour), was no lower than that reported for leavened white pan bread

Table 1—Formulation and composition of five Iranian flat breads

	Bread[a]				
	Barbari (78)	Lavash (82)	Taftoon (84)	Sangak (87)	Village (97)
Formula					
Wheat flour (g)	100	100	100	100	100
Compressed yeast (g)	1	0.5	0.5	0.125	–
Sour dough (g)	–	–	–	20	–
Salt (g)	2	2	1	1	1
Soda (g)	0.35	0.25	0.25	–	–
Date Syrup (g)	–	–	1.5	–	–
Water (ml)	60	45	60	85	48
Baking parameters					
Ferm. time (min.)	155	90	60	120	90
Baking Temp. (F°)	500	630	600	520	410
Baking Time (min.)	12.0	1.3	2.5	5.0	3.0
Composition[b]					
Protein (N x 5.7, %)	9.8(9.6,9.8)[c]	9.7(9.7,9.7)	10.0(9.8,10.1)	10.2(9.9,10.0)	10.6(10.6,10.6)
Ash (%)	2.81(2.76,2.76)	2.82(2.80,2.81)	1.74(1.71,1.78)	1.74(1.76,1.78)	2.24(2.22,2.20)
Ether extract (%)	0.42(0.69,0.63)	0.40(0.71,0.74)	0.35(0.77,0.79)	0.41(0.97,0.90)	0.76(1.26,1.31)
Crude fiber (%)	0.13(0.12,0.14)	0.29(0.24,0.22)	0.38(0.38,0.35)	0.93(0.74,0.77)	1.71(1.47,1.52)
NDF (%)[d]	0.58(0.51,0.50)	1.36(1.28,1.24)	2.11(1.98,1.89)	3.62(3.45,3.38)	6.32(5.96,6.03)
ADF (%)[e]	0.04(0.00,0.00)	0.45(0.38,0.33)	0.75(0.68,0.61)	1.27(1.07,1.05)	2.21(2.14,2.21)

[a] Numbers given in parenthesis below bread types indicate extraction rate of flour.
[b] Dry basis.
[c] Values within parenthesis refer to unfermented and fermented doughs respectively.
[d] Neutral detergent fiber (AOAC)
[e] Acid detergent fiber (AOAC)

Table 2—Growth responses and protein efficiency ratios (PER) of five Iranian flat breads with their corresponding unfermented and fermented doughs[a]

			Cumulative Weight Gain				PER	
Bread	Amount in diet (g)[b]	Diet intake (g)	1 wk (g)	2 wk (g)	3 wk (g)	4 wk (g)	Measured	Corrected
Barbari (78%)[c]								
Dough (UF)[d]	83.2	164.9±17.1	7.4±1	12.4±3	15.0±2	18.8±3	1.52±0.16	1.32±0.14
Dough (F)[e]	80.6	166.1±22.8	7.1±1	12.2±2	16.2±2	18.7±2	1.51±0.13	1.31±0.12
Bread	82.1	167.3±19.8	7.8±2	11.2±2	13.6±3	16.3±4	1.30±0.22	1.13±0.19
Lavash (82%)								
Dough (UF)	79.1	172.0±16.6	6.2±2	12.1±2	15.6±2	19.5±2	1.52±0.16	1.32±0.14
Dough (F)	76.5	175.6±17.6	8.3±2	13.4±3	17.7±3	23.6±3	1.79±0.16	1.56±0.14
Bread	79.8	186.0±18.5	6.0±2	11.8±2	15.0±3	18.3±2	1.31±0.10	1.14±0.09
Taftoon (84%)								
Dough (UF)	77.6	188.1±20.4	8.5±3	14.3±2	17.8±2	22.1±2	1.57±0.09	1.37±0.08
Dough (F)	77.9	176.3±18.7	7.7±2	13.0±2	17.2±2	22.1±3	1.68±0.13	1.46±0.11
Bread	78.9	176.0±20.0	8.2±2	13.2±2	16.7±3	19.4±2	1.48±0.14	1.29±0.12
Sangak (87%)								
Dough (UF)	70.7	201.9±13.8	8.1±1	16.0±2	21.9±3	27.9±3	1.85±0.19	1.61±0.17
Dough (F)	70.9	195.7±14.8	8.6±2	15.0±2	20.1±2	24.6±2	1.67±0.10	1.46±0.09
Bread	74.2	196.8±19.3	8.5±2	13.5±2	17.3±3	21.9±3	1.48±0.09	1.29±0.09
Village (97%)								
Dough (UF)	76.0	202.6±25.9	9.0±2	16.7±3	23.3±5	29.0±4	1.91±0.13	1.66±0.11
Dough (F)	76.1	207.2±13.6	8.1±1	16.0±1	22.7±2	27.9±2	1.80±0.07	1.56±0.07
Bread	80.2	211.4±26.6	7.9±2	15.6±2	20.7±3	25.3±3	1.61±0.16	1.39±0.14
Casein	8.49	223.2±26.9	14.3±4	27.3±2	38.9±5	48.0±5	2.87±0.10	2.50±0.09

[a] Values indicate average ± S.D. (10 rats/diet)
[b] Provided 7.5% protein in the diet
[c] Values within parenthesis indicate the extraction rate of flour used.
[d] Unfermented
[e] Fermented

(Ranhotra et al., 1977). The lower (7.5% vs 10%) protein content in test diets probably did not influence this. The concept that efficiency of protein utilization for growth improves continuously with decreasing dietary levels was shown to be erroneous (Hegsted and Neff, 1970). The PER value for other flat breads compared quite favorably with those for whole wheat or wheat protein-supplemented breads (Ranhotra et al., 1971).

Protein intake of Iranian rural dwellers is low. The daily per capita intake was 55–66g in villages where caloric intake was low (Hedayat and Sen-Gupta, 1967). The animal protein intake was exceedingly low. In one village 54% of the families consumed 5–10g of animal protein, daily; In another, 35% had 0–3g, daily. Thus with the combination of low protein diets and low quality protein, the stage is set for chronic protein malnutrition (Caughey, 1973).

—Continued on page 679

A Research Note

Isolation and Characterization of Invertase from Iraqi Date Fruit

BAHA A. MAROUF and LIHAD ZEKI

ABSTRACT

Soluble invertase was isolated and characterized from the pericarp of Iraqi date fruit, Sayer variety. The optimum pH of the soluble invertase was 4.0–4.7 and the optimum temperature was 50°C. The specific activity of the partially purified soluble invertase was 70.0 units per mg protein. The molecular weight of the soluble invertase was 70.0 units per mg protein. The molecular weight of the soluble invertase was probably more than 300,000 Daltons, and had high affinity for sucrose with a Km value of 3.33×10^{-3} mM. Sodium dodecyl sulfate (SDS) inhibited the activity of the soluble invertase.

INTRODUCTION

INVERTASE (D-fructofuranoside fructohydrolase, are widely distributed in higher plants (Hawker and Hatch, 1965). They can be classified into soluble invertase and insoluble (bound) invertases according to their association with cellular components. Soluble as well as insoluble invertases were found in the developing endosperm of maize (Jaynes and Nelson, 1971), and in carrot, potato, and red beet (Vaughan and MadDonald, 1967).

Invertases can also be classified, on the basis of their optimum pH, into acid invertases and neutral invertases. Sugar cane contains at least two invertases according to this classification. An acidic invertase with a pH optimum of 5.0–5.5 and a neutral invertase with optimum pH 7.0 (Glasziou, 1962). Similar results were reported in peas (Dick and Rees, 1976); however, only acidic invertase was reported to be present in tomato (Manning and Maw, 1975).

The present study deals with characterization of invertase from the pericarp tissue of date palm (*Phoenix dactylifera* L.) and the inhibitor of this enzyme.

MATERIALS & METHODS

Materials

Sample. Sayer date fruit were obtained from Zafarania Orchid located at Baghdad, Iraq.

Reagents. Sucrose was the product of Calbiochem, 3,5-dinitrosalicylic acid was obtained from BDH Chemicals, and bovine serum albumin was obtained from Serva.

Subcellular fractionation

The epicarps from date fruit were removed. The pericarps were homogenized in 20 mM borate buffer, pH 8.0, and 1N NaCl for 10 min at 4°C. The homogenate was filtered through No. 3 mesh sintered glass funnel. The filtrate was centrifuged at 20,000 rpm and 2°C for 30 min. The supernatant was used as crude soluble enzyme preparation. The pellet, after suspension in buffer, was used to check the presence of insoluble invertase.

Characterization of invertase

The supernatant obtained from centrifugation was applied to Amicon MMC Cell containing the following Diaflo Ultra filters, UM 10, PM 30, XM 50, XM 100A and XM 300 with 10,000, 30,000, 50,000, 100,000 and 300,000 molecular weight retentivities respectively. Extracts retained on filters were washed extensively with 20 mM acetate buffer at pH 4.6. Extract retained by 300 XM membrane filter was used for further studies.

The effect of pH on invertase was determined through pH 2.2–7.0 with 20 mM concentrations of glycin-HCl, citrate-phosphate and phosphate buffers with overlapping points. The temperature effect on invertase was determined by incubating at different temperatures at pH 4.6 for 30 min.

Assay procedure

Invertase activities were determined in 2 ml containing 1 mM sucrose, 20 mM acetate buffer pH 4.6, 0.1 ml date extract and distilled water. The incubation temperature was at 37°C. After 30 min, aliquots of 0.2 ml assay mixture were withdrawn, 0.5 ml of 3,5-dinitrosalicylic acid was added and the mixed solutions were boiled for 10 min in a boiling water bath. The color developed was measured at 540 nm, with a Carl Zeiss model PM4 Spectrometer. Concentrations of reducing sugars in assay tubes were determined using a glucose calibration curve (Bernfeld, 1955).

Protein contents of extracts were determined using Lowry protein determination (Lowry et al., 1951) with BSA as standard. One unit of enzyme activity was equal to one μmole reducing sugar per min under the conditions of the experiments. The specific activity was units per mg protein.

Effect of inhibitor

Sodium dodecyl sulfate (SDS) was incubated with the enzyme extracts. The reaction was started by the addition of the substrate, sucrose.

The kinetics of SDS inhibition of the soluble invertase was studied using various sucrose and inhibitor concentrations.

RESULTS & DISCUSSION

THERE IS only soluble invertase in the pericarp tissue of Sayer variety of dates. The specific activity of the enzyme increased 2.3 fold from 30 units/mg protein to 70 units/mg protein with the ultrafiltration step. The invertase activity was associated with the fraction retained by the Amicon XM 300 filter which indicated that the enzyme may have a larger molecular weight than 300,000. The high molecular weight of the soluble invertase indicates a possible subunit structure. Acid invertases from other sources had high molecular weights and subunits, as in sugar cane (Moretzki and Alexandar, 1967) and yeast (Neuman and Lampen, 1967).

No neutral or alkaline invertase activities were detectable in our samples; only acid invertases were present. No buffer effect on enzyme activities was observed. The optimum pH of soluble invertase was found to be 4.0–4.6. At low pH values, less than 3.4, no enzymic inversion of sucrose occurred. Optimum temperature of the enzyme was found to be 50°C (Fig. 1) and some activity was detected even at 60°C indicating a relatively thermostable enzyme.

The rate of soluble invertase reaction was determined as a function of sucrose concentration (4 μmoles–1000 μmoles). The enzyme showed a high substrate affinity for sucrose with Km value of 3.33×10^{-3} mmoles. Soluble invertase was competitively inhibited by SDS (Fig. 2). The SDS data indicated that SDS acted as competitive in-

Authors Marouf and Zeki are with the Nuclear Research Centre, Agriculture & Biology Dept., Tuwaitha, Baghdad, Iraq.

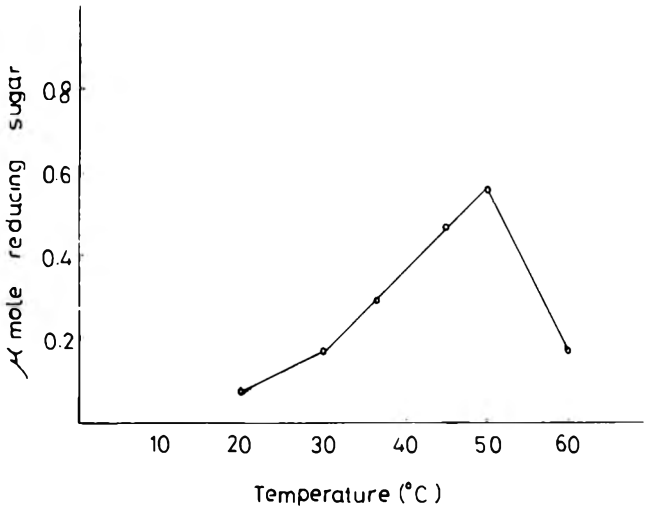


Fig. 1—Effect of temperature on the activity of soluble date invertase.

hibitor to invertase isolated from date fruit. In spite of our extensive review of literature we were able to locate similar competitive inhibition by SDS.

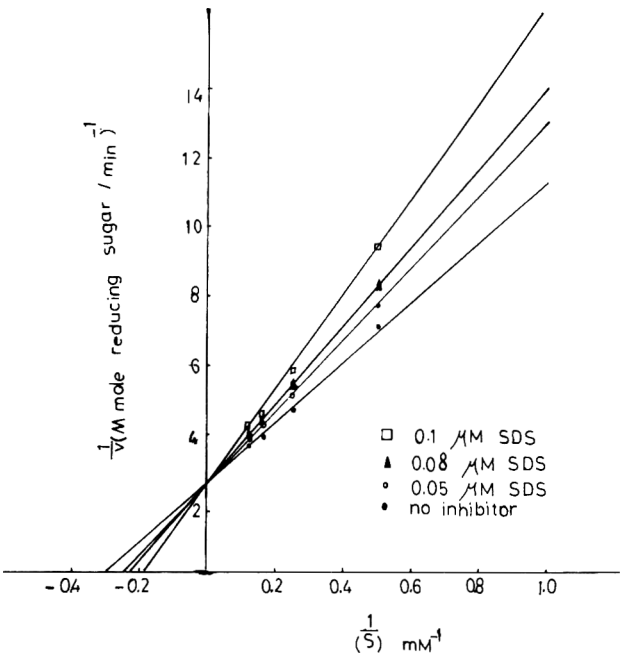


Fig. 2—Double reciprocal plots of ovelocity against sucrose concentration in the presence of SDS.

REFERENCES

Bernfeld, P. 1955. "Methods in Enzymol," Ed. Colowick, S.P. and Kaplan, N.O. Vol. 1, p. 49. Academic Press, Inc. New York.

Chin, C.K. and Weston, G.D. 1973. Distribution in excised Lycopersicum esculantum roots of the principle enzymes involved in sucrose metabolism. Phytochemistry 12: 229.

Dick, P.S. and Rees, T. 1976. Sucrose metabolism by roots of Pisum sativuum. Phytochemistry 15: 255.

Glasziou, K.T. 1962. Accumulation and transformation of sugars in sugar cane stalks: Mechanism of inversion of sucrose in inner space. Nature 193: 1100.

Hawker, J.S. and Hatch, M.D. 1965. Mechanism of sugar storage by mature stem tissue of sugar cane. Physiol. Plant 18: 444.

Janes, T.A. and Nelson, O.E. 1971. Invertase activity in normal and mutant maize endosperm during development. Plant Physiol. 47: 623.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randell, P.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265.

Manning, K. and Maw, C.A. 1975. Distribution of acid invertase in the tomato plant. Phytochemistry 14: 1965.

Moretzki, A. and Alexandar, A.G. 1967. Gel filtration studies of invertase from sugar cane meristem. Enzymol. Acta Biocatal. 35: 299.

Newman, N.P. and Lampen, J.O. 1967. Comparative study of the properties of the purified internal and external invertases from yeast (Saccharomyces strains) J. Biol. Chem. 243: 1573.

Vaughan, D. and MacDonald, I.R. 1967. Development of soluble and insoluble invertase activity in washed storage tissue slices. Plant Physiol. 42: 456.

Ms received 6/11/81; revised 10/10/81; accepted 10/12/81.

PROTEIN QUALITY OF IRANIAN FLAT BREADS . . . From page 677

There is evidence accumulating concerning the health advantages of a high cereal diet (Burkitt, 1973; Mickelsen, 1975; Carpenter, 1977; Faridi, 1981). However, since many Iranian villagers eat excessively high amounts of whole wheat, unleavened bread, their diets may induce hypoproteinemia and severe malnutrition.

REFERENCES

AOAC. 1975 "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.

Burkitt, D.P. 1973 Epidemiology of large bowel disease: The role of fiber. Proc. Nutr. Soc. 32: 145.

Carpenter, K.J. 1977 High-cereal diets for man. Proc. Nutr. Soc. 36: 149.

Caughey, J.E. 1973 Aetiological factors in adolescent malnutrition in Iran. new Zealand Med. J. 77: 90.

Eggum, B.O. and Duggal, S.K. 1977 The protein quality of some Indian dishes prepared from wheat. J. Sci. Food Agric. 28: 1052.

Faridi, H.A. 1981 Health advantages of high bread diet and approaches to U.S.-type flat bread production. Bakers Digest 55(3): 23.

Faridi, H.A., Finney, P.L., Rubenthaler, G.L., and Hubbard, J.D. 1982. Functional (Breadmaking) and compositional characteristics of Iranian flat breads. J. Food Sci. In press.

Hedayat, H. and Sen-Gupta, P.N. 1967 Nutrition survey in Iran. Iran Institute of Food and Nutrition. Mimiographs No. 1—10.

Hedayat, H., Shahbazi, H., Payan, R., Azar, M., Bavandi, M., and Donoso, G. 1973 The effect of lysine fortification of Iranian bread on the nutritional status of school children. Acta Pediat Scand 62: 297.

Hegsted, D.M. and Neff, R. 1970 Efficiency of protein utilization in young rats at various levels of intake. J. Nutr. 100: 1173.

Khan, M.A. and Eggum, B.O. 1978 Effect of baking on the nutritive value of Pakistani bread. J. Sci. Fd. Agric. 29: 1069.

Mickelsen, O. 1975 The nutrition value of bread. Cereal Food World 20: 308.

Ranhotra, G.S., Hepburn, F.N., and Bradley, W.B. 1971 Supplemental effect of wheat protein concentrate on the protein quality of white wheat flour. Cereal Chem. 48: 699.

Ranhotra, G.S., Loewe, R.T., and Lehmann, T.A. 1977 Breadmaking quality and nutritive value of sprouted wheat. J. Food Sci. 42: 1373.

Ronaghy, H.A. and Sen-Gupta, P.N. 1967 Nutrition survey in Iran. Iran Institute of Food Nutrition. Mimiographs No. 1—10.

Ronaghy, H.A., Gauhey, J.E., and Halsted, J.A. 1968. A study of growth in Iranian village children. J. Am. Clin. Nutr. 21: 488.

Shyamala, G. and Kennedy, B.M. 1962 Protein value of chapatis and puris. J. Am. Diet. Ass. 41: 115.

Steel, R.G.D. and Torrie, J.H. 1980. "Principles and Procedures of Statistics." McGraw-Hill, New York, NY.

Ms received 10/9/81; revised 11/25/81; accepted 11/30/81.

Presented at the 41st Annual Meeting of the Institute of Food Technologists, Atlanta, GA, June 7—10, 1981.

This project is funded in part by the Oregon and Washington Wheat Commissions. Their support is acknowledged.

Scientific Paper No. 5931, College of Agriculture Research Center, Washington State Univ., Pullman, WA 99164.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

A Research Note

A Method for the Quantitative Evaluation of Emulsion Stability in Coffee Whiteners

R. J. PEARCE and W. J. HARPER

ABSTRACT

A method is described for quantitatively measuring the extent of emulsion destabilization (feathering) when liquid coffee whiteners are added to hot coffee. Coffee whitener is added to standard hot coffee solution (pH 4.9, 90°C) in a 0.5% fat Babcock bottle. Feathering is observed on the surface of the whitened coffee in the wide portion of the bottle. Dense corn syrup solution is layered beneath to raise the level of the surface so that the coagulated material is concentrated into the graduated capillary tube. After standing to allow compaction, the length of the column of coagulated material is measured.

INTRODUCTION

A QUANTITATIVE ESTIMATION of functional response is a prerequisite for simultaneous evaluation of the interactions of the multiple components in a coffee whitener. Feathering was selected as an index of functionality relating to emulsion stability and behaviour of the protein. Evaluation tests described previously for feathering (Harper et al., 1980) determine only its presence or absence. In this paper a simple method is described which allows measurement of the extent of emulsion destabilization, exhibited as feathering, when liquid coffee whitener is added to hot coffee.

EXPERIMENTAL

Materials

Soy bean protein isolate was prepared from dehulled soy beans by water extraction of defatted flour and isoelectric precipitation of the protein. Other ingredients were: hydrogenated coconut oil (melting range 33.4–39°C, Capital City Products), corn syrup containing 75% carbohydrate (Caro, Best Foods, sodium stearoyl-2-lactolate (SSL) (Grinsted Products, Inc.), mono- and di-glycerides (Atmos 150, ICI America Inc.), polysorbate (Tween 60, ICI America Inc.), dipotassium hydrogen phosphate (K_2HPO_4) (Fisher Scientific).

Preparation of liquid coffee whitener

Soy bean protein isolate, and mono- and di-glycerides, polysorbate and SSL were dispersed in the melted coconut oil. K_2HPO_4 and corn syrup were dissolved in water at 71°C and added, with vigorous stirring, to the oil phase at the same temperature. After pasteurization at 71°C for 15 min, the macroemulsion was homogenized at 55°C with a laboratory-scale homogenizer (Foss America, N.Y.) utilizing four stages. The prepared coffee whitener was immediately cooled in an ice bath and allowed to stand at 4°C overnight before evaluation.

Estimation of the extent of feathering

A suspension of instant coffee, 2% w/v (Maxwell House), pH 4.9, was prepared and, while being continuously stirred, aliquots of 25 ml were removed by pipette and transferred to Babcock bottles (0.5% fat), previously thoroughly cleaned with chromic acid and copiously rinsed. These bottles, together with a flask containing more of the same coffee suspension, were placed in a covered water bath and heated to 90 ± 1°C. To each bottle was added 3 ml of coffee whitener in a slow, steady stream, with gentle swirling of the coffee, followed immediately by a further 2 ml of hot (90°C) coffee, to wash all the coffee whitener into the body of the bottle. After standing for about 5 min at room temperature, during which time any feathering became apparent at the surface, the bottles were carefully placed in another water bath at 55°C at a depth sufficient to heat as much of the bottle as possible. Corn syrup, diluted 1:10 with water at room temperature, was added in a steady, continuous stream so that it layered underneath the coffee until the coagulated material was concentrated just below the capillary. Finally, and more slowly, further diluted corn syrup was added to force all the surface material into the capillary. The bottles were then allowed to stand for 4 hr at 55°C to allow the solid, buoyant material to compact in the capillary. The height of the column of this material was measured and recorded.

RESULTS & DISCUSSION

AFTER ADDITION of the coffee whitener to the hot coffee, the appearance of feathering was rapid as soon as the solution became stationary. If the coffee whitener emulsion was very unstable, substantial coagulation tended to occur throughout the coffee. Table 1 shows the formulations for experimental coffee whiteners, which were designed to give a range of products varying in stability. The coffee whiteners E and B coagulated within 30 sec. Normally, samples behaving in this manner would not be treated further. The other samples showed no coagulation through the coffee but a layer of feather-like material was observed in all except A and C.

Forcing the feathered material into the capillary tube resulted in some initial redistribution of the material along the length of the capillary tube. However, on standing, the material floated back towards the surface and eventually formed a compact plug. Since little further compaction was observed after standing for 4 hr, the length of the plug was measured after this time. The results for formulations A to

Table 1—Concentrations of independent variables in liquid coffee whitener formulations with corresponding feathering evaluation data. At constant concentration were: hydrogenated coconut oil, 9.5 g/100g; K_2HPO_4, 0.1 g/100g; corn syrup 12.4 g/100g. Water was the dependent variable.

Bottle code	Concentrations of independent variables g/100g				Height of feather (mm)
	SSL	ATMOS 150	TWEEN 60	Soy isolate	
A	0.10	0.35	0.56	0.70	0
B	0.10	0.20	0.30	0.60	coagulated
C	0.25	0.35	0.56	0.60	1.5
D	0.40	0.35	0.56	0.70	11
E	0.40	0.275	0.44	0.70	coagulated
F	0.40	0.275	0.44	0.60	27
G	0.25	0.275	0.44	0.80	42
H	0.25	0.275	0.44	0.60	23
I	0.25	0.35	0.56	0.70	18

This work was performed at the Dept. of Food Science & Nutrition, Ohio State Univ., Fyffe Road, Columbus, OH 43210. Author Pearce is now with the CSIRO Division of Food Research, Dairy Research Laboratory, PO Box 20, Highett, Victoria 3190, Australia. Author Harper is now with the New Zealand Dairy Research Institute, Private Bag, Palmerston North, New Zealand.

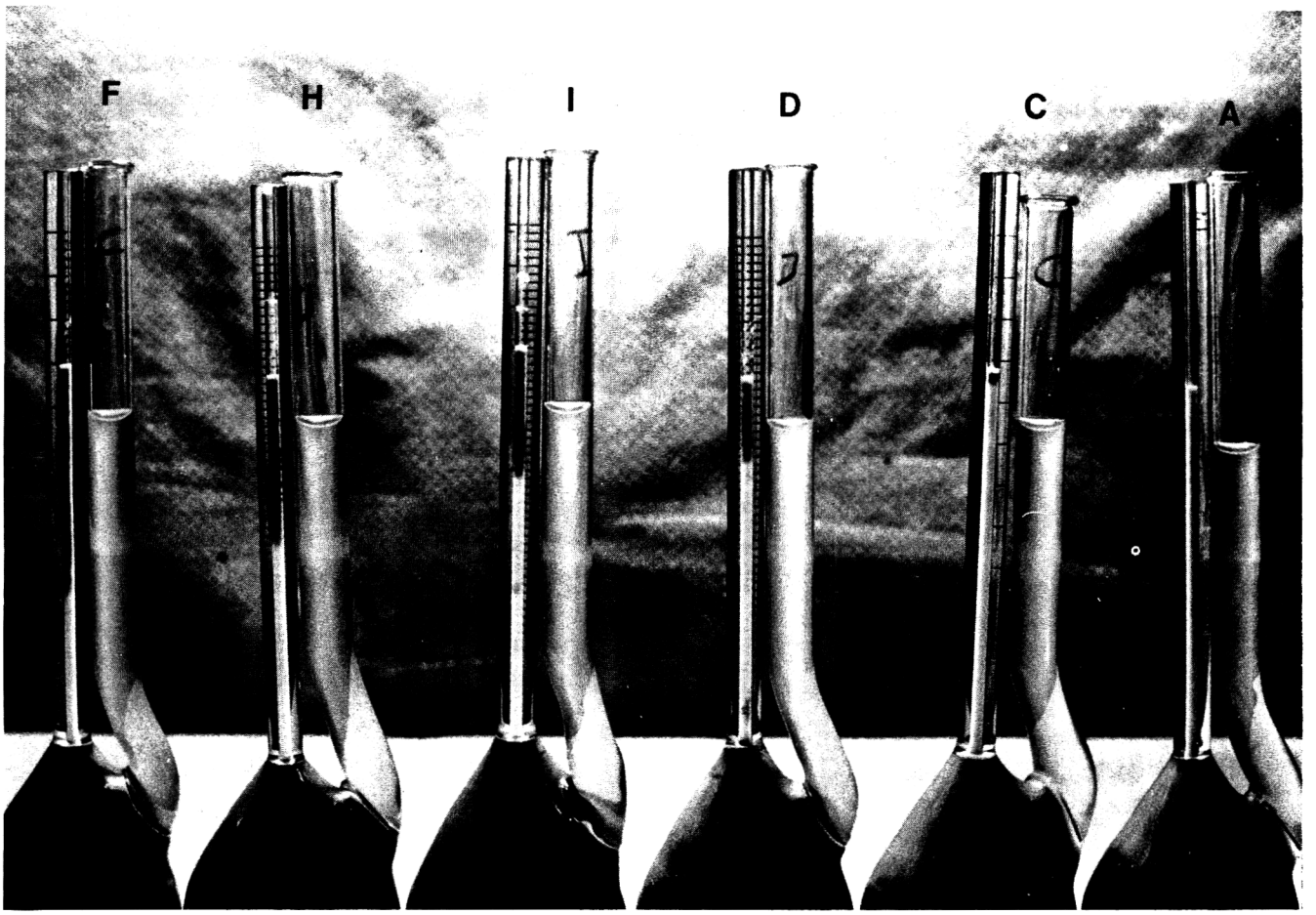


Fig. 1—Appearance of coffee in Babcock bottles at the completion of the test.

E are shown in Table 1. Routinely, coffee whiteners were tested in duplicate; under the test conditions described, variation between duplicates was less than 10%. In a series of six replicates of a single coffee whitener formulation, a mean value of 10.67 mm with a standard deviation of 0.82 mm was obtained.

After compaction of the feathered material on standing, different heights of material in the capillary tube were observed. The capillary tubes of Bottles B and E were completely full and those of Bottle G nearly full and were recorded as coagulated or >50 mm. The remaining bottles are shown in Fig. 1 where it may be seen that, in Bottles F to A, feathering decreased to zero (A). Even bottle C, which did not appear to have feathered in Fig. 1, showed that a little emulsion destabilization had occurred. Only coffee whitener was completely stable. The complete test, therefore, was more sensitive for observing emulsion destabilization in whitened coffee than was visual examination after the first stage. In addition, the complete test provided a quantitative measurement of the effect.

This type of functionality test, yielding quantitative, functional response data, provides an indirect route to establishing the component interactions in a real food system rather than in a simple model from which it is difficult to extrapolate to more complex systems. The test is of particular value in assessing the suitability of an ingredient and the level at which it should be added for optimum performance of a coffee whitener. It can also be useful for comparison of the functionalities of various protein preparations.

REFERENCE

Harper, W.J. Peltonen, R., and Hayes, J. 1980. Model food systems yield clearer utility evaluation of whey protein. Food Prod. Dev. 14(10): 52.
Ms received 5/9/81; revised 9/25/81; accepted 9/28/81.

A Research Note

An Improved Method for Preparation of Nitric Oxide Myoglobin

AHMAD REZA KAMAREI and MARCUS KAREL

ABSTRACT

We developed an improved method for preparation of bovine nitric oxide myoglobin using the following starting materials: 0.1 mM purified metmyoglobin; 0.1 mM (7 ppm) sodium nitrite; and 1.76 mM (350 ppm) sodium ascorbate. The method requires complete deoxygenation of the reacting system. NOMb prepared in this manner is a source of cured meat pigment which contains a minimum of impurities, since the nitrite reacts quantitatively with the myoglobin in this method.

INTRODUCTION

IN NITRIC OXIDE MYOGLOBIN (NOMb) one molecule of nitric oxide binds, via nitrogen atom, with the sixth coordination site of heme iron. This results in the bright red color of NOMb (Antonini and Brunori, 1971; Smith, 1975; Giddings, 1977; Livingston and Brown, 1981). Heating NOMb denatures the globin moiety, while nitric oxide remains with the heme iron. Consequently, the attractive, relatively stable pink color of cured meat—denatured nitric oxide myoglobin (DNOMb)—is formed (Reith and Szakaly, 1967a, b).

Research on cured meat pigment nitric oxide myoglobin (NOMb) in model systems usually requires preparation of the pigment solution, in high purity. We present an improved method for preparation of NOMb with high purity.

MATERIALS & METHODS

PREPARATION of pure metmyoglobin (metMb), the raw material for NOMb synthesis, is the first step in NOMb preparation. For this purpose, we combined the methods of Hardman et al. (1966) and Awad et al. (1963) with slight modification. Pure NOMb can be synthesized using gaseous nitric oxide. In fact, some workers have suggested industrial applications for NO-curing of meat products (Harper, 1960; Shank, 1965). Our previous work (unpublished) showed that when highly purified NO is bubbled through pure deoxygenated metMb, NOMb is formed by reductive nitrosylation. Similar findings were reported for nitric oxide hemoglobin (Keilin and Hartree, 1937; Chien, 1969). The use of gaseous nitric oxide, however, has limited usefulness because it requires a large excess concentration of NO in solution and head space, which makes the product unsuitable for subsequent studies (Shieh et al., 1979). NOMb can be formed by reduction of nitrite (to nitric oxide) in myoglobin solution. The usual reducing agent used in meat industry is ascorbate and/or isoascorbate.

There is no scientific consensus about the mechanism of NOMb formation with nitrite and ascorbate. It seems however, that dinitrogen trioxide (N_2O_3), as nitrosating agent, is first formed in a rate-determining reaction from two molecules of nitrous acid ($pK_a = 3.4$). N_2O_3 attack on ascorbate forms nitrite ester which breaks down to yield the semiquinone and nitric oxide. Reaction of the semiquinone with an additional mole of N_2O_3 completes the oxidation of ascorbate to dehydroascorbate (Bunton et al., 1959; Dahn et al., 1960; Archer et al., 1975). NO, then reacts with Mb or metMb to form NOMb. The latter is believed to auto reduce with time via internal electronic rearrangement (Giddings, 1977).

Authors Kamarei and Karel are with the Dept. of Nutrition & Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

To synthesize NOMb Fox and Thomson (1963) used 100-fold molar excess of nitrite and ascorbate. Paul and Kumta (1975) found that the optimum condition for NOMb synthesis is 0.05 mM metMb, and 50 mM nitrite and ascorbate at pH 5.5. Presence of very large excesses of nitrite and ascorbate can interfere in subsequent experimental studies and therefore negate, to some degree, the advantages of this method. Removing the extra nitrite and ascorbate by dialysis or gel filtration causes conversion of NOMb to metMb by exposure to air. We attempted, therefore, NOMb synthesis with minimum amounts of nitrite and ascorbate, which do not need to be removed. For this purpose, 104 ml fractions of purified 0.1 mM (A_{505} = 0.97) bovine metMb solution in 0.05M phosphate buffer pH 5.5 (simulating natural pH of beef) were completely deoxygenated with argon in bubbling flasks for 2 h at room temperature. Deoxygenation was found to be a critical condition, as reported by Fox and Ackerman (1968). Traces of cxygen were washed out of argon according to the method of Armor (1970).

To find the minimum required nitrite and ascorbate, we conducted the following experiments:

(a) The weighed fractions of sodium nitrite, each with 550 ppm (2.77 mM) sodium ascorbate, were introduced into the metMb solutions (four-ml samples for zero time were drawn just before this step). The applied concentrations of nitrite were 25 ppm (0.36 mM), 16 ppm (0.23 mM), and 7 ppm (0.1 mM), which is equimolar to metMb. To prevent NO escape and oxygen diffusion into the system, the bubbling rate of Ar was drastically decreased to maintain just a slight positive pressure. This also caused slow, but constant, mixing of solution. Four-ml aliquots, at 1-hr time intervals, were drawn by 5-ml syringes, using 30-cm needles, and immediately filtered (S&S filter paper #595) into cuvettes (1-cm path length) and inserted in a Perkin-Elmer (Hitachi 200) spectrophotometer. Absorption spectra in the 0–2 range were recorded, using 120 nm/min scan speed, for characteristic peaks between 700–460 nm, and Soret band (upon 10 times dilution with buffer) between 460–360 nm with the buffer as the blank.

(b) Keeping the nitrite concentration at minimum, i.e., 7 ppm (0.1 mM), we then tried to minimize the concentration of ascorbate. For this purpose, we used 450 ppm (2.27 mM), 350 ppm (1.76 mM), and 250 ppm (1.26 mM) ascorbate concentration for the NOMb synthesis. The absorption spectra were recorded in the same way as described above.

RESULTS & DISCUSSION

FIGURE 1 represents the typical absorption spectra of the gradual conversion of metMb to NOMb. The solution color also changed gradually, but clearly, from brown (metMb) to bright red (NOMb). The characteristic peaks of metMb (630 and 505 nm) were converted into characteristic peaks of NOMb (578 and 548 nm). A deep minimum wavelength at 508 nm is also characteristic of NOMb. The spectra of this gradual conversion show three isosbestic points at 603, 524, and 482 nm. The maximum wavelength for the Soret band also shifted—from 410 nm (metMb) to 421 nm (NOMb) – just after a sharp decrease in Soret absorption (probably metMb conversion to Mb) and during its gradual increase (NOMb formation). The obtained Soret band characteristics for bovine NOMb is in good agreement with the values obtained by Romberg and Kassner (1979) for horse NOMb.

At a minimum nitrite concentration of 7 ppm (0.1mM), i.e., equimolar to metMb concentration (0.1 mM), there was almost complete conversion in several hours. Becasue we considered avoidance of excess nitrite as necessity for

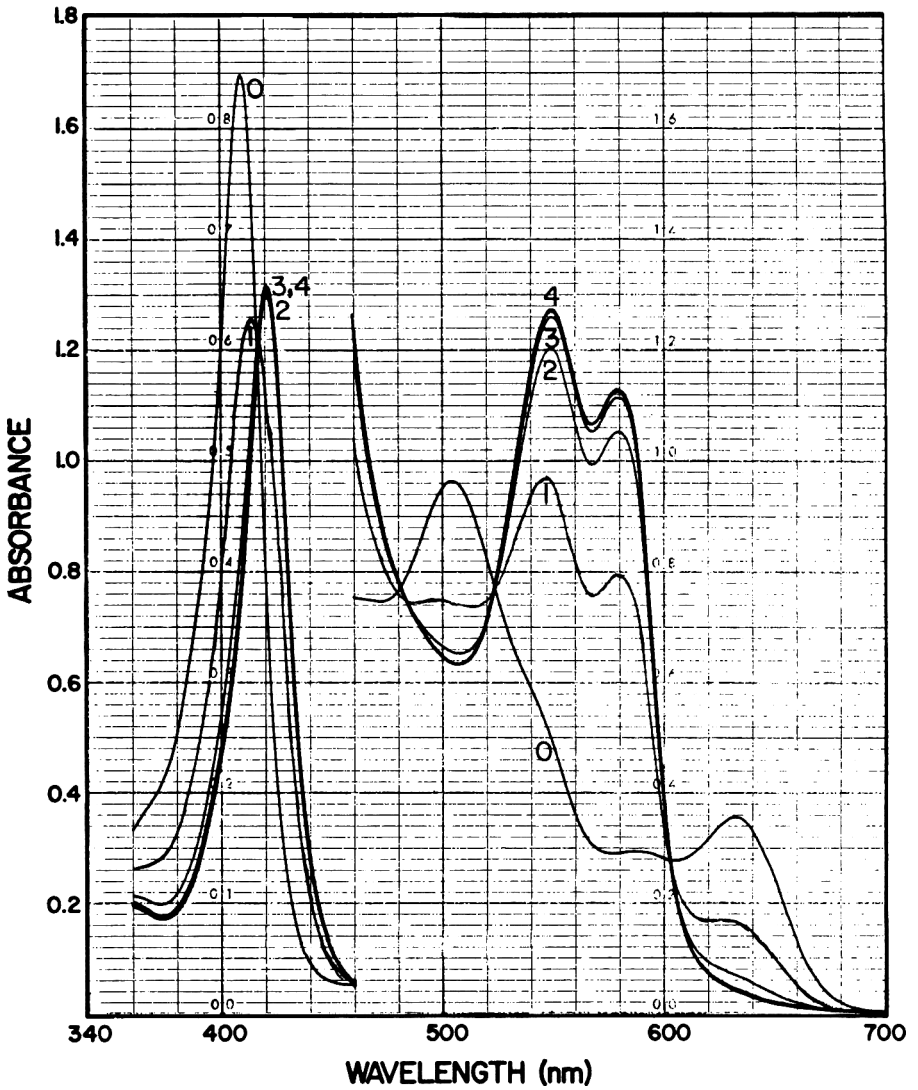


Fig. 1—Typical absorption spectra for preparation of bovine NOMb from 0.1 mM metMb. Spectra recorded in 1-hr intervals (0, 1, 2, 3, . . .) during this conversion. All absorptions, at 700 nm, are suppressed from 0.03 to zero.

Table 1—Molar absorptivities (ϵ x 10^{-3}; M^{-1} cm^{-1}) of bovine metMb and NOMb[a]

Wavelength (nm)	metMb	NOMb
505, β peak	9.7[b]	–
545–548, β peak	–	13.3[b] (13.0[c])
410, Soret (γ) band	170[c]	–
421, Soret (γ) band	–	137[c]

[a] The molar absorptivities of Soret (γ) bands are calculated based on the β peak absorptions of pigments.
[b] Fox and Thomson (1963)
[c] Current study (suppression of 0.03 absorbance unit, to obtain zero at 700 nm, has been taken into account).

studies on NOMb, this equimolar level was selected for nitrite concentration.

Assuming the molar absorptivities shown in Table 1, it was possible to monitor the percentage of NOMb formation as a function of time. The conversion rate from metMb to NOMb decreased sharply as ascorbate concentration (i.e., ratio of ascorbate to nitrite) decreased from 350 to 250 ppm. Consequently, we preferred an ascorbate concentration of 350 ppm (1.76 mM). This is the minimum possible amount of ascorbate, under our experimental conditions, adequate for synthesis in several hours. It is obvious, however, that by lowering the buffer pH from 5.5 the rate of NO formation is accelerated (Tannenbaum, 1976) and ascorbate molar concentration can be reduced theoretically to half that of nitrite, since, for each mole of oxidized ascorbate two moles of NO are produced (Bunton et al., 1959; Archer et al., 1975). This corresponds to 0.05 mM (10 ppm) ascorbate. This is possible only at the expense of deviation from simulated natural pH of beef. The slight excess of ascorbate, due to its reducing role improves NOMb stability.

In conclusion, synthesis of bovine NOMb under complete deoxygenation, using 0.1 mM purified metMb, 0.1 mM (7 ppm) sodium nitrite, and 1.76 mM (350 ppm) sodium ascorbate, at pH 5.5, provides an improved source of NOMb for further studies.

REFERENCES

Antonini, E. and Brunori, M. 1971. "Hemoglobin and Myoglobin and Their Reactions with Ligands." North Holland Publ. Co., Amsterdam.
Archer, M.C., Tannenbaum, S.R., Fan, T., and Weisman, M. 1975. Reaction of nitrite with ascorbate and its relation to nitrosamine formation. J. Natl. Cancer Inst. 54: 1203.
Armor, J.N. 1970. Studies in the reactivity of ruthenium amines. Ph.D. thesis, Stanford University, Stanford, CA.
Awad, E., Camerson, B., and Kotite, L. 1963. Chromatographic separation of hemoglobin and myoglobin on sephadex gel. Nature 198: 1201.
Bunton, C.A., Dahn, H., and Loewe, L. 1959. Oxidation of ascorbic acid and similar reductones by nitrous acid. Nature 183: 163.
Chien, J.C.W. 1969. Reactions of nitric oxide with methemoglobin. J. Amer. Chem. Soc. 91: 2166.
Dahn, H., Loewe, L., and Bunton, C.A. 1960. Uber die oxydation von Ascorbinsaure durch salpetrige saure Teil 6: Ubersicht und diskussion der ergebnisse. Helv. Chim. Acta 43: 320.
Fox, J.B. and Ackerman, S.A. 1968. Formation of nitric oxide myoglobin: Mechanism of the reaction with various reductants. J. Food Sci. 33: 364.
—Continued on page 685

A Research Note

Evaluation of the Quality of Cookies Supplemented with Distillers' Dried Grain Flours

CHO C. TSEN, WILLA EYESTONE, and JEANETTE L. WEBER

ABSTRACT

Distillers' dried grain (DDG) samples collected from various distillers differed in protein, ash, fat, and fiber contents, and colors. When distiller's dried grain flour (DDGF) was mixed with wheat flour at a 15 or 25% (w/w) replacement level for making sugar cookies, the replacement could decrease the width and thickness and darken the color of sugar cookies. DDGF was found suitable as a supplement for preparing dark-colored cookies to enrich their protein and fiber contents. Sensory evaluations of 15% DDGF supplemented bar, spice and chocolate cookies showed that they were all acceptable.

INTRODUCTION

DISTILLER'S DRIED GRAIN (DDG), a distiller's major by-product of alcohol fermentation, has been used widely in feeds. In the fermentation process, most grain starch is converted to alcohol, carbon dioxide, and other fermented products; the remaining nutrients (such as protein, fat, fiber, minerals, and vitamins) undergo almost a threefold concentration, mainly in DDG. Recent interest in the nutritional benefit of protein and fiber justifies exploring use of DDG as a protein and fiber supplement in bakery foods. Although there are several reports on the use of brewers' spent grain in bread and cookies (Prentice and D'Appolonia, 1977; Prentice et al., 1978; Kissell and Prentice, 1979), little information concerning DDG used as a bakery ingredient is available.

Most DDG samples are dark; they could be used as a partial replacement for wheat flour for preparing some dark-colored cookies such as bar, spice, and chocolate-chip cookies. DDG supplements would improve the nutritive value of the cookies by enriching their protein and fiber contents, and expand the use of the by-product from alcohol fermentation. For those reasons we evaluated the quality, acceptability, and taste of DDG-supplemented cookies. The results are reported here.

MATERIALS & METHODS

Materials

DDG samples, one each from six major U.S. distillers were used. Referred to as samples DDG-A, -B, -C, -D, -E, and -F, they were ground through an Alpine pin mill and then sifted once through a 28LW sieve for analyses and other tests. The flours thus obtained were designated as DDGF-A, -B, -C, -D, -E, and -F, respectively. One of the two soft wheat flours (SWF's) purchased for the study was bleached for preparing bar cookies; the other was unbleached for sugar, spice, and chocolate-chip cookies.

All analyses were by AACC standard Methods (1961–1975), except that fat was determined by AOCS Method Aa4-38 (1971) with petroleum ether as the extracting solvent. The colors of flour samples and cookies were evaluated with the Agtron multi-chromatic, abridged, reflectance spectrophotometer model M-500-A (for flour samples) and model M-300-A wide area viewer (for cookies). The instrument was standardized with standard discs M-33 and 00 at 640 nm (red mode) to read 100 and 0, respectively. AACC Approved Method 14-30 (1974) was used to evaluate the flour samples. The top center portion (circle with a diameter of 4.5 cm) of four cookies was exposed for reflectance measurements.

Authors Tsen, Eyestone, and Weber are with the Dept. of Grain Science & Industry, Kansas State Univ., Manhattan, KS 66506.

Preparation of cookies

Sugar cookie samples with wheat flour or wheat flour supplemented with 15% or 25% DDGF (% - replacement level of DDGF for wheat flour), were prepared according to the AACC Method 10-50D (1975). Cookie spread and the spread ratio (W/T - where W is the average diameter and T the average thickness) were measured, as previously reported (Tsen et al., 1975). Formulations for making other cookies from wheat flour and wheat flour supplemented with 15% DDGF are listed in Table 1.

Acceptability evaluation

Acceptability and taste of cookies were evaluated at about 3:00 p.m. by 99 students (untrained judges of both sexes aged 11–12) in classrooms under normal light at an elementary school in Manhattan, KS. Bar cookies were prepared in the morning for the afternoon evaluation. Spice and chocolate-chip cookies were prepared the day before the evaluation. For tasting, each of the randomly selected students was given one cookie of each sample sealed in a plastic bag. Water was provided for rinsing. Students were not told what the cookies were made of and were asked only to rank the cookies from excellent (1) to very poor (6), as described and statistically treated by Larmond (1970).

RESULTS & DISCUSSION

Compositional and color differences of DDGF samples

There were considerable variations in the composition and color among DDGF samples (Table 2). The differences, especially in color, indicate that DDGF samples, obtained from various distillers, were processed from different raw materials or by different processing methods or conditions, particularly drying.

Table 1—Formulations for cookies from wheat flour and wheat flour supplemented with 15% DDGF

Ingredients	Cookies		
	Bar (g)	Spice (g)	Chocolate-chip (g)
Wheat flour or wheat flour-DDGF mixture	170.0	400.0	200.0
Sugar	412.0	100.0	75.0
Dark-brown sugar	–	–	75.0
Shortening	200.0	100.0	56.0
Margarine	–	–	56.0
Salt	3.0	4.0	3.0
Baking powder	5.2	5.0	–
Soda	–	5.0	2.0
Vanilla	9.8	–	1.9
Egg	212.0	50.0	53.0
Corn syrup	40.0	–	–
Cocoa	74.0	–	–
Molasses	–	160.0	–
Cinnamon	–	1.4	–
Ginger	–	2.0	–
Water	–	–	1.0
Chocolate chips	–	–	83.0

Effect of supplementing DDGF on cookie spread and thickness

Supplementing 15 or 25% DDGF reduced the average cookie width from 8.47 cm (regular cookies-the controls) to 8.18 or 8.20 cm and average cookie thickness from 0.88 cm (the controls) to 0.77 or 0.76 cm, respectively. Largely because of the reduction in thickness, the spread ratio was improved by the supplement. But cookies supplemented with DDGF samples all appeared much darker than the control. The color readings varied from 84 Agtron units for 30% DDGF-A supplemented cookies to 30 Agtron units for 30% DDGF-B supplemented cookies, as compared to 100 units for the control.

Table 2—Chemical characteristics and colors of wheat flour and distillers' dried grain flours (DDGF)

	Moisture (%)	Protein[a] (%)	Ash (%)	Fat (%)	Fiber (%)	Color (Agtron unit)
Soft wheat flour (unbleached)	11.7	8.7	0.5	0.96	–	>100
Soft wheat flour (bleached)	11.8	9.0	0.4	0.90	–	>100
DDGF-A	6.7	28.0	2.4	10.9	11.2	85.0
DDGF-B	9.5	26.6	4.3	10.4	7.6	23.5
DDGF-C	11.3	27.7	4.0	8.2	6.8	35.0
DDGF-D	10.1	28.3	4.3	8.0	7.2	3.5
DDGF-E	7.7	26.1	4.4	9.3	6.9	30.5
DDGF-F	8.8	26.9	4.0	7.7	7.5	92.0

[a] Protein content was calculated as (% N X 5.7) for wheat flour and (% N X 6.25) for DDG flour.

Table 3—Taste panel results from bar, spice, and chocolate-chip cookies prepared from wheat flour supplemented with 15% distiller's dried grain flour (DDGF-B or DDGF-F)

	Type of cookies		
DDGF	Bar (mean)[a,b]	Spice (mean)[a,b]	Chocolate chip (mean)[a,b]
O	2.07 C	2.27 B	2.23 B
F	2.45 B	3.32 A	2.37 AB
B	2.72 A	3.58 A	2.65 A

[a] Means without a common letter in each column differ significantly ($P < 0.05$).
[b] More than 4 was not acceptable.

Evaluation of bar, spice, and chocolate-chip cookies supplemented with DDGF samples

In view of the darkness of DDGF samples and sugar cookies supplemented with DDGF samples, it seemed that DDGF would be an appropriate supplement for dark-colored cookies. Studies were thus conducted to evaluate the taste and acceptability of bar, spice, and chocolate-chip cookies made from wheat flour supplemented with 15% DDGF-B or DDGF-F. DDGF-B and DDGF-F were selected to represent dark and light DDG samples respectively for making the cookies. Statistically treated results (Table 3) indicate that bar, spice, and chocolate-chip cookies made from wheat flour supplemented with 15% DDGF-B or -F samples were all acceptable. Although the regular bar and spice cookies tasted significantly better than the supplemented cookies, no significant differences were found between regular chocolate-chip cookies and DDGF-F supplemented ones. DDGF-F sample was significantly better than DDGF-B as a supplement for making bar and chocolate-chip cookies.

REFERENCES

AACC. 1961-1975. "Approved Methods of AACC." Method 08-01, approved April, 1961; Method 44-15A, approved April, 1967; Method 46-11, approved Oct., 1976; Method 54-21, approved April, 1961; Method 32-15 approved April, 1961; Method 14-30 approved Oct., 1974; Method 10-50D approved Feb. 1975. The Association, St. Paul, MN.

AOCS. 1971. "Official and Tentative Methods," 3rd ed. American Oil Chemists' Society, Champaign, IL.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1.

Kissell, L.T. and Prentice, N. 1979. Protein and fiber enrichment of cookie flour with brewers' spent grain. Cereal Chem. 56: 261.

Larmond, E. 1970. Methods for sensory evaluation of food. Publication 1284, Canada Dept. of Agri., Ottawa.

Prentice, N. and D'Appolonia, B.L. 1977. High-fiber bread containing brewer's spent grain. Cereal Chem. 54: 1084.

Prentice, N., Kissel, L.T., Lindsay, R.C., and Yamazaki, W.T. 1978. High-fiber cookies containing brewers' spent grain. Cereal Chem. 55: 712.

Tsen, C.C., Bauck, L.J., and Hoover, W.J. 1975. Using surfactants to improve the quality of cookies made from hard wheat flours. Cereal Chem. 52: 629.

Contribution 81-393-j, Dept. of Grain Science & Industry, Kansas Agricultural Experiment Station, Kansas State Univ., Manhattan, KS 66506.

We thank the Distillers' Feed Research Council for its financial support and Dr. William N. Isgrigg for his helpful suggestions and for collecting samples.

IMPROVED NITRIC OXIDE MYOGLOBIN . . . From page 683

Fox, J.B. and Thomson, J. 1963. Formation of bovine nitrosylmyoglobin. Biochemistry 2: 465.

Giddings, G.G. 1977. The basis of color in muscle foods. J. Food Sci. 42: 288.

Hardman, K.D., Eylar, E.H., Ray, D.K., and Banasak, L.J. 1966. Isolation of sperm whale myoglobin by low temperature fractionation with ethanol and metallic ions. J. Biol. Chem. 241: 432.

Harper, R.H. 1960. Meat curing. U.S. Patent 2,930,703.

Keilin, D. and Hartree, E.F. 1937. Reaction of nitric oxide with hemoglobin and methemoglobin. Nature. 139: 548.

Livingston, D.J. and Brown, W.D. 1981. The chemistry of myoglobin and its reactions. Food Technol., 35(5): 244.

Paul, P. and Kumta, U.S. 1975. Reduced binding of nitric oxide in irradiated horse heart myoglobin. J. Agr. Food Chem. 23(1): 37.

Romberg, R.W. and Kassner, R.J. 1979. Nitric oxide and carbon monoxide equilibria of horse myoglobin and (N-methylimidazole) protoheme. Evidence for steric interaction with the distal residues. Biochemistry 18: 5387.

Reith, J.F. and Szakaly, M. 1967a. Formation and stability of nitric oxide myoglobin. 1. Studies with model systems. J. Food Sci. 32: 188.

Reith, J.R. and Szakaly, M. 1967b. Formation and stability of nitric oxide myoglobin. 2. Studies on meat. J. Food Sci. 32: 194.

Shank, J.L. 1965. Meat curing process. U.S. Patent 3,220,855.

Shieh, J.J., Sellers, R.M., Hoffman, M.Z., and Taub, I.A. 1979. Radiation chemistry of myoglobin, its derivatives, and model compounds in aqueous solution. In: "Radiation Biology and Chemistry," H.E. Edwards, S. Navaratnam, B.J. Parsons and G.O. Phillips, p. 179. Elsevier Scientific Publ. Co., New York.

Smith, K.M. (Ed) 1975. "Poryphyrins and metalloporphyrins." Elsevier Scientific Publ. Co., New York.

Tannenbaum, S.R. 1976. Vitamins and minerals. In "Principles of Food Science," Part I. Food Chemistry, p. 355, O.R. Fennema (Ed.). Marcel Dekker, Inc., New York.

Ms received 10/14/81; accepted 11/7/81.

A Research Note

Electrical Capacitance of Dark-cutting Beef

H. J. SWATLAND, P. WARD, and P. V. TARRANT

ABSTRACT

Electrical capacitance was measured on the exposed adductor muscles of sides of beef. Measurements were made with a 3 mV 100kHz current between two parallel needle electrodes in the meat. Capacitance decreased after slaughter, and was correlated with the pH of the longissimus dorsi muscles in both normal ($r = 0.94$, $P < 0.005$) and in dark-cutting carcasses ($r = 0.89$, $P < 0.01$). The normal decline of pH postmortem was curtailed in carcasses with dark-cutting meat. However, when adjusted for differences in pH, carcasses with dark-cutting meat had a lower ($P < 0.01$) capacitance than carcasses with normal meat.

INTRODUCTION

AN EXCESS OF MUSCLE activity or stress before slaughter may cause cattle to produce dark-cutting beef (Tarrant, 1981). This is due to an antemortem depletion of muscle glycogen and a consequent reduction of postmortem glycolysis. Dark cutting-beef has a high ultimate pH relative to normal beef.

The electrical capacitance of meat is probably due to the separation of electrolytes by the membrane systems of muscle fibers. The capacitance of meat declines postmortem, perhaps with some relationship to the decline in pH postmortem. In pale, soft, exudative (PSE) pork, the rapid rate and extent of pH decline is matched by a rapid decrease and loss of capacitance (Swatland, 1980). The brief study reported here was undertaken to find out if dark-cutting beef with a relatively high pH had normal or elevated capacitance.

MATERIALS & METHODS

DATA on the capacitance of the adductor muscle and the pH of the longissimus dorsi muscle were collected from abattoirs in Dublin and in Guelph. Data on live animals and exact slaughter times were not available and the subject of this research note is restricted to the relationship between capacitance and pH during the period between slaughter and 48-hr postmortem. In Dublin, data were collected from four dark-cutters and from four nearby normal carcasses in a storage cooler approximately 24 hr postmortem and from two carcasses with experimentally induced dark-cutting meat. Dark-cutting was induced by the subcutaneous injection of 50 ml of adrenaline solution (1 mg/L) in the flank and shoulder region at three times prior to slaughter (42, 24, and 18 hr). In Guelph, data were collected from four normal carcasses between slaughter and 48 hr postmortem. The data from the normal carcasses formed a continuous series, regardless of geographical origin.

The capacitance of the adductor muscle was measured on intact sides of beef hanging from an overhead rail. Measurements were made on exposed areas of the muscle, ventral to the pubis, using two parallel stainless steel needle electrodes (axial separation 11 mm, diameter 1.5 mm, penetration 2 cm). The adductor was chosen for capacitance measurements because this muscle can be measured rapidly without damaging the carcass. The eventual objective of research on meat capacitance is to develop a method for industrial use which can predict the properties of distant muscles such as the longissimus dorsi. A 100 kHz sinusoidal alternating current was generated from a battery-powered Wien bridge oscillator with a peak voltage in the meat of 3 mV. A feed-back stabilized amplifier and AC voltmeter were used to find a resistance and a capacitance in parallel which balanced that of the meat. The pH was measured with a Radiometer pH 29 meter which was equipped with a combined glass electrode (GK 2320C) and calibrated with buffers at pH 4.01 and 6.35. The electrode was inserted into the longissimus dorsi at the level of the third lumbar vertebra. A new site was used for subsequent readings on the same muscle.

The data reported here are based on 20 observations on 6 dark-cutters and on 20 observations on eight normal carcasses. The means for each carcass were analyzed with an intraclass regression model.

RESULTS & DISCUSSION

THE CAPACITANCE of the adductor and the pH of the longissimus dorsi were strongly correlated in both normal and dark-cutting carcasses ($r = 0.94$, $P < 0.005$ and $r = 0.89$, $P < 0.01$, respectively). The slopes of the regression lines for the mean values for each carcass were similar (Fig. 1).

—Continued on page 688

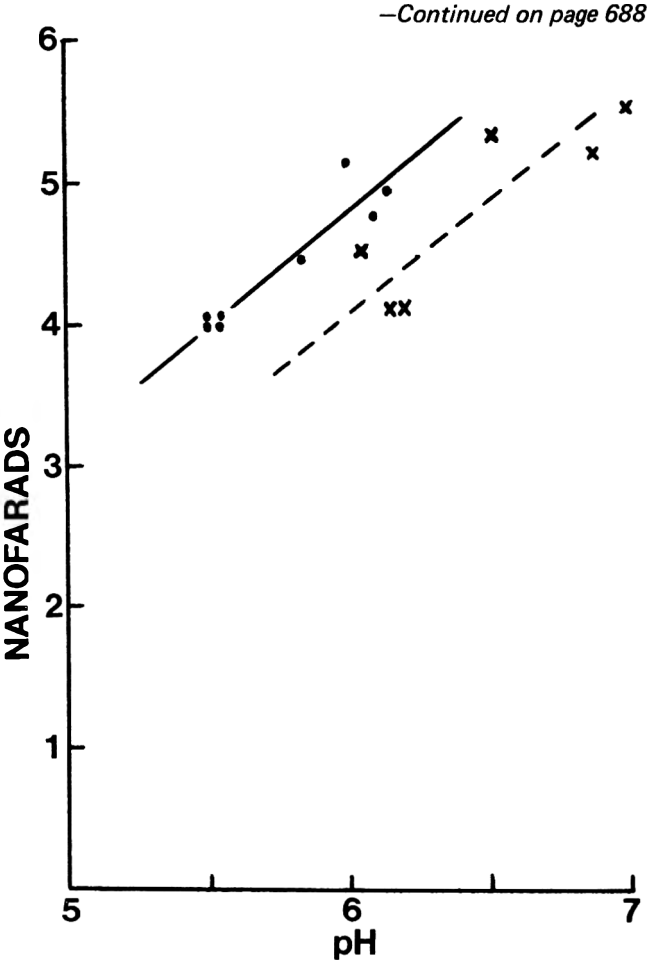


Fig. 1—Relationship between adductor muscle capacitance (nanofarads) and longissimus dorsi pH in normal (•) and dark-cutting carcasses (x). Each data point is a mean of several observations.

Author Swatland is with the Dept. of Animal & Poultry Science, Univ. of Guelph, Guelph, Ontario, N1G2W1, Canada. Authors Ward and Tarrant are with the Agricultural Institute, Dunsinea Research Centre, Castleknock, Co. Dublin, Ireland.

A Research Note

Effect of Electrical Stimulation and Steak Temperature at the Beginning of Cooking on Meat Tenderness and Cooking Loss

R. L. HOSTETLER, T. R. DUTSON, and G. C. SMITH

ABSTRACT

Six steaks from each of 24 loins (from electrically stimulated and control sides of each of 12 steers) were thawed to produce steak temperatures of 2, 7, 13, 14, 18 or 26°C just prior to cooking. After cooking to 70°C, steaks with a starting temperature of 26°C were 30% more tender than those with a starting temperature of 2°C. Electrical stimulation increased tenderness of steaks from all treatments (an average of 23%), and no interaction was found between electrical stimulation and starting steak temperature. Tenderness and cooking loss can be optimized by electrically stimulating carcasses and by starting cookery when steaks have high internal temperatures.

INTRODUCTION

THE EFFECT OF HEAT on the physical properties of meat, particularly tenderness, has been of interest to meat scientists for many years. Cooking of steaks to different final temperature has been shown to have a definite effect on tenderness and cooking loss of meat (Hostetler et al., 1976). However, the time it takes a steak to reach the same final temperature also has been shown to affect meat tenderness and cooking loss (Cover, 1937). Vail et al. (1943) reported that roasts thawed at oven temperature were less tender than those thawed at room temperature. Moody et al. (1978), using four methods of thawing and two methods of cooking, found that steaks and roasts thawed at room temperature were more tender, as measured by sensory panel and shear, than those thawed in a refrigerator at 3.3°C. Room temperature thawed roasts were also more tender than those placed in the oven while frozen or those defrosted and cooked in a microwave oven (Radarange). These studies suggest that the temperature of meat samples at the time cooking starts may have an influence on the tenderness of the cooked product.

The present study was conducted to determine if internal temperature of steaks at the start of cooking has an effect on cooked steak tenderness and cooking loss, and what influence electrical stimulation might have in conjunction with these parameters. Electrical stimulation (ES) has been shown to be an effective means of increasing the tenderness of meat (McKeith et al., 1981), but the relationship between ES and internal steak temperature prior to cooking with respect to tenderness, cooking loss and cooking time has yet to be defined.

EXPERIMENTAL

TWELVE ANIMALS were used in this study. One side of each carcass was electrically stimulated with 550 volts of AC current. Current was applied intermittently (2 sec on and 1 sec off) for 1 min using the Koch-Britton Stimulator, Model 350. The unstimulated side served as the control.

Loins were removed from both sides at 7 days postmortem, and six steaks (3 cm thick) were removed from each loin. These steaks were frozen and randomly assigned to one of six thawing groups. When thawed, the steaks had an internal temperature just prior to cooking of 2, 7, 13, 14, 18, or 26°C. The 13 and 14°C temperature groups were combined since no differences existed between the two groups.

Each of the steaks was then cooked to an internal temperature of 70°C using micro-thermocouples to monitor internal temperature according to the procedure of Hostetler and Dutson (1977). Cooking losses and cooking times were measured and steaks were allowed to cool to room temperature. At least eight cores of 1.25 cm diameter were taken from each cooked steak and shear force measurements were recorded using a Warner-Bratzler shear device.

Data were analyzed by analysis of variance and significance between means was determined using Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE TWO TREATMENTS involved in this study were starting internal temperature (2, 7, 14, 18 and 26°C), and electrical stimulation (stimulated and control). For the parameters measured (cooking times, cooking losses, and shear force values) there were no significant ($P > 0.05$) interactions between the two treatments. For this reason, the two tables presented give means for main effects only.

Cooking times (Table 1) among the various starting temperatures were different from each other except for two of the intermediate starting temperatures (14 and 18°C). The relationship of cooking time to starting internal temperature was such that a lower starting temperature resulted in a longer cooking time and vice versa. This is to be expected, since a starting internal temperature of 26°C is 37% of the change in temperature from 0 to 70°C, while 2°C is only 3% of the same temperature change. Each ten degree increase in starting internal temperature, up to room temperature (26°C), decreased the cooking time for each steak about 5 min. Electrical stimulation had no effect on cooking times (Table 2).

Cooking losses (Table 1) among starting internal temperature groups were different, again with the exception of the 14 and 18°C groups. Cooking loss was a function of cooking time, with each minute of cooking time resulting in an additional percentage point of cooking loss. Since thaw loss was not measured, the total loss could not be determined. This could explain the significantly lower ($P < 0.05$) cooking loss of 1.5% for the stimulated samples

Table 1—Cooking and shear parameters of steaks having different starting internal temperatures

Starting internal temp (C°)	Cooking times (min)	Cooking losses (%)	Shear force (kg)
2[a]	22[a]	27[a]	7.7[a]
7[b]	19[b]	24[b]	7.7[a]
14[c]	15[c]	20[c]	5.9[ab]
18[d]	14[c]	20[c]	6.3[ab]
26[e]	11[d]	18[d]	5.0[b]

abcde Means in the same column bearing a common superscript letter are not different ($P > 0.05$)

Authors Hostetler, Dutson and Smith are with the Meats & Muscle Biology Section, Dept. of Animal Science, Texas A&M Univ., College Station, TX 77843.

Table 2—Cooking and Warner-Bratzler shear parameters for electrically stimulated and unstimulated (control) steaks

Stimulation treatment	Starting internal temp (C°)	Cooking times (min)	Cooking losses (%)	Shear force (kg)
Unstimulated (Control)	13.6[a]	16.1[a]	22.1[a]	7.3[a]
Electrically stimulated	13.2[a]	16.1[a]	20.7[b]	5.4[b]

[ab] Means in the same column bearing a common superscript letter are not different ($P > 0.05$)

as compared to the unstimulated control samples. However, it is possible that, due to changes affected by electrical stimulation, more protein binding sites are available, resulting in more binding of water.

The effect of starting internal temperature on tenderness as measured by Warner-Bratzler shear force is presented in Table 1. Each four degree increase in starting internal temperature resulted in a decrease of approximately 0.5 kg in shear force. Thus, increasing the starting internal temperature from 2°C to 26°C (a total of 24°C) reduced shear force values by 2.72 kg. This is a sufficient decrease in shear force to be readily detected by a sensory panel as an improvement in tenderness. Electrical stimulation decreased shear force values at all starting internal temperatures (Table 2).

It has been shown (Hostetler et al., 1976) that cooking the longissimus muscle beyond 61°C tends to decrease tenderness, rather than improving tenderness. It is possible that, by increasing the starting internal temperature, steak from this muscle could be cooked to a higher final internal temperature (well done) without adversely affecting tenderness. Other muscles may respond similarly; however, more research is needed to determine the effect of internal temperature at the start of cooking on tenderness of different muscles.

REFERENCES

Cover, S. 1937. The effect of temperature and time of cooking on the tenderness of roasts. Texas Agric. Expt. Sta. Bull. 542.

Hostetler, R.L., Dutson, T.R., and Carpenter, Z.L. 1976. Effect of varying final internal temperature on shear values and sensory scores of muscles from carcasses suspended by two methods. J. Food Sci. 41: 421.

Hostetler, R.L. and Dutson, T.R. 1977. Effect of thermocouple wire size on the cooking time of meat samples. J. Food Sci. 42: 845.

McKeith, F.K., Smith, G.C., Savell, J.W., Dutson, T.R., Carpenter, Z.L., and Hammons, D.R. 1981. Effects of certain electrical stimulation parameters on quality and palatability of beef. J. Food Sci. 46: 13.

Moody, W.G., Bedau, C., and Langlois, B.E. 1978. Effect of thawing and cookery methods, time in storage and breed on the microbiology and palatability of beef cuts. J. Food Sci. 43: 834.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., New York, NY.

Vail, G.E., Jeffery, M., Forney, H., and Wiley, C. 1943. Effect of method of thawing upon losses, shear and press fluid of frozen beef steaks and pork roasts. Food Res. 8: 337.

Ms received 7/13/81; accepted 9/18/81.

T.A. 17003 of the Texas Agricultural Experiment Station. This research was funded in part by grants from the King Ranch Inc., Kingsville, TX and LeFiell Company, San Francisco, CA.

DARK-CUTTING BEEF . . . From page 686

However, when data were adjusted for differences in the pH of the longissimus dorsi, the adductor muscles of dark-cutting carcasses were found to have a lower capacitance than those of normal carcasses ($P < 0.01$).

The study produced an unexpected result since meat from dark-cutters had a low capacitance instead of a high or normal capacitance. The capacitance of meat is of interest for two reasons. Firstly, correlations between meat quality and capacitance might allow the development of electronic apparatus for the grading of meat quality. Secondly, an understanding of biophysical changes during post-mortem metabolism might help to explain the sources of variation in pH-dependent aspects of meat quality. From the first viewpoint, the results were discouraging, since it would be difficult to use low capacitance to detect dark-cutting carcasses. However, from the second viewpoint, the results are rather interesting since they show that capacitance changes are not a simple consequence of lactate-induced membrane damage.

In earlier studies on pork, lactate-induced membrane damage seemed to be a reasonable explanation of the correlation between capacitance and pH. However, recent work on intermuscular variation in the physical properties of pork (Swatland, 1982) has indicated that the statistical correlation between capacitance and pH might be based on an indirect relationship. The decline of capacitance and the rate of glycolysis might both be determined by a third factor, perhaps the metabolism of adenosine triphosphate (ATP). The results reported here give some support to this idea. The early absence of ATP is a feature which would be expected in both PSE pork, with a rapid rate of glycolysis, and in dark-cutting beef with a reduction in the extent of glycolysis. This might explain why, despite extreme differences in pH, both PSE pork and dark-cutting beef have a low capacitance relative to normal meat.

REFERENCES

Swatland, H.J. 1980. Postmortem changes in electrical capacitance and resistivity of pork. J. Anim. Sci. 51: 1108.

Swatland, H.J. 1982. Intermuscular variation in physical properties of pork. Can. Inst. Food Sci. Technol. J. (In press).

Tarrant, P.V. 1981. The occurrence, causes and economic consequences of dark-cutting in beef—a survey of current information. In "Current Topics In Veterinary Medicine and Animal Science," Vol. 10, Ed. Hood, D.E. and Tarrant, P.V. Martinus Nijhoff, The Hague.

Ms received 10/9/81; accepted 11/7/81.

A Research Note

Bread Compressibility as Affected by Slice Thickness

K. LORENZ and W. DILSAVER

ABSTRACT

White bread (pup loaves and 1-lb loaves) were wrapped in moisture-proof bags, which were sealed, and stored at 10°, 25°, and 50°C for 1 and 3 days, respectively. Compressibility measurements were made on 1, 1.5, and 2.5 cm thick slices of these bread with the Baker compressimeter. The temperature of storage and slice thickness affected compressibility values.

INTRODUCTION

THE SOFTNESS of a baked product is a very important quality characteristic. Laboratory measurements of the softness of breads and cakes are usually conducted with the Baker compressimeter (Platt and Powers, 1940; Noznick and Geddes, 1943; Crossland and Favor, 1950; Roewe-Smith et al., 1982). The American Association of Cereal Chemists has developed an official method for softness measurements using this instrument (AACC, 1969).

Softness or compressibility of a baked product varies with the product formulation, including the selection of an appropriate crumb softener (Stutz et al., 1973; Tenney, 1978), the length of storage of the product (Short and Roberts, 1971), the temperature of storage (Lorenz et al., 1982) and the position in a baked product where the measurement is made (Short and Roberts, 1971).

In previous studies, investigators have cut their breads or cakes into slices of various thickness for compressibility determinations. This makes comparisons of data from different studies very difficult, if not impossible, because slice thickness can influence compressibility readings as was shown in this study.

EXPERIMENTAL

THE PROXIMATE COMPOSITION of the flour and the bread formulation used in this study are given in Table 1. The breads were baked by the straight dough procedure. The doughs were mixed in a Hobart A-120 mixer with bowl and dough hook and fermented at 85°F and 85% R.H. for 1½ hr. Scaling weight was 500g per loaf for 1-lb loaves and 200g per loaf for pup loaves. After a 10 min floortime the loaves were mechanically molded and proofed at 100°F and 95% R.H. to 1 inch above the pan. Baking time at 425°F was 20 min for 1-lb loaves and 18 min for pup loaves.

Bread volume was measured by rapeseed displacement 1 hr after baking. Breads were wrapped in moisture-proof bags, which were sealed, and stored at 10°, 25°, and 50°C for 1 and 3 days, respectively, for softness determinations.

Bread softness (g force, 0.5 mm compression) was measured with the Baker Compressimeter using 1 cm, 1.5 cm and 2.5 cm thick slices. A 1-lb loaf provided either 17 1-cm thick slices, 13 1.5-cm thick slices or 7 2.5-cm thick slices. The number of slices from the pup loaves was 9, 6 and 4 for a slice thickness of 1 cm, 1.5 cm and 2.5 cm, respectively. Three softness measurements were made on each slice. Average softness values are based on duplicate bakes made on separate days.

The data were analyzed statistically, by computing an analysis of variance.

Authors Lorenz and Dilsaver are with the Dept. of Food Science & Nutrition, Colorado State Univ., Fort Collins, CO 80523.

RESULTS & DISCUSSION

Pound loaves

The temperature of storage produced considerable differences in compressibility. The higher the storage temperature the softer the bread. This confirms previous results by Roewe-Smith et al. (1981).

Slice thickness affected compressibility value. Slice thicknesses of 1 and 1.5 cm produced essentially the same compressibility values after storage of the bread for 1 day at 10° and 25°C, respectively. Compressibility values were significantly (α = 0.05) lower, however, when measurements were made on 2.5 cm thick slices. Since a lower compressibility value generally indicates greater softness, these 2.5 cm thick slices appear softer than the 1 or 1.5 cm thick slices. There should, however, not have been differences in bread softness. All the loaves came from the same batch and were processed identically. The selection of loaves for cutting into slices of a specific thickness was made at random (Table 2).

When 1 lb. loaves were stored for 1 and 3 days at 50°C, no differences in compressibility values were obtained when slice thickness was varied from 1–2.5 cm. Three-day storage of the 1-lb loaves at 10° and 25°C produced significant (α = 0.05) differences in compressibility of bread slices 1,1.5 and 2.5 cm thick. *–Continued on page 691*

Table 1–Bread formulation and proximate composition of flour.

Formulation		Flour analysis	
Ingredient	%	Component	%[a]
flour	100	Ash	0.50
water	as required	Crude fat	1.01
yeast	2.5	Nitrogen	1.96
yeast food	0.5	Protein	11.17
salt	2.0		
sugar	6.0		
nonfat dry milk	2.0		
shortening	3.0		
calcium propionate	0.3	Protein = N x 5.7	

[a] On 14% moisture basis

Table 2–Bread compressibility (g force, 0.5 mm compression)

Storage (days)	Storage temp (°C)	Lb loaves Slice thickness (cm) 1	1.5	2.5	Pup loaves Slice thickness (cm) 1	1.5	2.5
1	10	6.8	6.6	4.5	9.3	6.9	5.6
	25	4.6	4.4	3.4	6.2	4.1	3.7
	50	3.5	3.5	3.3	4.1	4.5	3.1
3	10	10.7	9.7	6.2	9.7	7.0	6.6
	25	8.1	7.4	4.8	10.8	7.0	5.8
	50	4.1	4.2	4.2	7.0	6.5	5.6

Average specific volume: 1-lb loaves = 5.14 cc/g
pup loaves = 4.61 cc/g

A Research Note

Bacterial Penetration of Muscle Tissue

C. O. GILL and N. PENNEY

ABSTRACT

Bacterial penetration of unprocessed post-rigor muscle tissue requires proteolytic degradation of the material between the muscle fibre and the fibrous layers of the surrounding endomysium.

INTRODUCTION

PREVIOUS OBSERVATIONS indicated that bacteria penetrate into muscle tissue by passing between muscle fibres only when they produce proteolytic enzymes (Gill and Penney, 1977). Both theoretical considerations and their own observations led Sikes and Maxcy (1980) to question our findings. We have therefore compared the methods used in the two studies to try to resolve the discrepancy between them.

MATERIALS & METHODS

CULTURES OF *Pseudomonas fluorescens*, NCIC 9053 (proteolytic), *P. fluorescens*, NCIC 8865 (nonproteolytic) and *Serratia marcescens*, NCCT 1377 (proteolytic) were maintained on nutrient agar and grown in shake culture in nutrient broth.

Samples of muscle tissue were prepared and inoculated as previously described by imbedding 2 cm^3 cubes in agar (Gill and Penney, 1977) or by painting with collodion (Sikes and Maxcy, 1980). Samples subjected to freezing were exposed to three cycles of freezing (−18°C) and thawing before preparation. Cooked samples (2 cm^3) were heated in foil for 15 min at 200°C. All inoculated samples were incubated overnight at 30°C. Micrographs were obtained from sections of meat samples which had been stained and embedded in epoxy resin as previously described (Gill and Penney, 1977).

RESULTS & DISCUSSION

ALL THREE ORGANISMS were present on the uninoculated surfaces of collodion-coated samples after overnight incubation. With agar imbedding this only occurred in blocks inoculated with the proteolytic *P. fluorescens* and *S. marcescens* while the nonproteolytic *P. fluorescens* was still confined to the inoculated surface after 4 days. This clearly confirmed the inability of the nonproteolytic organism to pass through meat and demonstrated that collodion coating did not prevent bacterial movement over the treated surfaces. Since such movement must be prevented when examining bacterial penetration, the technique was obviously inadequate and for subsequent experiments all samples were sealed in agar.

All three organisms penetrated cooked meat, an unsurprising result considering the visible gaps that appeared in cooked samples. However, frozen-thawed samples gave identical results to unfrozen samples. The claim of Sikes and Maxcy (1980) that freezing and thawing enhances penetration was therefore not substantiated, while the obvious destruction of tissue structure by cooking or mincing means that all their experimentation using such materials must be irrelevant to the problem of how bacteria penetrate unprocessed muscle.

Authors Gill and Penney are with the Meat Industry Research Institute of New Zealand (Inc.), P.O. Box 617, Hamilton, New Zealand.

Microscopic examination of muscle blocks penetrated by proteolytic bacteria showed that the organisms were usually confined between the muscle fibers and the surrounding endomysium. Neither of these structures appeared to have undergone any degradation, but in some sections bacteria were observed to lie within the endomysium (Fig. 1). The same appearance was presented by samples irrespective of whether the meat fibers were running horizontally or vertically to the direction of penetration.

The endomysium is a fine sheath of collagenous material surrounding each muscle fiber. We had suggested that disruption of this structure by bacterial proteases was necessary

Fig. 1—Muscle tissue penetrated by (a) a proteolytic strain of Pseudomonas fluorescens and (b) Serratia marcescens. Note the bacteria lying between the opposed layers of collagen at the top of (b), and the lack of obvious structural damage to either the muscle fibers or the organized components of the endomysium. Magnification, X1600.

for penetration (Gill and Penney, 1977) but Sikes and Maxcy (1980) pointed out that bacterial proteases have little or no collagenolytic activity. However, the endomysium is essentially the basement membrane of the muscle cells. It is composed of an amorphous ground substance containing fine collagenous fibres and is continuous between adjacent muscle cells (Gould, 1973). The ground substance is composed of cross-linked molecules of collagen and glycoprotein, but the collagen molecules contain nonhelical regions that are readily susceptible to proteolytic attack (Spiro, 1972; Kefalides, 1975). The micrographs (Fig. 1) indicate that the ground substance is degraded but that the fine layers of collagen fibers bounding the visible endomysial structure are refractory to proteolytic attack. The preferred area for invasion appears to be between the muscle fibres and the collagen fiber layers. This area could include a fiber free zone of the endomysium, the cell membrane and some muscle cell protoplasm. Unfortunately this region has been little studied in post-rigor muscle, and such studies would seem to be necessary for a full understanding of how bacteria penetrate into unprocessed muscle tissue.

REFERENCES

Gill, C.O. and Penney, N. 1977. Penetration of bacteria into meat. Appl. Environ. Microbiol. 33: 284.

Gould, R.P. 1973. The microanatomy of muscle. In "The Structure and Function of Muscle," Vol. 2, Ed. Bourne, G.H. Academic Press, New York.

Kefalides, N.A. 1975. Basement membranes: structural and biosynthetic considerations. J. Invest. Determatol. 65: 85.

Sikes, A. and Maxcy, R.B. 1980. Postmortem invasion of muscle food by a proteolytic bacterium, J. Food Sci. 45: 293.

Spiro, R.G. 1972. Basement membranes and collagens. In "Glycoproteins, Their Composition, Structure and Function," Vol. 5, Part B, Ed. Gottschalk, a. Elsevier, Amsterdam.

Ms received 8/29/81; accepted 9/28/81.

BREAD COMPRESSIBILITY AND SLICE THICKNESS . . . From page 689

Pup loaves

One- and three-day storage at 50°C produced no significant differences in compressibility when slice thickness was 1 or 1.5 cm. Compressibility was lower, however, when the bread slices were cut 2.5 cm thick.

Compressibility of bread was significantly (α = 0.05) lower when slice thickness was 1.5 cm compared to only 1 cm at storage temperatures of 10° and 25°C and storage times of 1 and 3 days, respectively. Differences in compressibility values were insignificant comparing 1.5 and 2.5 cm thick slices after 1 day at 25°C and after 3 days at 10°C (Table 2).

CONCLUSIONS

BAKER COMPRESSIMETER VALUES are affected by the thickness of the bread slice cut for a softness determination. For meaningful comparisons of softness data the thickness of slices of the product needs to be the same.

REFERENCES

AACC. 1969. "Approved Methods" (Method 74-10). America Association of Cereal Chemists, St. Paul, MN.

Crossland, L.B. and Favor, H.H. 1950. A study of the effects of various techniques on the measurement of the firmness of bread by the Baker Compressimeter, Cereal Chem. 27: 15.

Lorenz, K., Dilsaver, W., and Kulp, K. 1982. Comparative efficiencies of bread crumb softeners at various bread storage temperatures. J. Food Sci. - submitted.

Noznick, P.O. and Geddes, W.F. 1943. Application of the Baker Compressimeter to cake studies. Cereal Chem. 20: 463.

Platt, W. and Powers, R. 1940. Compressibility of bread crumb. Cereal Chem. 17: 601.

Roewe-Smith, P., Lorenz, K., and Kulp, K. 1982. Staling of variety breads. J. Food Sci. - submitted.

Short, A.L. and Roberts, E.A. 1971. Pattern of firmness within a bread loaf. J. Sci. Fd. Agric. 22: 470.

Stutz, R.L., Del Vecchio, A.J., and Tenney, F.J. 1973. The role of emulsifiers and dough conditioners in foods. Food Prod. Dev. 7(8): 52.

Tenney, R.J. 1978. Dough conditioner and bread softeners. The surfactants used in breadbaking. Bakers Dig. 52(4): 24.

Ms received 9/4/81; accepted 10/15/81.

A Research Note

Reduction in Free Fatty Acids Due to Parboiling of Paddy

S. ANTHONI RAJ and K. SINGARAVADIVEL

ABSTRACT

Free fatty acid (FFA) content of bran decreased due to parboiling of paddy. The reduction in the FFA was relatively more marked in paddy with higher FFA level and in fungal infected paddy. Among the various parboiling methods, pressure parboiling reduced the FFA to a larger extent. Steaming operation in the process of parboiling might have reduced the FFA.

INTRODUCTION

PARBOILING of paddy prior to milling is a common practice adopted commercially which improves the milling and cooking characteristic of paddy (Desikachar et al., 1969). However, in the course of parboiling, losses occur in the form of sugars or soluble carbohydrates, proteins and phenolic compounds present in the grains as well as those formed during soaking, which contribute to the loss of dry matter (Subrahmanyan and Dakshnamurthy, 1977; Singaravadivel et al., 1978; Anthoni Raj and Singaravadivel, 1979). The lower levels of the free fatty acid (FFA) content observed in parboiled bran just after milling, compared to the raw bran of the same lot of paddy, suggested a possible reduction in the FFA in the process of parboiling. Hence a study was undertaken to assess the extent of reduction in FFA due to various parboiling techniques adopted commercially in normal and high-moisture fungal-infected paddy.

MATERIALS & METHODS

SAMPLES of two paddy varieties, namely Co 25 and IR 20, were parboiled by pressure parboiling, hot soaking, and conventional single steaming methods. Pressure parboiling was done by rinsing the paddy with water for 5 min followed by steaming at 5 lb (0.3515 kg/cm^2) pressure for 20 min and then at 25 lb (1.7575 kg/cm^2) for 5 min. In the hot soaking method paddy was soaked in water at 65–70°C for 4 hr, the water drained and the paddy open-steamed for 10 min. In single steaming (cold soaking) paddy was soaked in cold water for 72 hr and then open-steamed for 10 min. The samples were quickly dried under air, shelled in a rubber roll sheller and milled in a McGill Miller No. 1 to 5% (± 0.1%) polish. The oil was extracted from the raw and parboiled bran with hexane immediately after milling and the FFA content determined by titrating against standard alkali (Karon and Altschul, 1944).

RESULTS & DISCUSSION

A REDUCTION in the FFA level in rice bran oil due to parboiling in general was observed, irrespective of the methods adopted. The amount of reduction was 0.20–0.71% and this varied with the initial FFA content in raw paddy (Table 1). The pressure parboiling of Co 25 and IR 20 paddy reduced the FFA in raw bran from 1.57% to 1.29% and 3.62% to 2.91%, respectively. A reduction of 0.20% and 0.41% in hot soaking and 0.25% and 0.51% in cold soaking methods was recorded for Co 25 and IR 20 paddy, respectively.

Authors Anthoni Raj and Singaravadivel are affiliated with the Paddy Processing Research Centre, Tamil Nadu Agricultural Univ., Tiruvarur, 610001, India.

High moisture paddy harvested during inclement weather show an increase in the FFA content in bran unless dried immediately. A similar increase was also observed whenever parboiled paddy was infected with fungi due to inadequate drying in humid, rainy weather. When such paddy lots (Co 25) were pressure parboiled there was a greater reduction in the FFA content. Mere open steaming for 10 min reduced the FFA content in bran oil from 20.17% to 15.33% while pressure parboiling reduced it to 7.60% (Table 2). However, the washing of adhering fungal spores reduced the FFA content by only 1.27%. A similar reduction was also observed in open steaming and pressure parboiling of fungal-infected Co 25 parboiled paddy. The practice of reprocessing the infected parboiled paddy spoiled by moulds, which offers a reduction in the FFA content in bran oil, is a corallary to the present finding.

The results clearly revealed that a reduction in the FFA content is brought about by parboiling. Kumaresan and Sreeramulu (1978) also reported a reduction in the FFA due to parboiling. While the control of FFA increase in storage of parboiled bran might be due to the inactivation of lipase during parboiling, as reported by Shaheen et al. (1975), the initial low FFA content in parboiled bran compared to the raw bran is solely due to the loss in the heating process of parboiling. The decrease in FFA during parboiling might be due to (1) volatalization during steaming; (2) degradation of unsaturated fatty acids; and/or (3) oxidation of fatty acids to carbonyl compounds, forming complexes with amylose. The presence of free fatty acids and other volatile acids in the steam distillate of bran re-

Table 1—Effect of different parboiling treatments on the free fatty acid content in rice bran oil

	Co 25 paddy[a]		IR 20 paddy[a]	
Method of parboiling	FFA content (%)	Reduction n FFA (%)	FFA content (%)	Reduction in FFA (%)
Raw	1.57	–	3.62	–
Pressure parboiled	1.29	0.28	2.91	0.71
Hot soaking	1.37	0.20	3.21	0.41
Cold soaking	1.32	0.25	3.11	0.51

[a] Average 3 experiments

Table 2—Effect of different parboiling treatments on the free fatty acid content in rice bran oil

	Raw paddy[a]		IR 20 paddy[a]	
Method of parboiling	FFA content (%)	Reduction in FFA (%)	FFA content (%)	Reduction in FFA (%)
Initial	20.17	–	30.44	–
Water washing	18.90	1.27	–	–
10 min steamed	15.33	4.84	22.75	7.69
Pressure parboiled	7.60	12.57	18.61	11.83

[a] Average 3 experiments

ported by Fujimaki et al. (1977) also indicates such a loss might occur when grains were steamed for parboiling. Free fatty acids were known to complex with amylose and becomes solvent-insoluble (Barber, 1972; Bolling et al., 1978). The loss of FFA component suggests that other volatile components of paddy might also get lost in parboiling and contribute partly to the dry matter loss encountered in parboiling. Loss in dry matter during steaming was also suggested earlier (Vellanki et al., 1977; Singaravadivel et al., 1978). Unlike the losses in the other constituents, the loss in FFA is a desirable feature of parboiling as this loss improves the quality of the bran.

REFERENCES

Anthoni Raj, S. and Singaravadivel, K. 1979. Influence of soaking and steaming on the loss of simpler constituents in paddy. J. Fd. Sci. Technol. 17: 141.

Barber, S. 1972. Milled rice and changes during aging. In "Rice: Chemistry and Technology," Ed. D.F. Houston, p. 215. Am. Assoc. of Cereal Chemists, St. Paul, MN.

Bolling, H., Hampel, G., and El Baya, A.W. 1978. Studies on Storage of milled rice for a long period. Fd. Chem. 3: 17.

Desikachar, H.S.R., Sowbhagya, C.M., Viraktamath, C.S., Indudhara Swamy, Y.M., and Bhashyam, M.K. 1969. Steaming of paddy for improved culinary, milling and storage properties. J. Fd. Sci. Technol. 6: 1.

Fujimaki, M., Tsugita, P., and Kurata, P. 1977. Fractionation and identification of volatile acids and phenols in steam distillate of rice bran. Agr. Biol. Chem. 41: 1721.

Karon, M.L. and Altschul, A.M. 1944. Effect of moisture and effect of pretreatments with acid and alkali on the rate of formation of free fatty acids in stored cotton seed. Plant Physiol. 19: 310.

Kumaresan, K. and Sreeramulu, U.S. 1978. Factors influencing the quantity and quality of rice bran oil. Il Riso 27: 341.

Shaheen, A.B., El Dash, A.A., and El Shirbeeny, A.E. 1975. Effect of parboiling rice on the rate of lipid hydrolysis and deterioration of rice bran. Cereal Chem. 52: 1.

Singaravadivel, K., Anthoni Raj, S., and Iengar, N.G.C. 1978. Loss of dry matter in parboiling of paddy. RPEC Reporter 4: 66.

Subramanyan, V. and Dhakshinamurthy, A. 1977. Nutritive losses during parboiling of rice. Il Riso, 26: 337.

Vellanki, R.R.V., Velupillai, L., Ramalingam, J., and Wikramanayake, W.E.A. 1977. A continuous steaming process for parboiling of paddy. Research Bull. 6/77, Rice Processing Development Centre, Anuradhapura, Sri Lanka, p. 14.

Ms received 7/27/81; accepted 9/15/81.

The authors are grateful to Mr. A.N. Dyaneswaran, Project Head, Paddy Processing Research Centre, Tiruvarur, for his keen interest in the studies.

ERRATA NOTICE

•*J. Food Sci.* 46(5): 1389–1393 (1981). Investigations on winged bean [*Psophocarpus tetragonolobus* (L.) DC] proteins and antinutritional factors by S.K. Sathe and D.K. Salunkhe. Under the subtitle "Polyacrylamide gel electrophoresis" on page 1390, the last line reads ". . . 19% (v/v) acetic acid." Please correct to read ". . . 10% (v/v) acetic acid."

•*J. Food Sci.* 46(5): 1557–1559 (1981). Changes in chlorophyll and pectin after storage and canning of kiwifruit by G.L. Robertson and D. Swinburne. Two of the columns in Table 2, page 1558, are incorrect. Please substitute the following corrected table:

Table 2–Chlorophyll and pheophytin pigments in fresh and canned kiwifruit

Sample		Total chlorophyll (mg/kg)	Chlorophyll a conversion (%)	Chlorophyll b conversion (%)	Total pheophytin (mg/kg)
A	F[a]	16.39	38	57	17.39
	C[b]	2.47	88	91	19.87
B	F	16.35	36	67	17.90
	C	1.87	91	91	18.67
C	F	16.53	28	71	18.67
	C	0.69	72	97	21.56
D	F	16.59	23	64	12.66
	C	1.63	89	86	25.58
E	F	15.89	39	56	13.90
	C	3.27	90	91	31.02

[a] F, fresh fruit
[b] C, canned slices

International Food Information Service
—Information Retrieval Services

What Is IFIS?

The International Food Information Service (IFIS) was created in 1968 to provide a number of services related to the retrieval of information from the literature of food science and technology.

IFIS's sponsors include the Commonwealth Agricultural Bureaux in England, the Gesellschaft für Information und Dokumentation in the Federal Republic of Germany, the Institute of Food Technologists in the U.S., and the Centrum voor Landbouwpublikaties in Landbouwdocumentatie in the Netherlands.

Primary Services

Food Science and Technology Abstracts (FSTA)

- *FSTA* is a monthly journal containing about 1,500 abstracts. Each issue also contains an Author Index as well as a Subject Index which contains extensive cross-references. All subscribers also receive an annual Author and Subject Index. To produce *FSTA,* IFIS regularly scans 1,400 journals from more than 75 countries, patents from 20 countries, and books in several languages. These abstracts are in English, but are taken from literature originally appearing in as many as 43 languages. A one-year subscription to *FSTA* costs approximately £ 280*.

Magnetic Tapes of *FSTA*

- Everything in *FSTA* is also available on seven- and nine-track IBM magnetic tapes which are for sale at about £ 2,000 per year.
- These tapes are also available for on-line retrieval via Lockheed Information Retrieval Service (USA); SDC Search Service (USA, Canada, France, and Japan); Information Retrieval System (IRS) (Frascati, Italy); or the German Institute for Medical Documentation and Information (DIMDI) (Cologne, Germany).

Secondary Services

Food Annotated Bibliographies

- Food Annotated Bibliographies are selected abstracts from *FSTA* on more than 50 important, specialized aspects of food science and technology. A partial listing of bibliographies available includes: Acidulants in Foods, Colorants, Aflatoxins, Coffee, Stabilizers and Emulsifiers in Foods, Synthetic Dairy Products, and Microwaves in Food Processing.
- Prices range from £ 3 to £ 15 per bibliography.

Current Awareness Service

- Current Awareness or selective dissemination of information services are regular updatings of specifically desired information from each updating of the *FSTA* data base.
- The information is available in three output formats and is carefully selected to match a user's requirements in virtually any aspect of food science and technology.
- Costs are from £ 10 per year for references; £ 15 per year for abstracts.

Retrospective Search Services

- Retrospective searches are searches of the literature to date (the complete data base) for citations and abstracts on specific subjects. More than 100,000 "documents" in *FSTA* are available for search by qualified food science graduates.
- The information is available in three output formats.
- Costs are from £ 5 per volume of *FSTA* searched for references; from £ 7.50 per volume for abstracts. Discounts are available with an annual contract.

Photocopying Service

- Photocopies of any article abstracted in *FSTA* can be quickly made available.
- Cost is 12p per page for single orders, with a minimum charge of £1.50; a deposit account service is also available.
- Translations will be done on request. Prices for this service vary depending upon length of the translation; ask for quote.

*All prices on this page are given in British pounds (£).

**An in-depth analysis of *FSTA* as well as Lockheed and SDC's search services can be found in the article "Retrieving Information from the Food Science Literature," which starts on p. 46 of the September 1977 *Food Technology* (reprints available from IFT at $1 per copy).

For Further Information Contact:

INSTITUTE OF FOOD TECHNOLOGISTS
SUITE 2120
221 N. LA SALLE STREET
CHICAGO, ILLINOIS 60601