4 July-August
Volume 43: Number 4

Coden: JFDAZ 43(4) 1043–1358 (1978)
ISSN: 0022–1147

JOURNAL of FOOD SCIENCE

APPLIED SCIENCE & ENGINEERING

Ascorbic acid content, pH, and flavor characteristics of acidified home canned tomatoes—*M.M. Skelton and J.A. Craig*

A rapid and sensitive sterility monitoring technique for aseptically processed bulk tomato paste—*C. Luster, III*

Acidulation of home canned tomatoes—*G.M. Sapers, J.G. Phillips, F.B. Talley, O. Panasiuk and J. Carré*

pH of tomatoes canned at home in Georgia—*J.J. Powers and D.R. Godwin*

Recovery of betalaines from red beets by a diffusion-extraction procedure—*R.C. Wiley and Y. Lee*

Some factors affecting pinto bean quality—*N.M. Quenzer, V.L. Huffman and E.E. Burns*

Temperatures of spaghetti after heating in convection ovens at service locations in a commissary foodservice system—*M.L. Cremer*

Quality control standards for cooked frozen green beans held on a steam table for varying holding times—*A.M. Brenner, M.J. Hitchcock, R.H. Lane, G.E. Goertz, A.M. Campbell and J.L. Collins*

Water-soluble vitamin content of some California wines—*M.N. Voigt, R.R. Eitenmiller, J.J. Powers and G.O. Ware*

Influence of orange juice composition on the thermal resistance of spoilage yeasts—*B.J. Juven, J. Kanner and H. Weisslowicz*

Effect of controlled atmosphere on flavor stability of almonds—*D.G. Guadagni, E.L. Soderstrom and C.L. Storey*

Functional properties of hydrolyzed lactose: Solubility, viscosity and humectant properties—*N.O. Shah and T.A. Nickerson*

Functional properties of hydrolyzed lactose: Crystallization—*N.O. Shah and T.A. Nickerson*

Mechanical interpretation of compressive stress-strain relationships of solid foods—*F.J. Calzada and M. Peleg*

Some mathematical aspects of mastication and its simulation by machines—*M. Peleg*

Short-cut method for the calculation of sterilization conditions yielding optimum quality retention for conduction-type heating of packaged foods—*H.A.C. Thijssen, P.J.A.M. Kerkhof and A.A.A. Liefkens*

Production of aflatoxins during storage of gamma-irradiated wheat—*A.G. Behere, A. Sharma, S.R. Padwaldesai and G.B. Nadkarni*

Feasibility of home fermentation to improve the amino acid balance of corn meal—*Y.D. Wang and M.L. Fields*

Available lysine in heated fortified rice meal—*T. Tsao, A.L. Frey and J.M. Harper*

Fermentation of peanut milk with *Lactobacillus bulgaricus* and *L. acidophilus*—*L.R. Beuchat and B.J. Nail*

Germination of corn and sorghum in the home to improve nutritive value—*Y.D. Wang and M.L. Fields*

Cornmeal/peanut flour blends and their characteristics—*G.N. Bookwalter, K. Warner, R.A. Anderson and E.B. Bagley*

Tensile strength of processed meats determined by an objective Instron technique—*T.A. Gillett, C.L. Brown, R.L. Leutzinger, R.D. Cassidy and S. Simon*

Factors associated with microbial growth in ground beef extended with varying levels of textured soy protein—*J.T. Keeton and C.C. Melton*

An assessment of the meat characteristics of pre-rigor leg-twisted mutton carcasses—*A. Okubanjo*

Some effects of selected plasticizing agents on the reversible compression of freeze-dried cooked beef cubes—*J.P. Morgan and D.F. Farkas*

Effects of preprocessing belly temperature and pumping pressure on bacon yields, salt residual and organoleptic quality—*R.P. Nusbaum and R.E. Rust*

Computerized cooking simulation of meat products—*R.J. Sarkin*

Yield characteristics, microbial load and chilling rates of skinned pork carcasses—*C.P. Salm, F.K. Ray, E.D. Aberle, M.R. Okos and M.D. Judge*

A survey of the malonaldehyde content of retail meats and fish—*G.M. Siu and H.H. Draper*

Consumer discrimination of pork color at the market place—*D. Wachholz, R.G. Kauffman, D. Henderson and J.V. Lochner*

Potential for water reuse in an Egyptian poultry processing plant—*A. Hamza, S. Saad and J. Witherow*

Bioconversion of shellfish chitin wastes: Process conception and selection of microorganisms—*P.A. Carroad and R.A. Tom*

Fatty acid compositions of 15 species of fish from tropical waters—*P.G. Viswanathan Nair and K. Gopakumar*

Definition of a prediction model for determination of the effect of processing and compositional parameters on the textural characteristics of fabricated shrimp—*H-M. Soo, E.H. Sander and D.W. Kess*

Effect of preprocess holding on the quality of canned Maine sardines—*B.M. Slabyj and R.H. True*

Contents continued on the inside of the front cover

A PUBLICATION OF
THE INSTITUTE OF FOOD TECHNOLOGISTS

1177 Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle—*R.D. Culler, F.C. Parrish Jr., G.C. Smith and H.R. Cross*

1181 Effect of tensile stress on the ultrastructure of bovine muscle—*R.J. Carroll, F.P. Rorer, S.B. Jones and J.R. Cavanaugh*

1188 Effects of pH, calcium ions and ATP on rigor contraction in glycerinated rabbit psoas muscle fiber—*K. Izumi, T. Ito and T. Fukazawa*

1191 Mechanisms of water loss of bovine semitendinosus muscle dry cooked from the frozen state—*C.C. Hung, E.A. Davis, J. Gordon and H.T. Davis*

1196 Bacterial flora of Antarctic krill (*Euphasia superba*) and some of their enzymatic properties—*M.D. Kelly, S. Lukaschewsky and C.G. Anderson*

1198 Flavor compounds related to the warmed-over flavor of turkey—*E.L. Ruenger, G.A. Reineccius and D.R. Thompson*

1201 Effect of cooking method on TBA numbers of stored beef—*W.H. Huang and B.E. Greene*

1204 Effects of heat treatment and protein concentration on the rheology of bovine plasma protein suspensions—*J.P. Harper, D.A. Suter, C.W. Dill and E.R. Jones*

1210 Chemical characterization of egg yolk myelin figures and low-density lipoproteins isolated from egg yolk granules—*T.D. Garland and W.D. Powrie*

1215 Characterization of plastein reaction products formed by pepsin, α-chymotrypsin, and papain treatment of egg albumin hydrolysates—*J.H. Edwards and W.F. Shipe*

1219 Influence of electrolytes on the solubility of rapeseed protein isolates—*L. Gillberg*

1224 An electrophoretic investigation of a peptic hydrolysate of cottonseed storage protein—*S.D. Cunningham, C.M. Cater and K.F. Mattil*

1229 Use of cluster analysis to evaluate sensory-objective relations of processed green beans—*D.R. Godwin, R.E. Bargmann and J.J. Powers*

1235 Tannin hazes in deproteinized apple juice—*J.P. Van Buren and R.D. Way*

1238 Folate levels in food—A comparison of microbiological assay and radioassay methods for measuring folate—*J.E. Ruddick, J. Vanderstoep and J.F. Richards*

1242 Lipid autoxidation and amino acid changes in protein-enriched sweet potato flakes—*W.M. Walter Jr., A.E. Purcell, M.W. Hoover and A.G. White*

1245 High pressure liquid chromatographic analysis of tyramine, phenylethylamine and tryptamine in sausage, cheese and chocolate—*P.E. Koehler and R.R. Eitenmiller*

1248 Synthesis of some unsaturated lactones and their relationship to deep-fat fried flavor—*W.A. May, R.J. Peterson and S.S. Chang*

1253 Effect of nitrogen source on growth and exoprotease production by *Clostridium perfringens*—*H.P. Blaschek and M. Solberg*

1257 Growth of *Bifidobacterium bifidum* var. Pennsylvanicus in laboratory media supplemented with amino sugars and spent broth from *Escherichia coli*—*Y.C. Jao, E.M. Mikolajcik and P.M.T. Hansen*

1261 Isolation, characterization and enzymic hydrolysis of pineapple gum—*K.L. Chenchin and H.Y. Yamamoto*

1264 Measurement of gel water-binding capacity by capillary suction potential—*T.P. Labuza and P.P. Lewicki*

1270 Emulsification properties of groundnut protein—*G. Ramanatham, L.H. Ran and L.N. Urs*

1274 Ultrastructural changes in defatted soy flour induced by nonextrusion texturization—*M.V. Taranto and K.C. Rhee*

1279 Control of chalkiness in soymilk—*D.A. Kuntz, A.I. Nelson, M.P. Steinberg and L.S. Wei*

1284 Effect of water activity on nuclease production by *Staphylococcus aureus*—*J.F. Kamman, S.R. Tatini and T.P. Labuza*

1287 Gas-liquid chromatographic determination of lenthionine in *Shiitake* mushroom (*Lentinus edodes*) with special reference to the relation between carbon disulfide and lenthionine—*Y. Ito, M. Toyoda, H. Suzuki and M. Iwaida*

1290 Gas-liquid chromatographic determination of carbon disulfide in *Shiitake* mushroom (*Lentinus edodes*)—*M. Toyoda, H. Suzuki, Y. Ito and M. Iwaida*

1293 Blood serum lipid patterns of adolescent boys fed corn oil, peanut oil or safflower oil—*C. Kies, M.E. Lopez-Rico, H.M. Fox, K.M. Stanek and A. Chan*

1297 A high pressure liquid chromatographic method for determining the stability of free methionine in methionine-fortified food systems—*L.S. O'Keefe and J.J. Warthesen*

1301 Innovations in sampling and culturing methods for bacteriological examination of broiler carcasses—*J.E. Thomson and J.S. Bailey*

1303 Fate of aflatoxin B_2 in a two-step microbial recovery system for food industry wastes—*K.M. Johnson and F.F. Busta*

1307 Determining kinetic parameters for thermal inactivation of heat-resistant and heat-labile isozymes from thermal destruction curves—*A.C. Ling and D.B. Lund*

1311 Ultrasonic peptization of soybean proteins from autoclaved flakes, alcohol-washed flakes and commercial samples—*L.C. Wang*

1314 Proximate analysis, fatty acid and amino acid composition of Nigella sativa L. seeds—*V.K. Babayan, D. Koottungal and G.A. Halaby*

1316 Changes in kunitz trypsin inhibitor during germination of soybeans: An immunoelectrophoresis assay system—*R.C. Freed and D.S. Ryan*

1320 Classification of wines by applying pattern recognition to chemical composition data—*W.O. Kwan and B.R. Kowalski*

1324 Changes in cytokinin activity in ripening tomato fruit—*N. Desai and G.W. Chism*

RESEARCH NOTES

1327 Storage life of pre-cut, reformed, vacuum packaged lamb loins—*J.D. Tatum, G.C. Smith and Z.L. Carpenter*

1328 Effect of various selective agars on the growth of rare and common Salmonellae—*D.L. Kroninger and G.J. Banwart*

1330 Aftereffect of submersion in water on greening and glycoalkaloid formation of potato tubers—*M.T. Wu and D.K. Salunkhe*

1332 Isolation of nonpathogenic clostridia from liquid cyclone processed cottonseed flour—*S.D. Goodwin and J.P. Cherry*

1334 Bread making properties of peanut flour produced by direct solvent extraction procedure—*M.N. Khan, D. Mulsow, K.C. Rhee and L.W. Rooney*

1336 Shear creep testing of demethylated pectin gel—*V.N.M. Rao, M.O. Gross and C.J.B. Smit*

1338 Change in regulation of myofibrillar ATPase activity by calcium during postmortem storage of muscle—*Y. Ikeuchi, T. Ito and T. Fukazawa*

1340 In vitro studies on saponin-vitamin complexation—*L.G. West and J.L. Greger*

1342 In vitro studies on saponin-mineral complexation—*L.G. West, J.L. Greger, A. White and B.J. Nonnamaker*

1344 Effect of cooking on vegetable fiber—*V. Matthee and H. Appledorf*

1346 Odor threshold of 2,3,6-trichloroanisole in water—*D.G. Guadagni and R.G. Buttery*

1348 Changes in the free fatty acid and ammonia content of beef during aging and processing—*G.M. Brooks and G.R. Ammerman*

1350 Nutritional evaluation of full-fat soy flour produced by dry heat roasting—*G.R. Jansen, J.M. Harper and L.A. O'Deen*

1352 Effect of two home canning procedures on fish and soy protein quality—*S.C. Keyes, I.D. Wolf, E.A. Zottola and P.V.J. Hegarty*

1354 Identification and quantitation of tryptophan and tyrosine metabolites in the banana by liquid chromatography with electrochemical detection—*T.M. Kenyhercz and P.T. Kissinger*

1355 Enzymatic estimation of available lysine—*L.E. McBee and R.T. Marshall*

1357 Effect of pH on volatile retention in freeze-dried model solutions—*T.G. Smyrl and M. LeMaguer*

1358 Errata Notice

REPRINTS of Articles from "Journal of Food Science"

☐ **SINGLE** *reprints are NOT available from* JOURNAL OF FOOD SCIENCE. *Send requests directly to the senior author (address appears near title).*

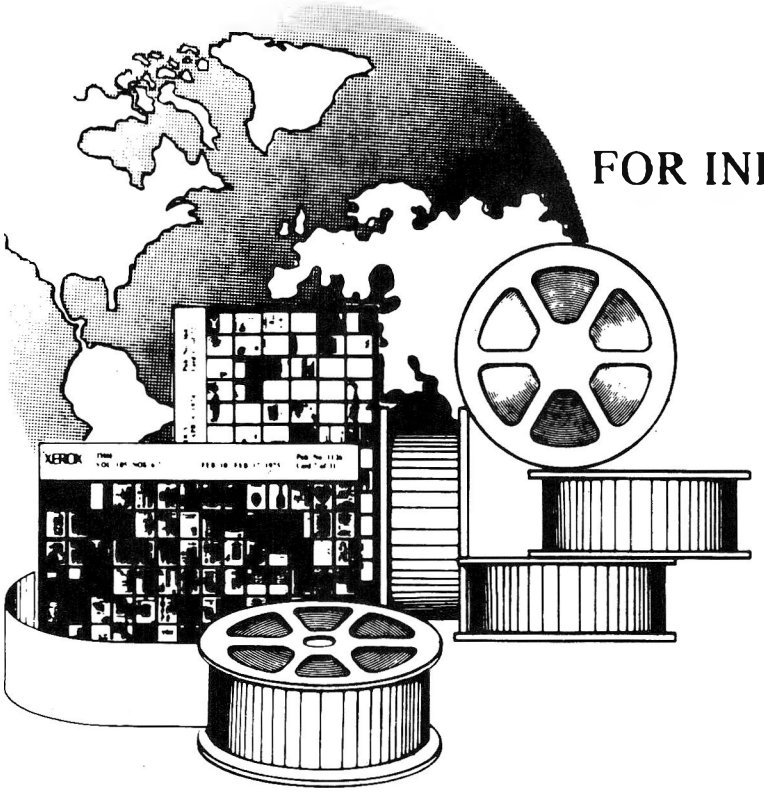
QUANTITY *reprints (minimum of 100 copies) can be ordered from IFT Subscription Department, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601. Price schedule available upon request.*

PAGE CHARGES for Publication of Research Papers

☐ *AT ITS March 1969 meeting, the Executive Committee of IFT voted to increase the page charge for research articles published in the* JOURNAL OF FOOD SCIENCE. *The page charge of $50 per printed page is effective for all research manuscripts received AFTER April 1, 1969. The page charge shall not constitute a bar to acceptance of research manuscripts because the author is unable to pay the charge.*

หอสมุด กรมวิทยาศาสตร์
15 ส.ค. 2521

This Publication is Available in MICROFORM



FOR INFORMATION WRITE:

University Microfilms International

Dept. F.A.
300 North Zeeb Road
Ann Arbor, MI 48106
U.S.A.

Dept. F.A.
18 Bedford Row
London, WC1R 4EJ
England

July-August 1978
Volume 43: Number 4

JOURNAL of FOOD SCIENCE

Scientific Editor
Bernard J. Liska

Asst. Scientific Editor
Anna May Schenck

Managing Editor
Bernard Schukraft

Director of Publications
John B. Klis

Publisher
Calvert L. Willey

Board of Editors

Z. Carpenter (79)
A. Clausi (79)
G. Flick (79)
L. Hackler (79)
J. Litchfield (79)
D. Lund (79)
P. Markakis (79)
R. Braddock (80)
B. Johnson (80)
N. Potter (80)
F. Sosulski (80)
H. Stone (80)
D. Wang (80)
A. Wasserman (80)
J. Augustin (81)
L. Beuchat (81)
F. Clydesdale (81)
H. Herring (81)
K. May (81)
H. Swaisgood (81)
R. Toledo (81)

- **MANUSCRIPTS** (3 copies) should be submitted to:
  Dr. Bernard J. Liska, IFT Scientific Editor
  P.O. Box 3067
  West Lafayette, IN 47906 USA

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*. Authors should request from the Director of Publications the revised leaflet "Style Guide for Research Papers." *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" was published in the September-October 1977 issue of *Journal of Food Science* (vol. 42, issue 4, pp. 1417–1420).

- **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, orders for back issues, and 100 or more repr nts should be sent to:

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

Member Subscriptions—$10 per year.

Non-Member Subscriptions—Accepted only on a calender year basis—no refunds. Rates include postage. Payment must accompany order. Domestic, Canada, Mexico, and PUAS—$30; all other destinations—$35. 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, $6 each; remittance to accompany order.

- **REPRINTS:** Request single reprints from AUTHORS. Price schedule for 100 or more reprints available upon request from Subscription Department.

- **COPYING FEES**—The appearance of the ISSN (International Standard Serial Number) code in the lower left corner of the first page of an article in *Journal of Food Science* indicates the copyright owner's consent that copies of the article may be made for personal or internal use. This consent is given on the condition, however, that the copier pay the stated per article copying fee ($2.25) through the Copyright Clearance Center, Inc., One Park Ave., New York, NY 10016 for copying beyond that permitted by Sections 107 and 108 of the U.S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promctional purposes, for creating new collective works, or for resale.

©Copyright 1978 by Institute of Food Technologists. All rights reserved. *JOURNAL of FOOD SCIENCE* (formerly *Food Research*) is published six times a year (bimonthly) by Institute of Food Technologists, Suite 2120, 221 N. LaSalle Street, Chicago, Illinois 60601 USA. Printed in USA. Second class postage paid at Chicago, IL and at additional mailing offices.

ifis Supplementary Services

Everyone knows about the International Food Information Service's *Food Science and Technology Abstracts* (FSTA), a monthly compilation of abstracts in 19 subject areas gleaned from more than 1,200 journals from 50 countries.

However, in addition to publishing, IFIS also has what are known as "Supplementary Services" which FSTA subscribers can avail themselves of. Here are a list of these services:

FOOD ANNOTATED BIBLIOGRAPHIES

- Food Annotated Bibliographies (FABS) are selected abstracts from FSTA on 51 important, specialized aspects of food science and technology. A partial listing of bibliographies available include: 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

RETROSPECTIVE SEARCH SERVICE

- More than 100,000 documents in FSTA searched
- State-of-the-art or "quicky" telephone requests accepted
- Annual contracts with discount
- Information available in three outpost formats
- Searches conducted by qualified food science graduates
- Cost from £5 per volume of FSTA for references; £ 7.50 per volume for abstracts

(Prices given are in British pounds)

CURRENT AWARENESS SERVICE

- Monthly searches of FSTA
- Personalized, computer-assisted service
- Information available in three output formats
- Information selected to match your requirements on any aspect of food science and technology
- Cost from £10 per year for references; £15 per year for abstracts

MAGNETIC TAPES

- Contain all information in FSTA plus bibliographic data, abstracts, author, and subject indexes
- Seven- and nine-track IBM tapes available
- "Clear" tapes suitable for computer retrieval
- Costs about £1,000 per year

PHOTOCOPYING SERVICE

- All journal articles abstracted in FSTA available promptly
- Cost 10p per page for single orders
- Deposit account service

For further information contact:

Calvert L. Willey, Executive Director
Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601
(or call 312-782-8424)

IFIS is sponsored by Commonwealth Agricultural Bureau, Institut für Dokumentationswesen, Institute of Food Technologists, Centrum voor landbouwpublikaties in Lanbouwdocumentatie, and Zentralstelle für maschinelle Dokumentation

ASCORBIC ACID CONTENT, pH AND FLAVOR CHARACTERISTICS OF ACIDIFIED HOME CANNED TOMATOES

MARILYN M. SKELTON and JEAN A. CRAIG

ABSTRACT

Two cultivars of tomatoes were home canned in a boiling water bath with various levels of citric acid and one level of sugar. After 7 months' storage, tomatoes to which 1g of citric acid had been added had the lowest ascorbic acid content. Addition of smaller quantities of citric acid, however, did not significantly affect ascorbic acid retention, compared with that of the nonacidified control. Adding 0.5g of citric acid or 1g of citric acid plus 1 tsp of sugar was found to be an acceptable method of decreasing the pH of tomatoes used in this study without seriously impairing the flavor or texture.

INTRODUCTION

SINCE AN OUTBREAK of botulism from home canned tomato juice in 1974, changes in procedure for home canning tomatoes have been suggested by a number of committees and agencies. There is no general agreement among these recommendations. Scientists from the Center for Disease Control, National Canners Association and the USDA (NCA, 1974), recommended adding citric acid to produce a definite acid taste and processing to a center temperature of 87.8°C for at least 2 min. The National Food Safety Committee, composed of food technologists from all states, met in St. Louis in April, 1975. They recommended that tomatoes be hot-packed and pint jars processed 35 min (45 min for quarts) in a boiling water bath or for 5 min at 10 lb pressure in a steam pressure canner (Johnson, 1975).

Many extension specialists have recommended acidification with lemon juice, vinegar, or citric acid in various quantities (Johnson, 1975). Wolf et al. (1977) reported the pH of 109 cultivars of tomatoes to be within the acid food range. Officials of the USDA studied 350 cultivars of tomatoes and reported that the fruit normally contains enough natural acid to prevent growth of *Clostridium botulinum* (Sapers, 1977). These conclusions based on mean pH ignored the considerable variation in pH of individual tomatoes within a given cultivar. Farrow (1963) found that 6 or 7% of individual usable tomatoes had a pH of 4.6 or greater.

The pH of tomatoes increases as they ripen from firm-ripe to soft-ripe (Villarreal et al., 1960), increasing the probability of a home canner's having a jar of tomatoes with pH > 4.6, the pH defining low-acid foods. The IFT Status Summary (1977) reported that tomatoes can be canned by the boiling water bath method, but did point out that canning overripe tomatoes can lead to serious problems.

Fields et al. (1977) measured the pH and studied the microorganisms in 292 containers of tomatoes home canned in Missouri. They found three samples with pH levels above 4.8 which could support the growth of *Clostridium botulinum* and found viable microorganisms in 116 samples. They recommended processing 10 min at 5 lb pressure. Results of a survey of home canners in three Kansas counties showed that only 28% of tomatoes canned were processed in the pressure canner (Phillips and Skelton, 1977).

Earlier work done here (Skelton and Marr, 1978) indicated that tomatoes home canned by the pressure method may have a poorer flavor, texture and appearance than those canned in a boiling water bath. Increasing the processing time from 10 to 35 min in the boiling water bath also produced undesirable changes. If a more stringent method than the 10 min boiling water bath processing of hot-packed tomatoes in pint jars recommended by the USDA (1975) is needed, then lowering the pH as is done in the commercial canning of tomatoes seemed to be a better alternative. However, adding the commonly recommended 1/4 tsp citric acid per pint resulted in a sour flavor and a significantly lower ascorbic acid content at a mean pH of 4.05. Lee et al. (1977) found that anaerobic destruction of ascorbic acid between pH 3.0 and 5.0 was greatest at 4.06.

In this paper we report the effects of several levels of citric acid, and 1 level of sugar on the pH, ascorbic acid content, texture and flavor of two cultivars of tomatoes canned in a boiling water bath.

EXPERIMENTAL

Materials

Two cultivars of tomatoes, Campbells 1327 and Jet Star, were grown on the Kansas State University Horticultural Farm, Manhattan, KS, and home canned at the firm ripe stage (when tomato first becomes bright red, but is still firm). These cultivars had the highest mean pH of the six which were studied in the previous season. Furthermore Jet Star is grown widely in Kansas and Campbells 1327 is a standard canning cultivar.

Treatment of raw tomatoes

The pH of longitudinal sections of 100 raw tomatoes of each cultivar was measured after each section was blended 1 min in a Waring Blendor. Total ascorbic acid content of samples from 100 raw tomatoes of each cultivar was determined by the method of Deutsch and Weeks (1965), slightly modified. Four grams acid-washed Norit A were used for each sample. The pH of the filtrate and boric/sodium acetate solution for the blank was adjusted to pH 7.5–8.0 to avoid excessively high blank readings. Fluorescence of the quinoxaline formed by the reaction of dehydroascorbic acid with 0-phenylenediamine was measured in a Coleman Model 12C Photofluorometer with B–1 and PC–1 filters.

Canning procedure

Tomatoes for canning were washed, then immersed in simmering water 30 sec and in cold water 30 sec to loosen the peel. Cores and peel were removed manually and tomatoes were quartered. The hot pack method was used exclusively because of the recommendation of the National Food Safety Committee (Johnson, 1975). Furthermore, shorter processing times are required when food is hot-packed than when cold-packed, thus decreasing the possibility of underprocessing by the homemaker. Tomatoes were heated in 4-pint lots to the boiling point, then packed in pint glass Mason jars that had been held in simmering water until used. The tomatoes were covered with hot juice to within 1/2 in. of the top of the jar. Additives (determined by treatment) were placed on tomatoes in the top of the jar before the jars were closed with standard 2-piece, self-sealing lids. Packs were processed in a boiling water bath.

Treatment variables

The additives to the tomato packs were: 0.065g, 0.205g, 0.5g, 0.75g, and 2.0g U.S.P. citric acid and 1g citric acid + 1 tsp sucrose (4.2g). Every pack contained 1/2 tsp (3g) canning salt. Each control

Author Craig is with the Dept. of Foods & Nutrition, Kansas State University, Manhattan, KS 66506. Author Skelton, formerly with Kansas State University, is now affiliated with Fitzsimons General Hospital, Building 111 No. 4, Denver, CO 80240.

0022-1147/78/0004-1043$02.25/0
©1978 Institute of Food Technologists

Table 1—Mean values[a] of pH, ascorbic acid content, sweet/sour flavor scores and texture scores for acidified home canned tomatoes

Treatment	Mean pH	Mean ascorbic acid content	Mean hedonic sweet/sour flavor score	Mean intensity sweet/sour flavor score	Mean texture
Control	4.20a	15.13b	4.19a	3.94a	3.87ab
0.065g citric acid	4.15ab	15.66b	4.15a	3.78a	3.75ab
0.205g citric acid	4.16ab	17.06ab	4.12a	3.74a	3.71abc
0.5g citric acid	4.08bc	15.49b	4.17a	3.37b	3.64bc
0.75g citric acid	4.00cd	16.36b	3.99a	3.19b	3.83ab
1.0g citric acid + 1 tsp sugar	3.85e	12.97c	3.99a	3.18b	4.04a
2.0g citric acid	3.64f	18.73a	3.05b	2.23c	3.36c
L.S.D.	0.099	1.98	0.40	0.326	0.387

[a] Different letters within a column indicate significant differences ($P < 0.05$).

pack contained only 1/2 tsp canning salt. Eight packs of each cultivar were used for each treatment.

All packs were processed 10 min in a boiling water bath. All jars were cooled to room temperature immediately after processing.

Storage and analyses

Tomatoes were stored 7 months at 22°C. After storage, samples were used from each jar for total ascorbic acid determination as described previously, pH measurements, and sensory tests.

An experienced 10-member taste panel rated the sweet/sour flavor balance on a 7-point intensity scale (7 = extremely sweet, 1 = extremely sour) and again on a 7-point hedonic scale (7 = like extremely, 1-dislike extremely). Texture was rated on a 7-point intensity scale (7 = extremely firm, 1 = extremely mushy).

A 2-way analysis of variance was used to determine differences in pH, ascorbic acid content, and sensory measurements of stored tomatoes attributable to cultivar and canning treatment. Mean separation procedure by Fisher's Least Significant Difference followed a significant F test.

Correlation coefficients for every pair of measurements were calculated.

RESULTS & DISCUSSION

pH

The mean pH values of 100 raw firm, evenly-ripened tomatoes of the Campbells 1327 and Jet Star cultivars were 4.50 and 4.53 with standard deveiations of 0.133 and 0.127, respectively. The corresponding ranges of values were 4.18–4.78 and 4.21–4.87. Twenty-six percent of the raw tomatoes of each cultivar had pH $\geq$ 4.6 even though no over-ripe tomatoes were included. This illustrates the importance of considering the pH of individual tomatoes rather than cultivar means when making recommendations for home canning methods.

Mean pH values of tomatoes with each additive are shown in Table 1. Corresponding ranges for each cultivar are shown in Table 2. The pH value of acidified tomatoes compared with that of the controls was lowered an average of 0.3 units per gram of citric acid added, which agreed with results of Leonard et al. (1959). Results of Gould et al. (1971) suggest that if the initial pH is above 4.5, the pH shift will be even greater. There was a significant positive correlation ($P < 0.01$) between the pH and the sourness as rated by the sensory panel (Table 3) and between the pH and the panel's preference for sourness.

Variation of ascorbic acid content with pH. The mean ascorbic acid content of the raw Campbells 1327 tomatoes was 22.44 mg/100g and of the Jet Stars was 25.87 mg/100g. The ascorbic acid content of canned tomatoes (Table 1) to which 1g of citric acid had been added was significantly lower ($P < 0.05$) than that of any of the other tomatoes. That was the same amount of citric acid without added sugar that Skelton and Marr (1977) found decreased ascorbic acid content com-

Table 2—Range of pH values for home canned tomatoes of two cultivars

Treatment	pH range	
	Campbells 1327	Jet Star
Control	4.13–4.25	4.04–4.39
0.065g citric acid	4.05–4.23	4.07–4.26
0.205g citric acid	4.04–4.18	4.06–4.65
0.5g citric acid	3.82–4.05	3.60–4.52
1.0g citric acid + 1 tsp sugar	3.76–3.91	3.80–4.04
2.0g citric acid	3.52–4.21	3.54–3.67

Table 3—Correlation coefficients for certain characteristics of home canned tomatoes

	pH	Ascorbic acid	Hedonic sweet/sour score	Intensity sweet/sour score	Texture
pH	1.0000**				
Ascorbic acid	−0.0011	1.0000**			
Hedonic sweet/sour score	0.4190**	−0.1349	1.0000**		
Intensity sweet/sour score	0.6272**	−0.1792*	0.6615**	1.0000**	
Texture	0.0616	−0.1781*	−0.2841**	−0.1417	1.0000**

* $P < 0.05$
** $P < 0.01$

Fig. 1—Variation in total ascorbic acid content with pH of home canned tomatoes.

pared with that of nonacidified tomatoes. However, the mean pH of the tomatoes acidified with 1g citric acid in this study was 3.85; in the earlier study it was 4.05. There was a significantly higher ($P < 0.05$) ascorbic acid retention in tomatoes to which 2.0g citric acid had been added than in any of the other tomatoes except those containing 0.205g citric acid. The general shape of the curve (Fig. 1) is the same as that obtained by Lee et al. (1977) in that ascorbic acid values in the pH range 4.0–4.2 did not differ significantly ($p < 0.05$). However, the lowest ascorbic acid retention occurred at pH 3.85 in this study, compared with 4.06 in that of Lee and coworkers.

The mean ascorbic acid content of all canned tomatoes in the study was 18 mg/100g which means that a 100g serving would furnish 40% of the NRC RDA of ascorbic acid for adults. Home canned tomatoes could be a significant source of the vitamin for families who consume them. Therefore, the conservation of ascorbic acid should be an important consideration in the recommendation of canning methods.

Flavor

There was a significant correlation ($P < 0.05$) between the sensory panel's preference for sweet/sour flavor balance and their sweet/sour intensity scores (Table 3). Sourest samples were rated lowest on the hedonic scale. Mean sweet/sour scores on the hedonic scale did not differ significantly except that the tomatoes to which 2g citric acid had been added were rated significantly lower ($P < 0.05$) than any of the others. Results indicated that 0.5g (1/8 tsp) citric acid or 1g (1/4 tsp) citric acid plus 1 tsp sugar might be added without affecting the flavor adversely, compared with that of nonacidified tomatoes. In our earlier study (Skelton and Marr, 1977), we found that 1/4 tsp citric acid alone significantly decreased flavor preference scores compared with those of nonacidified tomatoes.

The sourness on the intensity scale did not differ significantly ($P < 0.05$) among the nonacidified tomatoes and those with 0.065g or 0.205g citric acid per pint. Sourness intensity scores for tomatoes with 0.5g or 0.75g and those with 1g citric acid plus 1 tsp sugar did not differ significantly, but were significantly lower ($P < 0.05$) than those of the control and those containing less citric acid. Those containing 2g citric acid were significantly more sour ($P < 0.05$) than any of the others.

Texture

There appeared to be no definite relationship between levels of citric acid and texture scores.

SUMMARY

ADDING 0.5g citric acid or 1g citric acid plus 1 tsp sugar was found to be an acceptable method of decreasing the pH of tomatoes used in this study for home canning in the boiling water bath without seriously impairing the flavor. Texture of tomatoes treated by either method was not significantly ($P < 0.05$) different from that of the control. Adding 1g citric acid/pt resulted in the lowest ascorbic acid content in the tomatoes studied.

Results of this study and previous work (Skelton and Marr, 1977), suggest that the method that should be used to home-process tomatoes is the addition of 1/8 tsp U.S.P. citric acid/pt to hot packed tomatoes and processing pint packs 10 min in a boiling water bath. Further work is needed to confirm the effectiveness of this method for tomatoes with higher pH values.

REFERENCES

Deutsch, M.J. and Weeks, C.E. 1965. Microfluorometric assay for vitamin C. J. AOAC 8: 1248.

Farrow, R.P. 1963. A survey of pH variation in canning tomatoes. National Canners Association Research report no. 1–63, Washington, DC, Jan.

Fields, M.L., Zamora, A.F. and Bradsher, M. 1977. Microbiological analysis of home-canned tomatoes and green beans. J. Food Sci. 42: 931.

Gould, W.A., Mount, J., Hawiler, L. and Black, J. 1971. Effects of food additives on quality of canned tomatoes. Report, Dept. of Horticulture, Ohio State University, Columbus, OH.

IFT Expert Panel on Food Safety. 1977. Home canning. Food Technol. 31: 43.

Johnson, E.H. 1975. Extension service nutritionist, Washington, DC, Fact Sheet, Feb. 21.

Lee. Y.C., Kirk, J.R., Bedford, C.L. and Heldman, D.R. 1977. Kinetics and computer simulation of ascorbic acid stability of tomato juice as functions of temperature, pH and metal catalyst. J. Food Sci. 42: 640.

Leonard, S.J., Pangborn, R.M. and Luh, B.S. 1959. The pH problem in canned tomatoes. Food Technol. 13: 418.

National Canners Association. 1974. News release, Washington, DC, Aug. 5.

Phillips, D.E. and Skelton, M.M. 1977. Sources of information and home canning methods used by homemakers in three Kansas counties. Keeping Up With Research no. 35, Kansas State University Agr. Exp. Sta.

Sapers, G.M. 1977. News release. USDA/ARS, Philadelphia, PA, June 24.

Skelton, M.M and Marr, C.A. 1978. Ascorbic acid content, pH and acceptability of tomatoes processed by different home canning methods. Home Economics Research J. In press.

USDA. 1975. Home and Garden Bull. No. 8, United States Department of Agriculture, Washington, DC.

Villarreal, F., Luh, B.S. and Leonard, S.J. 1960. Influence of ripeness on organic acids in canned tomato juice. Food Technol. 14: 176.

Wolf, I.D. Schwartau, C.M., Larson, K.D., Davis, D.W. and Zottola, E.A. 1977. Home canning of food: Tomatoes, tomato products and other acid foods. Paper presented at 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 6.

Ms received 10/18/77; revised 2/4/78; accepted 2/8/78.

The authors thank Dr. Arthur Dayton, Head, Dept. of Statistics, KSU for statistical analysis and Dr. Charles Marr, Dept. of Horticulture & Forestry for providing tomatoes.

A RAPID AND SENSITIVE STERILITY MONITORING TECHNIQUE FOR ASEPTICALLY PROCESSED BULK TOMATO PASTE

CLAUDE LUSTER, III

ABSTRACT

A rapid, sensitive monitoring technique for the detection of microbial contamination of aseptic tomato paste systems has been developed. Samples are aseptically collected by means of a newly developed sampling technique and placed with media in incubation for variable time intervals and are analyzed for the presence of diacetyl or acetylmethylcarbinol by means of colorimetric assay or GLC. The technique can detect microbial spoilage in 4–12 hr compared to 72–96 hr using traditional methods. The technique also makes possible the ability to monitor bulk storage tanks automatically by sampling headspace gases above the product and injecting into a GLC.

INTRODUCTION

TOMATO PASTE serves as a base constituent or foundation for a large number of food products. Due to the unique time profile of the California tomato crop season, late July to middle October, it is somewhat difficult to precisely meet consumer market demand for particular tomato based products. However, by processing enough tomatoes into paste in large containers, it is possible to meet consumer demand for a particular product at any time interval between seasons by reprocessing the tomato paste into desired products. Processing large quantities of tomato paste by the traditional method yielded a product very poor in quality. However, by employing a method termed "aseptic processing," it was proven that it was possible to process large quantities of tomato paste without serious quality degradation. Along with aseptic processing, however, the problem of maintaining and determining the asepsis of the system evolved. Studies performed by Jones (1936) and Pederson (1949) attempted to identify causative organisms of microbial spoilage in conventionally processed canned tomato products. However, literature concerning causative organisms or monitoring of microbial spoilage of aseptic bulk storage systems is rare, if not nonexistent. Traditional techniques of determining the asepsis of the system are inadequate for a number of reasons which will be dealt with later. New rapid detection methods are unacceptable mainly because they are uneconomical and inconvenient. It was the purpose of this research to conceive an economical and sensitive monitoring procedure for aseptic tomato paste systems.

EXPERIMENTAL

Microorganisms

Lactic acid bacteria and yeasts isolated from the third effect of the Rossi-Catelli T-120 evaporation system and from spoiled tomato paste were used.

Sampling procedure

75–100g of tomato paste was collected aseptically into a presterilized 500 ml Erlermeyer flask containing 300 ml of culture medium using Luster's cup shroud technique as shown in Figure 1. The flasks were incubated at 32°C for variable time intervals.

Author Luster is with the Tri/Valley Growers, 12045 S. Ingomar Grade Road, Los Banos, CA 93635.

002-1147/78/0004-1046$02.25/0
© 1978 Institute of Food Technologists

Analytical

Ingredients for bacteriological media were from Difco (Detroit, MI). The culture medium contained: peptone–10g, yeast extract–7.5g, invert sugar–10g, and water to liter. The medium was adjusted to pH 4.2 ± 0.1 by the addition of small amounts of a 25% tartaric acid. Alpha-napthol, creatine, diacetyl acetylmethylcarbinol, invert sugar and tartaric acid were from J.T. Baker Chemical Company. Other chemicals were reagent grade from standard suppliers.

The Hewlett-Packard GC-Mass Spectral unit owned by National Canners Association in Berkeley was used to determine the peak fragmentation patterns of the predominent metabolic by-products of spoilage organisms. The Cyphernet Mass Spectral Search System of Ann Arbor, MI, linked through a teletype terminal at NCA, Berkeley, was used to identify by-products of spoilage organisms from data collected from the GC-Mass Spectral Unit.

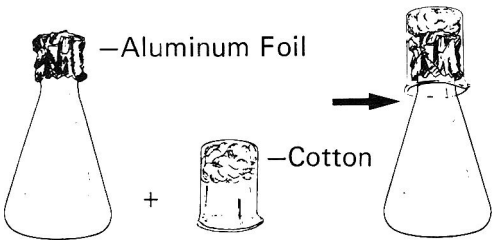
300 ml of tomato paste-medium mixture were added to a 1–L distillation flask which was then heated strongly enough to bring the contents to a boil in about 5 min. The distillate was allowed to drop through a filter of Whatman's No. 1 paper into a graduated cylinder. The filter removed oil and cloud interference. When 25–30 ml of distillate were collected, the sample was then refrigerated until transfer to the GC-Mass Spectral Unit at NCA. A 10 μl sample was then injected into the GC-Mass Spectral Unit and peak intensities measured for each compound detected. Known sterile samples were compared to known unsterile samples taken from the third effect evaporator and actual induced industrial spoilage incidents. Data concerning peaks and their intensities was fed into the computer for compound identification.

Upon identification of diacetyl (DA) and acetylmethylcarbinol (AMC) as the spoilage organisms chief by-products, a colormetric method was used for the determination of DA and AMC (Byer, 954): 40 ml of the distillate were poured into a 125 ml Erlermeyer flask and 10 ml of alpha-napthal reagent (5% alphanapthol in isopropyl alcohol) was added, followed by 4 ml of 7.1N potassium hydroxide. The flask was swirled vigorously for 30 sec and then about 10 ml of the reaction mixture was poured into a 13 × 100 mm glass test tube. Sixty seconds after the reagents were mixed the 540 nm wavelength was measured using a Bausch and Lomb Spectronic 20 Spectrophotometer. This reading is proportional to the diacetyl concentration in the sample. After 30 min a second reading was made. The difference between the 30-min and 60-sec reading is proportional to the AMC concentration. A blank solution was made by mixing water and the reagents. Each time an analysis is made, a control sample of 750 mg diacetyl is included to assure that the reagents are working properly.

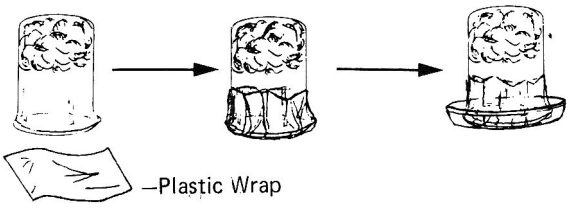
RESULTS & DISCUSSION

BEFORE DEALING WITH the discussion of the specific problem, it is necessary to discuss the basic process flow and control parameters in the aseptic tomato paste system in which the research was performed. It is important that the individual(s) monitoring the systems have a basic understanding of bacteriological principles along with a good understanding of the functional principles of the particular system involved. The tomatoes are brought in from the fields and are washed, sorted, processed through the hot breaks (pectic enzyme inactivation), filtered and concentrated from about 5.0° Brix to 31.0° Brix. The evaporation systems used were Rossi-Catelli T-120's triple effect evaporators. The "aseptic system" begins at the evaporator discharge where the paste as 31.0° Brix is pumped over to a holding tank. The holding tank discharges paste into the sterilization unit which sterilized the paste, in this case by using steam injection heating, by raising the temperature to about 96°C. The tomato paste then flows to the cooling unit via a temperature sensitive valve which senses

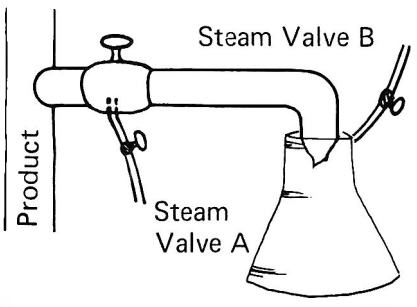
–Text continued on page 1048



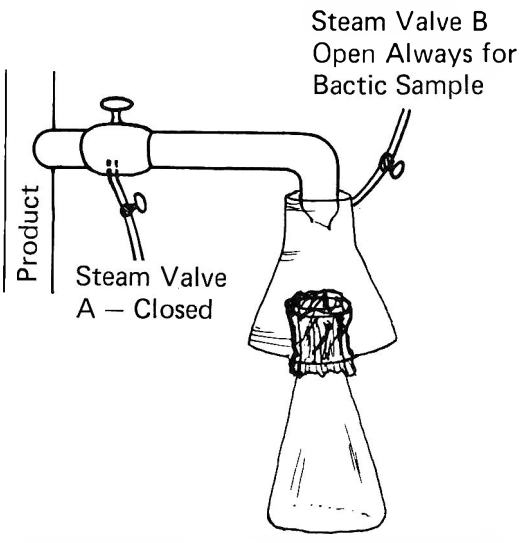
A. Sterilize media in 500 ml Erlenmeyer flask with aluminum foil cover along with 250 ml cotton plugged cover beaker.



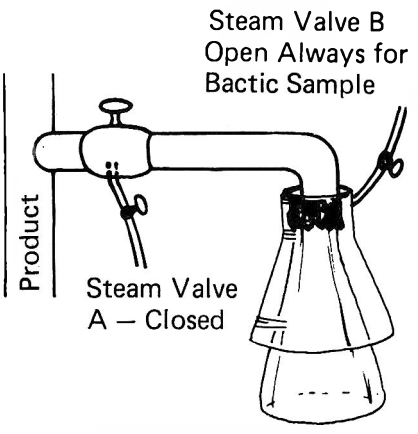
B. After sterilization, spray bacteriocidal solution into cover beaker and cover with a plastic wrap square previously sprayed heavily with bacteriocidal spray. Place, with wrap section down, into petri dish containing bacteriocidal solution.



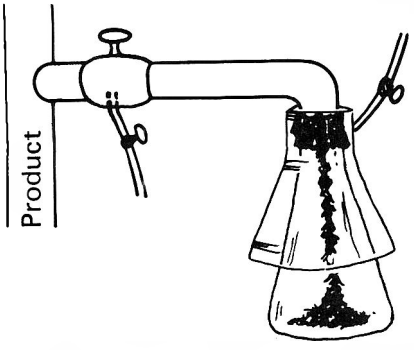
C. To sample, take petrie dish containing cover beaker and flask containing nutrient media out to cup shroud sampling device constructed as above.



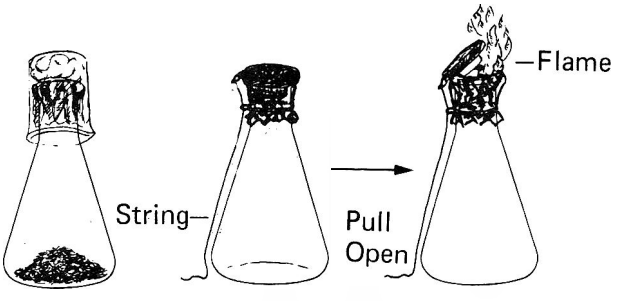
D. Pour alcohol on top of aluminum foil and light to ignite flame. Let flame burn for 3–5 sec. Take flask with flame on top and put under cup. When neck of flask has disappeared under cup, close steam valve A while simultaneously pushing flask flush into cup.



E. The aluminum foil covering has now been ruptured by the probe and the flask is being shrouded by steam via steam valve B into the side of the cup.



F. Open product valve and discharge tomato paste into flask making sure not to overfill flask past media line. Shut product sample valve off.



G. Quickly take cover beaker in free hand and put cover on flask immediately after taking flask from under the cup. Open steam valve A. In situations where wind contamination may be a problem, a special spring action cover attachment can be made to insure protection against wind contamination. The cover closes on top of the flask the second it clears the sample probe.

Fig. 1—Luster's cup shroud sampling technique.

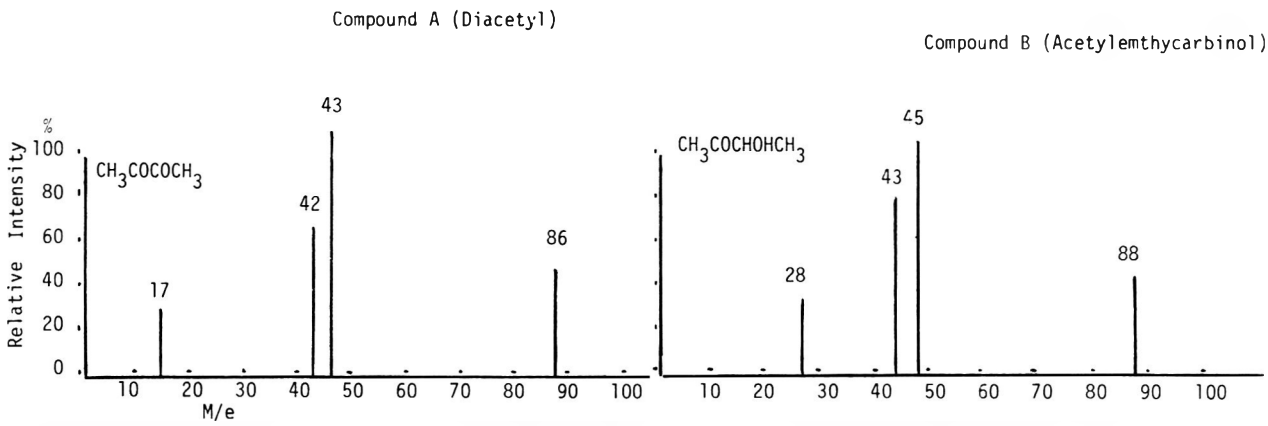


Fig. 2—Fragmentation patterns of Compounds A and B. (Note: Solutions of reagent grade DA and AMC yielded identical fragmentation pattern as Compound A and B, respectively.).

any tomato paste receiving inadequate heating and diverts it back into the holding tank, if such a condition exists. The cooling unit is composed of basically three sub-units: (1) hot holding tank, (2) separator, (3) condensor. The tomato paste, after proper sterilization, flows into what is termed a "hot holding tank" where it is held for 4-6 min to insure sterilization. The tomato paste is then fed into what is termed a "flash cooler" which flashes the paste down to 32–38°C using about 28.5 in. Hg vacuum created by a steam evaporator-condensor system. Upon cooling, the tomato paste is then filled into a previously sterilized container such as a 55 gal drum or a bulk storage tank (10,000–125,000 gal).

With a basic understanding of the system involved we can now discuss the problem and results obtained. One basic commandment of aseptic processing is positive pressure throughout the system. With positive pressure, if a leak develops the leak is inside out rather than outside in. It is obvious, therefore, that one of the most critical parts of the system as far as asepsis is concerned, is the flash cooler because of the fact that it is operated under vacuum. The third effect was chosen as an unsterile comparison because of similar operating conditions.

Present traditional methods of detecting the bacterial contamination have major deficiencies based on (1) sensitivity and (2) rapidity. These deficiencies are further compounded by the fact that the predominant causative organisms (lactic acid bacterium) are low ATP producers, therefore, very slow growing organisms. Newer rapid detection methods have major drawbacks in terms of economical investment and lack of flexibility.

The strategy of devising the detection system was to isolate the types of organisms involved in a system contaminated and to go from there. What was needed were samples from an unsterile flash cooler on a continuous basis. This, as probably reasoned, was impossible to arrange because of the economics involved. It was later agreed that the system in fact already possessed an unsterile comparison in the third effect of the Rossi-Catelli T-120 evaporation system. The third effect processing parameters were 32–43°C and 28.5 in. Hg which were almost exactly the same as the sterile flash cooler. It was then reasoned that the same type of organisms which grow in the third effect of the evaporators were the type which could live in the flash cooler. The samples taken showed the predominant type organisms were: (1) Lactobacillus, (2) Leuconostoc and (3) yeast. Tomato paste is a very selective medium because of its low pH (4.2–4.4) for particular types of organisms. Using this information, the biochemical by-products of the spoilage organisms in tomato paste were tested. Samples of tomato paste from the third effect evaporators and induced industrial spoilage incidences were cultured in the enrichment medium for time intervals of 2, 4, 16 and 24 hr.

The steam distillate of the third effect sample showed the significant presence of two predominent compounds when run on the GLC. Samples obtained from incidences of industrial spoilage conditions also showed basically the presence of the same two compounds. Pure cultures of bacteria were selected from both the third effect inoculum and the spoiled sample inoculum. The results showed that the bacterium produced Compound A in greater abundance than Compound B and that yeast produced Compound B in greater abundance than Compound A. With retention times of the two compounds known, the identification of the compounds could be done by Mass Spectroscopy. The fragmentation pattern of Compounds A and B respectively, was as shown in Figure 2. Upon feeding the peak fragmentation patterns into the Cyphernet Mass Spectral Search System the two compounds were identified as (1) diacetyl (DA) and (2) acetylmethylcarbinol (AMC). By employing a colormetric method (Byer, 1954) to analyze for the concentrations of DA and AMC, the use of GLC was avoided without any meaningful loss in sensitivity. Although tomatoes naturally contain some DA or AMC, most of it is lost during the low pressure evaporative processes. The residual normally never exceeds 0.02 mg per gram of paste; however, this would depend on the bacterial load in certain stages of the evaporative system such as the third effect in this particular system. When a sample exceeded a concentration of 0.05 mg per gram of paste or greater, it was considered as suspect spoilage. When this condition existed, duplicate samples were taken and run at different intervals such as 0, 2, 6, 12 and 24 hr. Significant relative increases in DA or AMC production signify that the system is contaminated. The 0.05 mg per gram action level varies in correlation to cell count depending on the metabolic capabilities of the particular organisms; however, it was found that the maximum cell count correlated with this level was about 4×10^5 cells per gram in 4–6 hr. The 6-hr concentration parameter seems to be a sufficient compromise between speed and sensitivity. These parameters yield results which are about 100–150 times more sensitive then simple visual inspection of signs of spoilage.

The ability of certain bacteria and yeasts to tolerate low pH values (4.2–4.4) gives them a tremendous selective advantage over other organisms in tomato paste. It is reasonable to assume that this selective factor favors the growth of a small number of organisms, with the pH being the most critical factor. The biochemical justification or logic confirming DA and AMC can obviously be seen in that selective pressure would favor those organisms which could shunt away from acidic end-product fermentation (since the pH is already 4.2–4.4), to neutral end-product fermentations such as DA and AMC.

—Continued on page 1052

ACIDULATION OF HOME CANNED TOMATOES

G. M. SAPERS, J. G. PHILLIPS, F. B. TALLEY, O. PANASIUK and J. CARRE

ABSTRACT

The feasibility of acidifying home canned tomatoes was determined. Citric acid, lemon juice, or vinegar were added at three concentrations to tomatoes which were canned by the raw pack method. The pH of low acid products was lowered effectively by acidulation with lg citric acid monohydrate or 1 tbsp lemon juice per pint. Vinegar was less effective than the other acidulants and also contributed an off-flavor at all levels. Acidulants equilibrated more rapidly when added to filled jars rather than to empty jars before filling. Alternative acidulation recommendations were compared by use of data derived from canning studies and from measurements of the response of high pH raw tomatoes to acidulation.

INTRODUCTION

THE POSSIBILITY that tomatoes having pH values high enough to permit the growth of *Clostridium botulinum* might be used by home canners has prompted canning specialists to consider the need for acidulation. Recently published data indicate that the occurrence of tomatoes having pH values of 4.8 or above is very rare (Sapers et al., 1977, Powers, 1976). Nevertheless recommendations for the addition of acidulants to home canned tomatoes have been issued from a number of sources including state extension specialists (Gould and Gray, 1974), popular magazines (Carper, 1976), home canning cookbooks (Better Homes and Gardens, 1973), and manufacturers of home canning equipment (Anon., 1975a). These recommendations are in some cases inconsistent. For example, the addition of 1/2 tsp lemon juice or 1/4 tsp citric acid per pint container were suggested as alternative (equivalent) means of acidulation although they differed in citric acid content by a factor of 10 (Gould and Gray, 1974). We have found other recommendations which call for the addition of 1 tsp of lemon juice or vinegar per pint (York, 1976) to be of questionable effectiveness, lowering product pH by less than 0.1 pH unit (Sapers et al., 1977). Misleading information of this type has been widely disseminated by the media.

Acidulants may be added to commercially canned tomatoes to reduce the risk of microbiological spoilage in accordance with 21 CFR Part 53.40 (Code of Federal Regulations, 1973). This practice is described in detail by Powers in his recent review (Powers, 1976). The Food and Drug Administration has recently proposed a change in the Good Manufacturing Practice for thermally processed low acid foods (Gardner, 1976) which would classify tomatoes having a pH above 4.6 as a low acid food unless they were acidified to pH 4.6 or below. The current upper limit for tomatoes, pH 4.7, does not provide an adequate safety factor with respect to the ability of *C. botulinum* to grow and produce toxin (Gardner, 1976).

Studies conducted by the USDA at the Eastern Regional Research Center (ERRC) have focused on the occurrence of low acid tomatoes (Sapers et al., 1977, 1978b) and on their response to canning (Sapers et al., 1978a). At this time we report the results of acidulation experiments conducted with low acid tomatoes to evaluate the effectiveness of three acidulants and their compatibility with home canning practices.

Authors Sapers, Talley, Panasiuk and Carré are with the USDA Eastern Regional Research Center, ARS, 600 E. Mermaid Lane, Philadelphia, PA 19118. Author Phillips is with the USDA Biometrical and Statistical Services, Northeast Region and is assigned to the Eastern Regional Research Center.

EXPERIMENTAL

Materials

Samples of 19 cultivars, representing both low and high acid tomatoes, were obtained from Beltsville, MD, Doylestown, PA, Mississippi State, MS. Sodus, MI, and Santa Paula, CA. Tomatoes were transported, ripened, and prepared for analysis and canning as described previously (Sapers et al., 1977; 1978a, b).

Canning and acidulation

Canning experiments were carried out with both ripe and overripe tomato samples which varied widely in acidity. The tomatoes were canned in pint jars by a modification of the USDA raw pack procedure (USDA, 1975) as described previously. Replicate filled jars were acidified after being filled and before being sealed and thermal processed by the addition of acidulants at three concentrations (in duplicate) together with 3g NaCl. Each canning experiment consisted of the six acidified jars, a control (containing 3g NaCl), and a raw composite for analysis. Acidulants included citric acid monohydrate (0.45, 0.72 and 1.00g per 450g product), bottled lemon juice (equivalent to 10, 15 and 20 ml of 5% citric acid solution per 450g product), and distilled white vinegar (equivalent to 10, 20 and 30 ml of 5% acetic acid solution per 450g product). Exact levels of addition of lemon juice and vinegar were calculated from the acidity of each individual bottle, determined by titration with 0.1N NaOH. The quantity of tomatoes in the jars containing lemon juice and vinegar was reduced by a weight equal to the volume of added acidulant so that the net weight and fill of container would be approximately constant. In addition to these acidulants, commercial acidulant tablets developed for home canners (Morton Salt Co., Chicago, IL) also were evaluated. The tablets, which contain 0.454g citric acid (anhydrous) and 2.0g NaCl, were added to the filled jars using one tablet per pint; accompanying controls also contained 2.0g NaCl. All jars were processed in a boiling water bath (100°C) for 35 min.

Canned products were allowed to equilibrate for 1 month at room temperature prior to analysis and sensory evaluation.

Equilibration experiments

Tomatoes were acidified and canned by the standard procedure described above (top addition) and also by adding acidulants and salt to empty jars and then filling with tomato pieces (bottom addition). After being thermally processed, the jars were equilibrated at room temperature for 1, 3 and 6 days. They were opened, and the contents were carefully removed with a small ladle as three 140g portions representing the top, middle, and bottom thirds of the product. Each portion was blended and analyzed separately.

Analyses

Immediately after each set of canning jars was filled, the accompanying raw composite was blended and analyzed for pH, titratable acidity, and response to acidification with 5% citric acid solution, as described previously (Sapers et al., 1977, 1978b). After equilibration, the appearance of canned products was noted, and the samples were blended and analyzed for pH and titratable acidity (Sapers et al., 1978a).

Sensory evaluation

A panel consisting of 16–18 members, screened for ability to recognize different acidulants and levels of acidity, evaluated the canned tomatoes. The tomatoes were blended at high speed for 2 min and served to panelists at room temperature in 2-oz portions. Each panelist was provided with a complete set of coded samples including a control and the acidified products. Panelists were asked to rate the samples using a standard 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). No high pH canned

หอสมุด กรมวิทยาศาสตร

products (composite pH > 4.5) were given to the panel to avoid any possible risk of botulism. The significance of differences in flavor scores between samples was determined by analysis of variance and Duncan's multiple range test.

RESULTS & DISCUSSION

Effectiveness of acidulants

The response of canned tomatoes to acidulation with citric acid monohydrate was determined with samples representing both high and low pH cultivars (Table 1). The lowest level of addition, 0.45 g/pint, (approx 1/8 tsp/pint) reduced the pH of the canned high pH tomatoes by about 0.2 unit. The highest level, 1g per pint (approx 1/4 tsp/pint), reduced the pH by 0.3–0.4 units. Similar results were reported by Leonard et al. (1960), Lamb et al. (1962), and Pray and Powers (1966). pH reductions at all levels of acidulation were smaller in low pH canned tomatoes than in the higher pH samples.

Table 1–Acidulation of canned tomatoes with citric acid monohydrate

			pH			
			Level of addition (g/pint)			
Cultivar	Source	Ripeness[a]	0	0.45	0.72	1.00
Ace	MI	R	4.59	4.42	4.36	4.27
Ace55VF	PA	OR	4.68	4.44	4.36	4.21
Cal Ace	MI	R	4.60	4.40	4.36	4.28
Garden State	MD	R	4.70	4.52	4.40	4.30
MD122	MD	R	4.64	4.36	4.29	4.18
Nova	MD	OR	4.59	4.34	4.28	4.20
Valiant	PA	OR	4.58	4.38	4.28	4.22
Big Girl	PA	R	4.34	4.18	4.10	4.04
Jet Star	PA	OR	4.26	4.12	4.04	3.96
Jubilee	PA	R	4.16	4.10	4.06	3.96
VF10	CA	R	4.23	4.08	4.00	3.95

[a] R = ripe, OR = overripe

Table 2–pH reduction in tomatoes canned with citric acid monohydrate, lemon juice and vinegar

	pH reduction[a]					
	Citric acid (g/pint)		Lemon juice (ml/pint)		Vinegar (ml/pint)	
Cultivar	0.45	1.00	10	20	10	20
Ace55VF	0.20	0.46	0.20	0.37	0.17	0.30
Big Girl	0.16	0.30	0.17	0.32	0.09	0.17
Fireball	0.16	0.33	0.21	0.38	0.13	0.24
Jet Star	0.18	0.38	0.20	0.37	0.17	0.24
San Marzano	0.16	0.28	0.18	0.35	0.21	0.27
Valiant	0.20	0.36	0.19	0.37	0.16	0.25

[a] Acidulants at approx equal concentrations (g acid/pint)

Table 3–Comparison of acidulants for home canned tomatoes

		Effectiveness[a]	
Acidulant	No. sets	Meq basis	Wt basis
Citric acid monohydrate	52	1.02	1.02
Lemon juice	12	1.01	1.01
Vinegar	26	0.55	0.64
Acidulant tablet[b]	3	1.10	1.10

[a] pH reduction with acidulant/pH reduction in composite titrated with 5% citric acid monohydrate solution
[b] Contains 0.454g citric acid (anhydrous)

We have compared citric acid (powder and tablets), lemon juice, and vinegar, the principal acidulants proposed for home canned tomatoes, at different levels of addition in both high and low acid canned tomatoes to determine their relative effectiveness (Table 2). Vinegar is a weaker acidulant on a molar basis than citric acid or lemon juice, which are similar. Citric acid is the major organic acid in lemon juice (Clements, 1964). We have found bottled lemon juice to be relatively constant in acidity: 5 samples representing 3 manufacturers contained between 5.2 and 5.6% titratable acidity, calculated as citric acid. In previous studies (Sapers et al., 1977), we reported a linear relationship between pH and added acid for acidified raw tomatoes. The same relationship applies to canned tomatoes; initial pH values and slopes for regression equations describing acidification with citric acid were the same (no significant differences at 0.05 level) for cans and corresponding raw composites which were titrated with 5% citric acid solution. We calculated acidification curve slopes for all sets of acidulation data and compared each acidulant to citric acid monohydrate by determining the ratio of slopes for cans and composites (Table 3). This method of comparison avoids the problem of batchwise differences in raw material pH and jar-to-jar variation which complicate the direct comparison of acidulants (Table 2). Citric acid and lemon juice are identical in effectiveness, both having slope ratios close to unity, and vinegar is about 2/3 as effective as citric acid, on an acid weight basis (Table 3). Citric and acetic acids have similar equivalent weights; however, citric acid is the stronger acid, its pk_1 value being 3.14 in contrast to 4.76, the pk for acetic acid (at 20°C). The acidulant tablets appear to be slightly more potent than would be predicted from their specified citric acid content; this discrepancy was subsequently confirmed by analysis of the tablets for titratable acidity.

Equilibration of acidulants

Canned tomatoes acidified with citric acid monohydrate or acidulant tablets, added to the top of the filled jars, equilibrated very quickly, probably as a result of convection during processing (Table 4). The top and bottom portions of the contents of each jar differed in pH by less than 0.1 unit within 1–3 days. However, products which were acidulated by the addition of acid to the bottom of the canning jars before filling, equilibrated much more slowly. Even after 6 days, the top portions of the acidified jars were about 0.3 pH unit higher than the bottom portions. The low pH and appearance of the bottom portions suggest that the acidulants dissolved but did not diffuse from the bottom serum layer through the

Table 4–Equilibration of canned acidified Ace55VF tomatoes

			pH		
Acidulant	Method of addition	Day	Top	Bottom	Difference
Citric acid monohydrate[a]	Top	1	3.95	4.06	–0.11
		3	4.03	4.12	–0.09
		6	4.06	4.08	–0.02
	Bottom	1	4.06	3.84	0.22
		3	4.11	3.80	0.32
		6	4.14	3.82	0.32
Tablet[b]	Top	1	4.18	4.26	–0.08
		3	4.18	4.24	–0.06
		6	4.22	4.14	0.08
	Bottom	1	4.32	4.00	0.32
		3	4.29	3.95	0.34
		6	4.28	3.94	0.34
Control[c]	Top	6	4.38	4.39	–0.01
	Bottom	6	4.40	4.32	0.08

[a] 1g per pint
[b] 1 tablet = 0.454g citric acid per pint
[c] 3g salt per pint

dense mass of tomato pieces above. Some acidulation recommendations (Anon., 1975b; Anon., 1976) have specified that acidulants should be added to the jar before the tomatoes in order to achieve an even distribution of acidity. Our results, with the raw pack method of canning, lead us to the opposite conclusion: acidulants should be added after the jars are filled.

Effect of acidulation on acceptability

Sensory evaluation of canned low pH tomatoes to which acidulants had been added indicated that product flavor was not affected adversely by acidulation with citric acid monohydrate or lemon juice, even at the highest levels of addition (Table 5). On the other hand, vinegar was objectionable in some samples at the lowest level and in all samples at higher levels. We obtained similar results with higher pH canned tomatoes prepared from other cultivars including Ace, Ace 55VF, Big Boy, Fireball, Jet Star, Jubilee, San Marzano and Valiant. A number of studies have demonstrated that acidulation with citric acid, at levels comparable to those in our study, did not adversely affect tomato flavor (Powers, 1976). However, off-flavors due to vinegar have been reported by Klippstein (1976) and by Powers (1976). Since vinegar is a weaker acid than citric acid, it would have to be used at higher levels in canned tomatoes to achieve the desired pH reduction. At effective levels, vinegar would probably yield a product with an unacceptable flavor.

Acidulation requirements for high pH tomatoes

Published recommendations for the acidulation of canned tomatoes generally have been based on the results of studies with tomatoes which were not excessively high in pH, i.e., pH 4.3–4.6 (Lopez and Schoenemann, 1971; Pray and Powers, 1966; Leonard et al., 1959; Lamb et al., 1962; Kattan et al., 1956). Only Gould (see Powers, 1976) reported data on the acidulation of higher pH tomatoes (pH 4.5–4.77).

In parallel with the canning studies which yielded data on the acidulation of samples as high in pH as 4.74, we also obtained data on the acidulation of individual raw tomatoes from similar samples having even higher pH values, some as high as 5.1. Linear regression equations describing the titration of 54 such tomatoes with 5% citric acid solution were obtained. Since we previously established that acidulation curves for canned and raw tomatoes were identical, we have used regression coefficients derived from these equations to estimate acidulation requirements if the high pH tomatoes were to be canned. The acidulation requirement, defined as the quantity of acidulant required to lower the tomato pH from its original value to a target pH, can be calculated by dividing the difference between these pH values by the slope of the acidulation curve.

Acidulation requirements for the high pH tomatoes are summarized in Table 6. Three target pH values were used: 4.6, representing the upper pH limit for canned tomatoes proposed by the Food and Drug Administration to minimize the risk of growth and toxin production by *C. botulinum* (Gardner, 1976); 4.3 and 4.4, the latter pH values bracketing the lower limit for the germination of spores of *Bacillus coagulans*, an organism responsible for flat sour spoilage in canned tomato products (Rice and Pederson, 1954).

Acidulation requirements for high pH tomatoes vary widely within and between cultivars. A conservative estimate of the acidulation requirement for all high pH tomatoes should be based, not on the mean, but rather, on a level of acidulation which reduces a given percentage of the high pH tomato population below the target pH. That percentage can be determined from the proposed level of acidulation, the mean acidulation requirement for all cultivars (see Table 6), and the standard deviation for the acidulation requirement, assuming that the acidulation requirement for the high pH tomatoes is normally distributed. We compared a number of recent acidulation recommendations on that basis (Table 7).

Table 5—Sensory evaluation of high acid tomatoes canned with acidulants

Cultivar	Acidulant	Composite pH	Flavor score[a] Control	Low level[b]	High level[c]
Big Girl	Citric acid	4.30	5.50	6.00	5.61
	Lemon juice	4.31	6.58	6.35	6.17
	Vinegar	4.31	6.58	4.82[d]	3.17[e]
Heinz 1350	Citric acid	4.36	6.11	5.94	5.00[e]
	Vinegar	4.28	6.12	4.25[e]	3.43[e]
Manalucie	Citric acid	4.41	6.52	6.41	5.74[d]
	Lemon juice	4.26	5.87	6.12	5.62
	Vinegar	4.30	6.26	5.36	3.31[e]

[a] Nine-point hedonic scale
[b] 0.45g citric acid monohydrate, 10 ml lemon juice or 10 ml vinegar per pint
[c] 1.00g citric acid monohydrate, 20 ml lemon juice or 20 ml vinegar per pint
[d] Significantly different at 0.05 level
[e] Significantly differetn at 0.01 level

Table 6—Acidulation requirements for high pH tomatoes

Cultivar	No. tomatoes pH ⩾ 4.75	Mean acidulation requirement[a] pH ⩽ 4.6	pH ⩽ 4.4	pH ⩽ 4.3
Ace	5	0.32	0.67	0.95
Ace55VF	8	0.44	0.87	1.08
Cal Ace	10	0.36	0.61	0.74
Fireball	6	0.44	0.74	0.90
Garden State	20	0.48	0.84	1.02
Others	5	0.45	0.77	0.93
All cultivars	54	0.43	0.77	0.95
	Std Dev	0.12	0.15	0.19

[a] g citric acid monohydrate per pint tomatoes

Table 7—Effectiveness of different levels of acidulation with high pH tomatoes

Acidulant	Level per pint	Percent of high pH[d] tomatoes reduced to ⩽ pH 4.6	⩽ pH 4.4	⩽ pH 4.3
Citric acid monohydrate	1/4 tsp	99.9	95	60
	1/8 tsp	75	5	1
Citric acid tablet[a]	1	60	2	0
Lemon juice[b]	1 tbsp	99	45	15
	2 tsp	75	5	1
	1 tsp	10	0	0
Vinegar[c]	1 tbsp	75	5	1
	2 tsp	20	0	0
	1 tsp	1	0	0

[a] Containing 0.454g anhydrous citric acid
[b] Containing 5% anhydrous citric acid
[c] Containing 5% acetic acid equivalent to citric acid X 0.64
[d] Assuming acidulation requirements for individual high pH tomatoes (Table 6) to be normally distributed.

Only the highest recommended levels of citric acid and lemon juice would reduce essentially all of the high pH tomatoes represented by our data (Table 6) to pH 4.6 or below. Substantial protection against microbiological spoilage would also be achieved, especially with the citric acid recommendation. The lower level of lemon juice and the two lowest levels of vinegar would be virtually worthless in reducing high

pH tomatoes to or below either target pH (pH 4.6 or pH 4.3–4.4). Other levels of acidulation compared on Table 7 would lower the pH of most high pH tomatoes to 4.6 but not to pH 4.3–4.4. Consequently, if one wished to assure the absence of canned tomatoes exceeding pH 4.6 and also provide protection against spoilage, an acidulation level of 1/4 tsp citric acid monohydrate or one tbsp bottled lemon juice (containing 5% citric acid) per pint would be required. Higher levels of acidulation would reduce the probability of spoilage but would entail the use of inconvenient units of measure, i.e., 4 tsp, and might have an adverse effect on product flavor.

REFERENCES

Anon. 1975a. How to hot pack or cold pack with your home canner. General Housewares Corp., Terre Haute, IN.

Anon. 1975b. Suggest raising acidity when canning tomatoes (Press release). Purdue University, West Lafayette, IN. Sept. 4.

Anon. 1976. Label for Morton New Tomato Canning Tablets. Morton Salt Co., Chicago, IL.

Better Homes and Garden. 1973. "Home Canning Cookbook," p. 44. Bantam Books, Inc., New York.

Carper, J. 1976. Home Canning: The proper way. Readers Digest, August, p. 145.

Clements, R.L. 1964. Organic acids in citrus fruits. 1. Varietal differences. J. Food Sci. 29: 276.

Code of Federal Regulations. 1975. Title 21, Food and Drugs, Section 53.40 Canned Tomatoes; identity; label statement of optional ingredients.

Gardner, S. 1976. Pickled, fermented, acidified and low acid foods. Proposed good manufacturing practices. Federal Register 41(143): 30442.

Gould, W.A. and Gray, E.J. 1974. Canning tomatoes in the home. Cooperative State Extension Service. The Ohio State University. 6/74/10M.

Kattan, A.A., Ogle, W.L. and Kramer, A. 1956. Effect of process variables on quality of canned tomato juice. Proc. Am Soc. Hort. Sci. 68: 470.

Klippstein, R. 1976. Cornell University. Personal communications.

Lamb, F.C., Lewis, L.D. and Kimball, J.R. 1962. Factors affecting the pH of tomatoes. Research Report 61-C-44. National Canners Assoc., Berkeley, CA.

Leonard, S.J., Pangborn, R.M. and Luh, B.S. 1959. The pH problem in canned tomatoes. Food Technol. 13: 418.

Leonard, S.J., Luh, B.S. and Pangborn, R.M. 1960. Effect of sodium chloride, citric acid, and sucrose on pH and palatability of canned tomatoes. Food Technol. 14: 433.

Lopez, A. and Schoenemann, D.E. 1971. Updating developments in acidification of canned tomatoes. Canning Trade 93(19): 8.

Powers, J.J. 1976. Effect of acidification of canned tomatoes on quality and shelf life. Critical Reviews in Food Science and Nutrition, p. 371.

Pray, L.W. and Powers, J.J. 1966. Acidification of canned tomatoes. Food Technol. 20(1): 87.

Rice, A.C. and Pederson, C.S. 1954. Factors affecting growth of Bacillus coagulans in canned tomato juice. 2. Acidic constituents of tomato juice and specific organic acids. Food Research 19: 124.

Sapers, G.M., Panasiuk, O. and Carré, J. 1978a. Effects of thermal processing and salt on the pH and acidity of home canned tomatoes. J. Food Sci. 43: 951.

Sapers, G.M., Phillips, J.G., Panasiuk, O., Carré, J., Stoner, A.K. and Barksdale, T. 1978b. Factors affecting the acidity of tomatoes for home canning. HortScience 13(2): 000.

Sapers, G.M., Stoner, A.K. and Phillips, J.G. 1977. Tomato acidity and the safety of home canned tomatoes. HortScience 12: 204.

U.S. Department of Agriculture. 1975. Home canning of fruits and vegetables. Home and Garden Bull. No. 8. March 1975. Washington, D.C.

York, G.K. 1976. Things to know before you can fruits and vegetables (part 2). (Press release) University of California, Davis, April 19.

Ms received 8/26/77; revised 11/28/77; accepted 12/1/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

The authors thank Professors Hugh C. Price and Theodore Wishnetsky of Michigan State University, Professors Milo Burnham and Gale R. Ammerman of Mississippi State University, Theodore C. Torrey and Lois Stringer of the W. Atlee Burpee Co., and Dr. Allan K. Stoner, USDA Beltsville Agricultural Research Center, ARS, for their help in providing tomatoes for our canning studies. We also acknowledge the assistance of Sandra P. Graham, Statistical Clerk at ERRC, and William M. Brooks, Paula Civitillo, Anthony Dixon, and Cindy Oshman, Summer employees.

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.

MONITORING TECHNIQUE FOR BULK TOMATO PASTE . . . From page 1048

In conclusion, determination of diacetyl and acetylmethylcarbinol production in conjunction with a minimal of traditional bacteriological testing offers a rapid, sensitive, and economical means of monitoring the sterility of an aseptic tomato paste system. Media costs, laboratory technician manpower hours, and obvious savings of tons of finished product almost make this monitoring parameter a necessity in an aseptic system of this type.

REFERENCES

Byer, E.M. 1954. Visual detection of either diacetyl or acetylmethylcarbinol in frozen concentrated orange juice. Food Technol. 8: 173.

Jones, A.H. 1936. A study of organisms causing gaseous spoilage of canned tomato products. Canadian Canners & Food Manufacturer 7: 9.

Pederson, C.S. 1949. The type of organisms found in spoiled tomato products. NY State Agric. Expt. Stat. Tech. Bull. 285.

Ms received 4/7/77; revised 8/7/77; accepted 8/12/77.

The author thanks Dr. Jack Ralls of National Canners Association at Berkeley, CA, for valuable discussions and suggestions throughout the course of this investigation. The author also thanks Dr. Ralls and Mr. Richard Lane for instructions in use of NCA's GLC-Mass Spectroscopy set-up.

pH OF TOMATOES CANNED AT HOME IN GEORGIA

JOHN J. POWERS and DAVID R. GODWIN

ABSTRACT

Of 387 jars of tomatoes or tomato juice canned at home in Georgia, seven showed microbial growth indicative of underprocessing or seal failure. Of the remaining 380 jars, the pH of the contents of one jar was 4.65; otherwise all the pH values were below 4.6. Extreme-value analysis was applied to the pH data to estimate the likelihood of the pH of the content of an individual jar exceeding 4.8. The probability was 0.0014. The total acidity, buffering capacity, ascorbic acid content, grams malic acid required to lower the pH to 4.3 and 4.1, and the vacuum of the container were also determined.

INTRODUCTION

ABOUT 20 YR AGO concern began to be expressed that the pH of tomatoes might be so high at times as to permit *Clostridium botulinum* to produce toxin in the canned products (Powers, 1976). The National Canners Association (Farrow, 1963) met the issue head on by determining the pH of 14,728 individual fruits in 1959 and 1961. Approximately 6% of the tomatoes were above 4.6, and 0.46% were above pH 4.8. Spores of *Cl. botulinum* have been shown to grow out and produce toxin at pH 4.8. and above (Townsend et al., 1954). In theory, one tomato high in pH might provide a zone in a can where spores of *Cl. botulinum* could grow, but judging from the billions of cans of tomatoes packed commercially over the years without incidence of botulism, this task is too much for the organism at a borderline pH level after having been subjected to the lethal effect of the thermal process. Nelson (1956) and Smolka et al. (1974) reported, for example, that the pH range for recovery by heat-damaged cells and spores is narrower than for growth by nonstressed organisms.

In recent years there have been five outbreaks of botulism from either home-canned tomatoes or tomato juice (CDC, 1974) and over the years, 19 outbreaks in the United States and Canada (Powers, 1976). There are at least three reasons which may account for the distressful experience of home-canners as compared with commercial canners. One is that home gardeners use a greater number of varieties and some of these may be higher in pH (Sapers et al., 1976; Powers, 1976: Scientific Status Summary, 1977) than those used by commercial canners. Secondly, the lethality of thermal processes used at home may be less, and thirdly, simply because there are so many more home canners there is greater likelihood that some would fail to use sound procedures. This report is intended to cast some light on the first and third of these possibilities.

Sapers et al. (1976) reported that of 55 varieties screened, only the pear type and the "standard red" ranged above pH 4.6. Wolf et al. (1977) examined 109 varieties. None was above pH 4.6. their study included the canned product as well as the raw tomatoes, and they made over 1,000 determinations. Fields et al. (1977) also studied the canned product. They found that of 176 jars not containing "viable" microorganisms, one jar was at pH 4.7 and one at 4.8. Of 116 jars containing viable microorganisms, the content of one jar was at pH 5.0.

This study reports the pH of 380 jars of tomatoes canned at home in Georgia. The total acidity, buffering capacity, reduced ascorbic acid content, mg malic acid required to lower the pH to 4.1 and jar vacuum were also determined.

Authors Powers and Godwin are with the Dept. of Food Science, University of Georgia, Athens, GA 30602.

0022-1147/78/0004-1053$02.25/0
© 1978 Institute of Food Technologists

EXPERIMENTAL

ONE JAR of tomatoes or tomato juice from each of 387 homemakers in Georgia was secured for us by Mrs. Catherine Sigman, Extension Home Economist. The homemakers donated the tomatoes and they provided information about the variety, processing method used, and the time of season when packed if they were sure of the facts for the particular jar donated.

A vacuum gauge was used to measure the vacuum. The pH was measured electrometrically after standardizing the pH meter against reference buffers at pH 4.0 and 7.0. Simply because we had the product on hand, we decided to make a crude estimate of the reduced ascorbic acid content. This was done by visually titrating the serum (after centrifuging down the solids) with 2,6-dichlorophenol-indophenol standardized against pure ascorbic acid (AOAC, 1970). Total acidity and the buffering capacity (van Slyke, 1922; Sane et al., 1950) were determined by titrating 25 ml of the serum to pH 8.35 with a standardized 0.01N NaOH solution. To estimate the amount of acid which would be required to lower the pH to 4.1, if acidification were to be used, 25 ml of serum was titrated with a 0.63N solution of malic acid.

RESULTS & DISCUSSION

OF THE 387 JARS collected, seven showed visible mold growth or evidence of other microbial activity. The content of one jar was at pH 7.0. The implications of this will be discussed below.

Table 1 lists the means and ranges for total acidity, buffering capacity, reduced ascorbic acid content, grams malic acid required to lower the pH to 4.3 and 4.1, and the vacuum of the 380 sound containers. Although there was a significant correlation, $P = < 0.001$, between the vacuum and the ascorbic acid content, r was only 0.25. Long storage (some of the tomatoes donated were from the 1972, 1974 and 1975 seasons), high storage temperatures, excessive headspace and perhaps rather low ascorbic acid content to start with probably account for most of the low values. According to the record

Table 1—Mean, range and standard deviation for variables measured

Variable	Mean	Range	Standard deviation
pH	4.22	3.43–4.65	0.143
Total acidity (as % citric acid)	0.39	0.22–0.65	0.110
Buffering capacity (as milliequivalents)	0.30	0.05–0.61	0.10
Reduced ascorbic acid, mg %	11.97	0.0–24.6	5.04
Malic acid required to lower pH to 4.3, g/100g tomato	0.084	0.00–0.198	0.057
Malic acid required to lower pH to 4.1, g/100g tomato	1.31	0.00–3.29	0.99
Vacuum, inches Hg	16.2	0.00–27.1	5.45

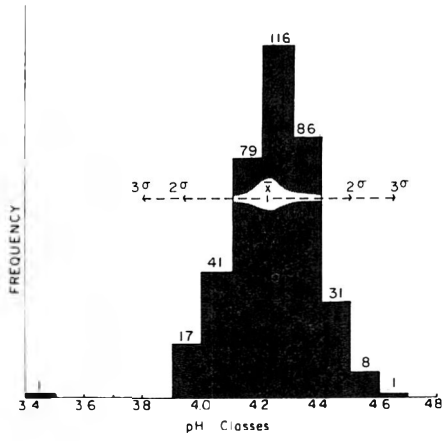


Fig. 1—Histogram for the pH of the contents of 380 jars of home-canned tomatoes or tomato juice classified at 0.10 intervals. The figure at the top of each column shows the number of values within that pH range. The scale superimposed on the histogram shows where the mean pH occurred and the 2σ and 3σ ranges.

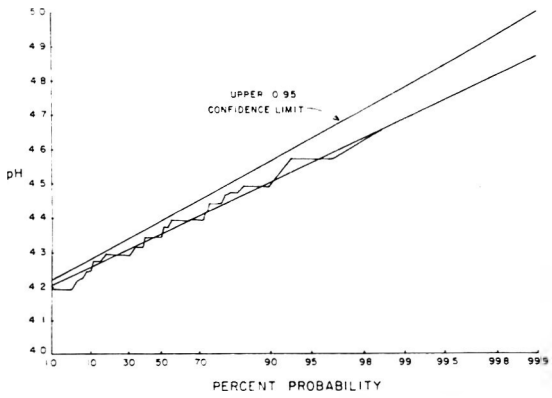


Fig. 2—Extreme-value regression plot for the pH of tomatoes and the upper 0.95 confidence limit.

furnished, one homemaker had added ascorbic acid; this accounts for the high upper value. The ascorbic acid content was otherwise within the range Brecht et al. (1976) reported for fresh tomatoes. The correlation between the buffering capacity and pH was -0.3252 ($P = < 0.001$), a consequence of which is that tomatoes already high in pH would be the least resistant to an increase in pH. In general, about 1.3g of malic acid per 100g of tomato was required to lower the pH to 4.1. This amount is in good agreement with prior studies (Pray and Powers, 1966). Since citric acid and malic acid are so much alike in their properties, the amount of malic acid needed also is a good indication of the amount of citric acid which would be required. The titrations were carried to pH 3,8, so estimates could be made to other pH levels.

pH Range

Figure 1 shows a histogram for the pH values of the 380 sound jars. One value was pH 4.65; otherwise no values were above 4.6. The jars examined probably were representative of those canned in this state since they had come from all sections of the state and thus our three major soil-climate zones were accounted for. Over 20 varieties of tomatoes were included. In some cases, the homemaker did not know the variety. Instead of the pH being higher today as some claim, the average pH was slightly lower than Bigelow and Cathcart (1921) reported 56 yr ago.

With respect to pH, the Food and Drug Administration in issuing proposed regulations for pickled, fermented, and acidified foods (Federal Register, 1976) is in the process of removing an exception tomatoes have. At present, the pH can be as high as 4.7 before the Good Manufacturing Practices for low-acid, thermally-processed foods apply to tomatoes. Under a re-alignment of the new acid-food regulations and the low-acid ones, the upper pH limit for tomatoes will be 4.6, the same as for other low-acid, thermally-processed foods. With one slight exception, all the canned tomatoes we examined were thus within the acid-food catagory.

Estimate of higher pH

To estimate the likelihood of even higher pH values existing, extreme-value analysis (Powers et al., 1962; Powers and Ware, 1976) was applied to the highest pH value among each six pH determinations made. Figure 2 shows an extreme-value plot and the upper 0.95 confidence level based on 63 values. Approximately 99.16% of future "highest" values may be expected to be below pH 4.8 or, in other words, only 8.4 times out of 1,000 sets of six jars each, might the pH be above pH 4.8. The probability of a single container being over 4.8 is thus 0.0014. This estimate is somewhat lower than the results of the NCA (Farrow, 1963) based on actual measurement of the pH of 14,728 individual raw fruits but in line with its results (Farrow, 1976) when 12,861 composite samples of raw tomatoes, 7,460 cans of tomato juice and 3,921 cans of tomatoes were examined (Powers, 1976).

One precaution which should be kept in mind in estimating the pH of tomatoes is that soft, over-ripe tomatoes (Villarreal et al., 1960; Sapers et al., 1976; Scientific Status Summary, 1977) are generally higher in pH than firm, ripe ones.

pH and botulism outbreaks

A question which naturally arises is whether there is a connection between the rare occurrence of high pH tomatoes and the rare occurrence of outbreaks of botulism from home-canned tomato products. The question cannot be answered, of course, but it is unlikely that pH alone is the cause. Fields et al. (1977) pointed out that the contents of the jar they encountered having a pH of 5.0 contained viable microorganisms. The tomatoes had been prepared by an open-kettle method, which involved no thermal processing and which method is not recommended by the Missouri Extension Service. In our state, a 10-min water-bath process is specified for hot-packed tomatoes (Sigman, 1977). This process is based on the Federal recommendations (USDA, 1972) still in force as of December 1977. The spoiled tomatoes (at pH 7.0) which we encountered possessed such a nauseous odor that they would not have been eaten. They had been hot-packed but did not recieve the 10-min process specified. Both the high-pH tomatoes described by Fields et al. (1977) and the spoiled tomatoes we observed were packed in violation of the processing recommendations of the two respective Extension Services. Fields et al. (1977) stated that many of the cold-packed, water-bathed jars of tomatoes they examined must not have received the water-bathed processes recommended by the U.S. Department of Agriculture. Powers (1976) pointed out that some of the reports of botulism outbreaks from home-canned tomatoes clearly indicated that recommended practices had not been followed. The fact that 6 of the 7 jars of spoiled tomatoes we encountered has mold growth (the tomatoes at pH 7.0 contained a mixed culture) indicates that the homemakers had not processed the tomatoes sufficiently to destroy vegetative cells, seal failure had occurred, or else the homemaker had erred in some other way. No matter what the pH of tomatoes is, failure to use processes which will destroy vegetative cells is grave indeed. There is a little evidence that vegetative cells of *Cl. botulinum* can grow down to pH 4.1. Nikolaava and Mazokhina (1972) reported that vegetative cells of *Cl. botulinum* were capable of multiplying and developing 1,000

MLD toxin in mushroom sauce at pH 4.1–4.35. Spore germination was inhibited below pH 5.0. If the process is not long enough to destroy vegetative cells, then there is a possibility *Cl. botulinum* can grow directly, and if not, that other vegetative cells may flourish and raise the pH so that *Cl. botulinum* can grow and produce toxin. If recommended processes are used, growth of *Cl. botulinum* either directly or indirectly by this means is very unlikely.

The question really is whether there is evidence that the pH is inherently high enough at times for spores to germinate. The experience of the commercial canners and an overwhelming number of home canners is that *Cl. botulinum* does not grow in tomatoes given recommended thermal processes at boiling-water temperature. The outbreaks of botulism which have occurred probably represent underprocessing, not growth because of high pH. The pH is above 4.7 or 4.8 sufficiently often so that more outbreaks would have occurred if pH alone were enough to have set the stage for growth. The combined action of a borderline pH level and the lethal effect of the heat from the processes traditionally used seem to hold the spores in check. If one assumes Rozanova's (1974) results are universally valid, spores can even grow out at pH 4.7. He reported that when 1450 spores per ml were in tomato juice, toxin was formed at pH 4.7. If the spore count were lower, toxin was not formed even after 13 months. Considering that about 6% of the tomatoes canned are above 4.6, present thermal processes must be adequate; otherwise, there would have been many more outbreaks of botulism. The outbreaks probably cannot be attributed to a matter of high pH, but of failure to abide by recommended process times so as to destroy vegetative cells and heat-damage the spores so that they cannot grow out. This is not a pH problem, but one of education: to get people to follow directions and to understand why they should.

Aside from practical experience, there are scientific grounds to explain why outbreaks of botulism from tomato products are so rare in spite of the pH being above 4.6 at times. While the thermal resistance of spores increases (up to a point) as the pH increases, the percentage of tomatoes being at or above a given pH level decreases. Xezones and Hutchings (1965) reported a D value of 0.788 at 110°C for spores of *Cl. botulinum* heated in a spaghetti-tomato-cheese substrate at 4.6. At pH 5.0, the D value was 1.0. Their data probably over-estimate the thermal resistance of *Cl. botulinum* in tomatoes because they subcultured into a good bacteriological medium and their food product was a better substrate to start with than are tomatoes, but their data and ours can be used to illustrate the general relationship. Using their D values, if enough heat were applied at pH 4.6 to produce a lethality of 12D, the combined probability of survival and of tomatoes being above 4.6 (see Fig 2) would be $(1 \times 10^{-1\,2})$ (0.063) or $6.3 \times 10^{-1\,4}$. If the same amount of heat were applied but the pH were 5.0, the joint probability would be $(1 \times 10^{-9.5})$ (1×10^{-3}) or $1 \times 10^{-1\,2.5}$. The risk has increased somewhat but not inordinately. However, the risk might be quite great for some other product, the average pH of which is higher or the frequency distribution of which is skewed in a different manner so that a greater percentage of values would be above 4.6.

Though we and Wolf et al. (1977) did not encounter samples above pH 4.6, there is a need to examine the pH of tomatoes more exhaustively. Homemakers need unequivocal directions. For several years they have been bombarded with all types of alarmist articles in popular periodicals and some of the recommendations from food technologists have also left them in doubt. Our state, for example, following the lead of the USDA gives 35- and 45-min water-bath processes for pints and quarts of tomatoes, but it also cautions homemakers that they should use 45- and 55-min processes if the tomatoes are "low-acid." A NCA Press Release (Anonymous, 1974) advised homemakers to add citric acid until the tomatoes *taste* acid. Currently, there are recommendations to pressure can tomatoes, to acidify them, and to use longer processing times. There are good reasons for having more than one procedure, but the homemaker should not have to guess what the pH of her tomatoes is in order to select the process to be used. Scientists should resolve that matter for her.

REFERENCES

Anonymous. 1974 NCA Information Letter No. 2469, National Canners Assn., Washington, DC.

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

Bigelow, W.D. and Cathcart, P.H. 1921. Relation of processing to the acidity of canned foods. Bull. 17-L, National Canners Assns, Washington, DC.

Brecht, P.E., Keng, L., Bisogni, C.A. and Munger, H.M. 1976. Effect of fruit portion, stage of ripeness and growth habit on chemical composition of fresh tomatoes. J. Food Sci. 41: 945.

CDC. 1974. Botulism in the United States, 1899—1973. DHEW Publ. No. (CDC) 74-8279, Center for Disease Control, Atlanta, GA 30333.

Farrow, R.P. 1963. A survey of pH variation in canning tomatoes. Research Rept. No. 1—63, National Canners Assn., Washington, DC.

Farrow, R.P. 1976. Private communication to John Powers, March 4, 1976.

Federal Register. 1976. Proposed Good Manufacturing Practices. Pickled Fermented, Acidified and Low-acid Foods. Federal Regist. 41: 30221.

Fields, M.L., Zamora, A.F. and Bradsher, M. 1977. Microbiological analysis of home-canned tomatoes and green beans. J. Food Sci. 42: 931.

Nelson, F.E. 1956. The influence of the pH of the plating medium upon enumeration of heat-treated bacteria. Bact. Proc. 1956: 40.

Nikolaeva, S.A. and Mazokhina, N.N. 1972. Multiplication of Clostridium botulinum type B in preserved mushroom sauce (in Russian). Vopr. Pitan. 2:83—86. [In Chem. Abstr. (1972) 77: 30027w.]

Powers, J.J. 1976. Effect of acidification of canned tomatoes on quality and shelf life. CRC Critical Reviews in Food Sci. & Nutr. 7: 371.

Powers, J.J., Pratt, D.E., Carmon, J.L., Sommatmadja, D. and Fortson, J.C. 1962. Application of extreme-value methods and other statistical procedures to heat-penetration data. Food Technol. 16: 80.

Powers, J.J. and Ware, G.O. 1976. Comparison of SIGMPLOT, prohibit and extreme-value methods for the analysis of threshold data. Chemical Senses & Flavor 2: 241.

Pray, L.W. and Powers, J.J. 1966. Acidification of canned tomatoes. Food Technol. 20: 87.

Rozanova, L.I. 1974. The development of microorganisms causing botulism in preserved tomato products (in Russian). Trudy, Vsesoyuznyi Nauchno-Issledovatel "Skii Institut Konservnoi I Ovoshchesushil" Noi Promyshlennosti 21: 54.

Sane, R.H., Powers, J.J., Morse, R.E. and Mills, W.C. 1950. The pH and total acidity of raw and canned pimientos. Food Technol. 4: 279.

Sapers, G.M., Huhtanen, C.N. and Phillips, J.G. 1976. ERRC Research on Tomato Acidity. Report to USDA cooperators and others, Eastern Reg. Res. Center, USDA, Philadelphia, PA.

Scientific Status Summary. 1977. Home canning. Institute of Food Technologists, Chicago, IL.

Sigman, C.C. 1977. So Easy to Can. Cooperative Extension Ser., University of Georgia College of Agriculture, Athens, GA.

Smolka, L.R., Nelson, F.E. and Kelley, L.M. 1974. Interaction of pH and NaCl on enumeration of heat-stressed Staphylococcus aureus. Appl. Microbiol. 27: 443

Townsend, C.T., Yee, L. and Mercer, W.A. 1954. Inhibition of the growth of Clostridium botulinum by acidification. Food Res. 19: 536.

USDA. 1972. Home canning of fruits and vegetables. Home and Garden Bull. No. 8, U.S. Dept. of Agriculture, Washington, DC.

van Slyke, D.D. 1922. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. J. Biol. Chem. 52: 525.

Villarreal, F., Luh, B.S. and Leonard, S.J. 1960. Influence of ripeness levels on organic acids in canned tomato juice. Food Technol. 14: 176.

Wolf, I.C., Schwartau, C.M., Karson, K.C., Davis, D.W. and Zottola, E.A. 1977. Home canning of food: tomatoes, tomato products and other acid foods. Abstract 206, Program Abstracts, 37th Annual Meeting of the Institute of Food Technologists, Chicago, IL.

Xezones, H. and Hutchings, I.J. 1965. Thermal resistance of Clostridium botulinum (62A) spores as affected by fundamental food constituents. 1. Effect of pH. Food Technol. 19: 1003.

Ms received 9/28/77; revised 12/24/77; accepted 12/30/77.

RECOVERY OF BETALAINES FROM RED BEETS BY A DIFFUSION-EXTRACTION PROCEDURE

ROBERT C. WILEY and YA-NIEN LEE

ABSTRACT

A continuous counter-current diffusion apparatus was used for the extraction of betalaines from red beets. The apparatus was used with an incline of 4.0° and 4.5° and was operated at temperatures from 70–74°C. The efficiency of water and of citric acid solutions (0.050–0.125%) to extract betalaines was investigated. The solvent to sliced beet ratios were approximately 1.5/1. Best recoveries of about 71% betanine were found in the more acid systems at 4.5° incline angle. The betacyanine/betaxanthine ratio could be varied and was influenced by temperature and pH of the various processing operations used to produce beet juice powders i.e. diffusion-extraction, concentration and spray drying.

INTRODUCTION

RECENT PROBLEMS with the safety of artificial dyes have stimulated research on natural plant pigments and pigments of microbial origin. As a substitute for the red synthetic dyes there has been considerable interest in the red colorants from such plant sources as beet, grape, roselle, cranberry and other (Riboh, 1977). The red beetroot (*Beta vulgaris* L.) shows promise as a natural red colorant, and depending on cultivar can contain various proportions betacyanines and betaxanthines (red and yellow colorants). The yellow beetroot (*Beta vulgaris* L. cv Burpee's Golden) is a source of natural water soluble yellow colorants (betaxanthines). The main red pigment in red beetroots is betanine and the dominant yellow pigment is vulgaxanthine-I, which is also the main pigment in the yellow beetroot. The hue of betanine is not greatly affected by pH in the range from 3.0–7.0 according to Nilsson (1970) and Riboh (1977). However, out of this pH range marked color changes are associated with pH change. Unfortunately the beet betalaines are heat labile and according to von Elbe et al., (1974) in degradation studies of betanine in model systems, the degradation follows first order reaction kinetics and at 50°C and pH 5.0 the half-life of a betanine solution is 312 ± 30 min.

Although purification of plant pigments has progressed rapidly and a great deal of research on beet pigments has been conducted by Nilsson (1970) and Adams and von Elbe (1977) and others using column chromotography, there has been less recent information concerning recovery methods of plant pigments. Most research has centered around pressing and centrifugal methods (Philip, 1974), and these operations are generally followed by an extraction procedure (Chiriboga and Francis, 1970). The literature does not adequately report the recovery procedures used for beet powder preparations now commercially available on the market, although the manufacturers indicate the powders were initially recovered by a pressing operation. One such press, the Willmes press, Stollenverk (New Jersey) using an expandable internal rubber tube has been used to press the juice from steam-blanched sliced beet tissue. The yield of the betanine from this press procedure was about 45–50% (Lee, 1976).

Authors Wiley and Lee are with the Food Science Program, Dept. of Horticulture, University of Maryland, College Park, MD 20742.

0022-1147/78/0004-1056$02.25/0
© 1978 Institute of Food Technologists

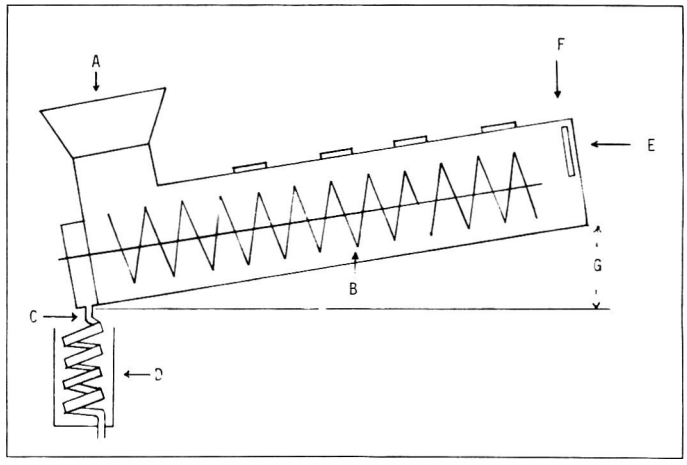


Fig. 1—Schematic of DDS diffusion-extraction unit used to recover betalaines from sliced beets. (A) Sliced beet inlet; (B) Helix 7 flights; (C) Beet juice outlet; (D) Beet juice cooler; (E) Extracted beet cossette discharge; (F) Acidified water inlet; (G) Adjustable incline.

Although extraction and diffusion-extraction procedures have been available for many years they have been automated recently for recovery of sugar from sugar beets (Brüniche-Olsen, 1962) and apple juice from crinkle cut apples (Laursen, 1974; Lüthi and Glunk, 1976).

This research was initiated to determine the feasibility of adapting a diffusion apparatus to continuous counter-current extraction of betalaines from raw beet tissue.

EXPERIMENTAL

Raw Product

Samples of red beets were grown in New York State and in California. The beet cultivars studied were Dark Red Detroit and Garnet and were held in 2°C storage at 85% relative humidity for up to 2 months prior to processing.

Apparatus and procedure

De Danske Sukkerfabrikker (DDS) pilot model B stainless-steel diffusion apparatus which employs a continuous stepless counter-current extraction principle was used to extract betalaines from beet tissue. Figure 1 shows a schematic of the diffusion-extraction equipment. The sliced raw beet cossettes are filled at the lower end of the diffuser at a prescribed rate in the presence of N_2 (A). A 7-flight helix (B) carries the raw material counter-current to the extracting liquid. The extract of beet juice is drawn from the lower end of the diffusion apparatus (C) and chilled (D) and the extracted cossettes are discharged from the upper end of the apparatus (E). The apparatus includes steam-jacketed chambers to heat the raw material and break the tissue at the time that it is placed in the machine. The extraction solvent is introduced from the top (F). The slope of the diffuser is controlled by adjustment of legs (G). Variables which were considered in preliminary operations of the slope diffuser described in Figure 1 were as follows: slope or angle of the diffuser, rpm's of the helix, temperature of the three steam-jacketed sections, flow rate of the extracting solvent, temperature of the extracting solvent, input rate of the crinkle sliced beet cossettes, cossette thickness, and atmosphere of the diffuser. The results of three preliminary operations, six hours in length, which were varied continuously resulted in the optimized conditions which are reported in Table 1.

The slicer used to prepare the raw beets was a stainless steel centrif-

ugal type Urschel model GKS slicer (Valparaiso, IN) which will produce crinkle cuts from 1.5 mm to 15.8 mm thick. The cuts used in the various experiments were varied but 3.0 mm gave the best results.

The major variable in the experiments was acidity of the extracting systems which was 0.000%, 0.050%, 0.090% and 0.125% citric acid solutions in experiments 1–4 respectively.

The raw beet juice extract was treated in opaque 5-gal containers with 4 ml the pectinase enzyme concentrate Spark-L, Miles Laboratory, (Elkhart, IN), for 36 hr at 2°C. The enzyme clarified juice was pressure filtered through fine diatomaceous earth using pressures up to 10 lb/in^2. The resulting beet juice showed no cloud.

The extracted beet liquids from Experiments 3 and 4 were concentrated in a Buchler Flash Evaporator (FE-2) at 50–55°C for 1 hr to ca 26° Brix. Spray-dried samples were handled in portable Niro Atomizer at inlet tempertures ranging from 135–150°C. A hydrolyzed cereal solid was added as drying aid to equalize solids in the concentrates to 30% at the start of the spray drying operation.

Analytical

According to Nilsson, (1970) there was little error involved in calculation of pigment concentrations if all betacyanines were considered betanine and all betaxanthines were considered vulgaxanthine-I since betanine, isobetanine and vulgaxanthine-I comprised over 95% of the color in the beetroot. Determination of betanine (betacyanines) and vulgaxanthine-I (betaxanthines) in beet juice: Ten grams of extracted beet juice were added to 0.1M pH 5.0 McIlvaine's citric-phosphate buffer solution made up to 1000 ml. The diluted juice was filtered through Whatman No. 1 paper if it were not clear. To determine the absorbance of the diluted juice the McIlvaine buffer was also used as blank sample. The absorption at 537 nm, 476 nm and 600 nm was recorded, respectively. The percentage of betanine and vulgaxanthine-I was calculated from the equations (Nilsson, 1970) as follows:

$$x = 1.095\ (A_{537} - A_{600})$$
$$y = -0.258\ A_{537} + A_{476} - 0.742\ A_{600}$$

where "x" is the absorption of betanine and "y" is the absorption of vulgaxanthine-I. Thus, the concentrations of betanine and vulgaxanthine-I can be calculated, as $E^{1\ cm}_{1\%} = 1120$ for betanine and $E^{1\ cm}_{1\%} = 750$ for vulgaxanthine-I.

Determination of betanine (betacyanines) and vulgaxanthine-I (bextaxanthines) in raw beet tissue or extracted beet cossettes: Representative samples of beetroot or beet cossettes from each lot were selected. The raw beets were washed and trimmed to excise the growing crown. Equal weights (1 kg/L) of beet tissue were added to McIlvaine citric-phosphate 0.1M pH 5.0 buffer solution. The mix was blended for 2 min. Ten grams of the blended samples were diluted with distilled water to 1L and then filtered through Whatman No. 1 paper. The absorbance of the filtrate at 537 nm, 476 nm and 600 nm was determined as before. The blending and mixing steps should be completed under a nitrogen atmosphere at 2°C in order to prevent the degradation of the pigments. The total solids were determined by oven method and the refractive index of the soluble solids of the beet juice was determined with an Abbey and an ATAGO N1 hand refractometer.

The data for the diffusion-extraction method were collected on a time basis, i.e., on an hourly basis. The betanine recovered in juice or loss in cossettes was calculated from the amount of betanine in the hourly obtained juice or in the hourly discharged cossettes divided by the amount of betanine in the hourly input raw beet. The same procedure was followed for vulgaxanthine-I.

Table 1–Optimum operating conditions of the DDS slope diffuser Model B for Experiments 1–4

Diffuser slope 4.0–4.5°	Extracting liquid flow rate 18 1/hr
Diffuser temp 70–74°C	Liquid temp 35°C
Helix rpm 0.12–0.16	Cossettes retention 56 min
Diffuser atmosphere, N_2	Juice retention 51 min
Raw cossette feed rate 12 kg/hr	Raw cossette thickness 3.0 mm

Table 2–Pigments, °Brix and pH conditions in the beet cossettes across the 7 helix flights of the diffuser

Helix flight	Zone	% Betanine	% Vulgaxanthine-I	Red/yellow ratio	°Brix	pH
1	Cossette inlet	0.026	0.024	1.08	6.2	6.03
2	–	0.024	0.021	1.14	5.8	5.95
3	–	0.018	0.016	1.13	4.8	5.90
4	Middle	0.014	0.011	1.27	3.8	5.82
5	–	0.010	0.008	1.25	3.0	5.65
6	–	0.007	0.006	1.16	2.0	5.28
7	Cossette discharge	0.004	0.003	1.33	1.2	4.58

RESULTS & DISCUSSION

BETANINE RECOVERY was emphasized in the 4 main experiments, each of approximately 5 hr duration, and was based on the optimized operating parameters developed from 3 preliminary diffusion-extraction operations. The significant variable studied in the 4 experiments was acidity level of the extracting liquid used to stabilize and preserve the betanine and vulgaxanthine-I. The operating parameters used in the 4 experiments are presented in Table 1.

It was obvious during the preliminary experiments that all of the variables in Table 1 could not be studied in detail because the time of a typical diffuser operation may vary from 5–24 hr or more in duration. Each operating variable at a rather slow helix speed was studied for an hour or more to determine best conditions. It was clear that much more operation time would be required to determine optimum conditions for each of the variables studied and that a wide range of different beet cultivars should be used if possible. The diffuser slope should be increased to reduce retention time of the juice to approximately 30 min. Brüniche-Olsen (1962) felt a 7° incline was best for extraction of sugar beets. It was also very important that the raw beet tissue be heated as quickly as possible in the diffuser to inactive endogenous oxidase enzyme systems since the sliced cossettes or raw beets were not blanched prior to extraction. Blanching was a possibliity although an increased recovery would have to off-set the pigment losses during blanching induced by heat and leaching.

The data in Table 2 show the betanine, vulgaxanthine-I, brix and pH gradients in cossettes in the 7 flights of the diffuser resulting from an acidified operation. Results indicated that the betanine and vulgaxanthine-I concentrations dropped steadily as the beet cossettes moved through the diffuser. Brix of the tissue was greatly reduced while the pH of the tissue gradually became more acid as the cossettes moved countercurrent to the acidified water. The red/yellow ratios in Table 2 show that heat and fairly low acidity are more damaging to yellow than red pigments in the cossettes and that the more acid pH's at the cossette discharge end of a diffusion-extraction operation produce a beet juice more violet in color because of the destruction of the yellow colorant.

The betanine recovery in Table 3 is based on the best hourly operation in the 4 main experiments. The greatest yields of betanine were found in Experiments 2 and 3 at an incline of 4.5° and a fairly low level of acidity in the extracting solvent. It was expected that the recovery in Experiment 4 (0.125%) would be higher but because of a longer retention time of the juice in the diffuser at the 4.0° incline yields were lower. In Experiment 4 the cossette pH was slightly lower than necessary (pH 3.8) and this may have stimulated the betacyanine decolorizing enzyme reported by Soboleva et al. (1976), who found the pH optimum of this system to be pH 3.4. There was a significant increase in betanine yield in all acidified extractions as compared with a potable H_2O system. This increase appeared to be from 10–20% over the potable H_2O extraction. Acidification of the extracting liquid needs to be studied further to ascertain if there may be increases in extraction efficiencies from the use of citric acid solutions greater than those studied. Acidifying compounds such as acetic and malic acids and others should also be investigated.

Although recovery of only 71.1% of the betanine was re-

Table 3—Betanine recovery information and operation conditions for the four main experiments

Raw product		Operations		Juice			Cossettes			
Experiment cultivar	% Betanine raw beet	Diffuser slope	% Citric acid in extracting liquid	pH	% Betanine	% Betanine recovery	pH	% Betanine	% Betanine loss	% Betanine loss during processing
1. Dark Red Detroit	0.0976	4.5°	0.000	6.3	0.033	53.8	6.3	0.014	11.8	34.4
2. Dark Red Detroit	0.0976	4.5°	0.050	5.2	0.046	70.6	5.3	0.008	3.0	26.4
3. Garnet	0.0700	4.5°	0.090	5.5	0.030	71.1	4.0	0.003	0.2	28.7
4. Garnet	0.0979	4.0°	0.125	5.5	0.035	62.5	3.8	0.001	0.3	37.2

corded in these experiments, it should be possible by further study of acidifying solvents, enzyme and atmosphere control, and reduction of retention time of juice in the diffuser to recover around 90% of the betanine in the raw beet tissue.

Table 4 shows that the concentration operation to approximately 26° Brix caused only about 1 7% loss in betanine in 1 hr at 55°C. Preliminary indications were that this recovery level and an apparent increased color stability to heat appeared to be due to molecular modification by acid hydrolysis and heat during diffusion-extraction, enzyme inactivation during initial stages of the extraction or to the protective mechanism reported by von Elbe et al. (1974) and Pasch and von Elbe (1975) who indicated certain foods as well as beet juice and puree itself help reduce betanine destruction. The diffusion-extraction procedure appeared to remove water-soluble low molecular weight substances from the beet cossettes and probably would include more of these substances than juice produced by the press and/or centrifugal methods. Brüniche-Olsen (1962) in their studies found a large number of diffusible substances in the sugar-beetroot including salts of organic and inorganic acids and undissociated or slightly dissociated substances like pectins and proteins. It is not known which diffusible extract provides betanine and/or vulgaxanthine-I protection in natural beet juice and pulp.

An additional factor which was helpful in the concentrating step was that the diffusion-extracted beet juices showed low levels of cellular tissue which was expressed as insoluble solids. In Experiments 3 and 4 they amounted to 0.28 and 0.20%, respectively. Low levels of cellular tissue and/or insoluble solids were a distinct advantage in enzyme clarification, filtration and spray-drying operations. These levels of insoluble solids appeared to reduce beet-like aroma reactions in the spray-dried powders.

Results of the spray drying tests in Table 5 show that pH has a definite influence on the betacyonine/betaxanthine ratio, with the more acid juice concentrates providing a powder with more violet and less yellow colorants. The average recovery of betanine in pH 5.0 juices after concentrating and spray drying was about 87.3%. If the extraction efficiency shown in Experiment 2 (70.6%) was combined with the recovery by pH 5.0 spray-dried powder (87.3%) the overall recovery of betanine in the spray-dried form was approximately 62%.

During this research emphasis was placed on betanine and red-violet colorants but it was noticed in the spray-dried powders that vulgaxanthine-I was important where blood-red and more orange hues were required as a food colorant. Red/yellow ratios should be the best method to report beet juice colorants for use in food applications. This approach is possible according to Nilsson (1973), who has reported that red/yellow ratios may vary with cultivars, harvests and even among beetroots. Results reported here provide red/yellow ratios from juice samples from about 100 kg raw beets per diffuser operation and show direction with respect to variants in processing operations not necessarily absolute values.

REFERENCES

Adams, J.P. and von Elbe, J.H. 1977. Betanine separation and quantification by chromotography on gels. J. Food Sci. 42: 410.

Brüniche-Olsen, H. 1962. "Solid-liquid extraction." NYT Nordisk Forlag. Arnold Busck Copenhagen, Denmark.

Chiriboga, C. and Francis, F.J. 1970. An anthocyanin recovery system from cranberry pomace. J. Amer. Soc. Hort. Sci. 95: 233.

Laursen, J. 1974. Herstellung von Apfelsaft mit des DDS-Diffusion. Flüssges Obst 7: 284.

Lee, Y. 1976. Private communication, University of Maryland, College Park, MD. 20742.

Lüthi, H.R. and Glunk, U. 1976. Unpublished Data. Production of apple juice by continuous extraction. Institut für Lebensmittelwissenschaft Laboratorium für Verfahrenstechnik der Eidg. Techn. Hoehschule, Zurich.

Nilsson, T. 1970. Studies into the pigments in beetroot. Lantbr Hogsk 36: 179.

Nilsson, T. 1973. The pigment content in beetroot with regard to cultivar, development, growth and growing conditions. Swedish J. Agric. Res 3: 187.

Pasch, J.H. and von Elbe, J.H. 1975. Betalaines as natrual food colorants. Food Prod. Dev. 9: 38.

Philip, T. 1974. An anthocyanin recovery system from grape wastes. J. Food Sci. 39: 859.

Riboh, M. 1977. Natural color: what works what doesn't. Food Eng. 49: 66.

Soboleva, G.A., Ul'yanova, M.S., Zakharova, N.S. and Bokuchava, M.A. 1976. Betacyanin-decolorizing enzyme. Biokhimiga 41: 968.

von Elbe, J.H., Sy, S.H., Maing I-Y and Gabelman, W.H. 1972. Quantitative analysis of betacyanins in red table beets (Beta vulgaris L.) J. Food Sci. 37: 932.

von Elbe, J.H. 1975. Stability of betalaines as food colors. Food Technol. 29: 42.

von Elbe, J.H., Maing, I-Y and Amundson, C.H. 1974. Color stability of betanin. J. Food Sci. 39: 334.

Ms received 6/20/77; revised 12/13/77; accepted 12/20/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA June 5-8, 1977.

The authors acknowledge the help of J. Laursen and Don Lewis in processing and analytical work. Thanks also go to De Danske Sukkerfabrikker, Copenhagen for use of the slope diffuser, the Urschel Company and the G.L. Webster Co. for use of the GKS Slicer.

Scientific article no. A-2400, Contribution No. 5416, Maryland Agricultural Experiment Station, Food Science Program, Dept. of Horticulture.

Table 4—Betanine recovery after concentration beet juice extract to 26° Brix

Original juice pH	% Betanine juice extract	% Betanine conc	% Betanine recovery
5.5	0.033	0.12	93.4
5.0	0.033	0.11	89.4
4.5	0.033	0.13	93.4
4.0	0.070	0.12	93.2

Table 5—Pigments in spray-dried beet juice powder with inlet temperatures ranging from 135–150°C

Sample pH	% Betanine	% Vulgaxanthine-I	Red/yellow ratio
5.5	0.354	0.349	1.01
5.0	0.359	0.323	1.11
4.5	0.383	0.335	1.14
4.0	0.380	0.323	1.18
Raw beet juice	–	–	1.04

SOME FACTORS AFFECTING PINTO BEAN QUALITY

N. M. QUENZER, V. L. HUFFMAN and E. E. BURNS

ABSTRACT

This study was designed to objectively describe the chemical and physical attributes of three pinto cultivars grown at Thrall and El Paso, TX, and Twin Falls, ID. The relationships of these attributes were established through analysis of variance and correlation coefficients. Shear values were found to be the more reliable quality attribute. The expected relationship between rehydration and shear was not found. Maximum imbibition did not yield the most tender product. The amount of water imbibed varied by location. Beans from El Paso that suffered a late frost failed to imbibe water until heat treatment was applied. Firmness was found to be related to calcium and magnesium contents. Sensory preference scores rated the Texas-grown cultivars over the Idaho-grown cultivars. Whole, reddish-brown beans of a mild distinguishable flavor were preferred.

INTRODUCTION

DRY BEANS provide an economical source of calories and protein to the American diet. As a vegetable food, beans provide protein, iron, calcium, magnesium, phosphorous, and are a rich source of the B vitamins (de Moraes and Angelucci, 1971; Rockland et al., 1977). Pinto beans are available in the dried state and must be rehydrated prior to processing.

The practice of soaking beans insures product tenderness and uniform expansion while increasing product yield (Burr et al., 1968; Hoff and Nelson, 1965). Dry beans are commercially hydrated for 10–18 hr. The extent of this hydration has a direct influence on the subsequent cooking time and palatability. Hydration at elevated temperatures is effective in reducing processing times; however, a purple-brown color may develop (Rockland, 1974). Dawson et al. (1952) showed that a short method of soaking in which the beans were added to boiling water, boiled 2 min, removed from the heat and allowed to soak 1 hr gave satisfactory results. However, a cooking time of 15–30 min longer was required to reach the same degree of tenderness as comparable bean samples soaked for 18 hr in cold water.

The passage of water through the general surface of the bean is limited by the permeability of the seed coat (Snyder, 1936). Snyder also observed that imbibition was lower in weak acid solutions than in water. Additions of polyphosphates tend to increase the level of water uptake while sodium chloride has a slightly depressing effect. Pintos soaked overnight with sodium bicarbonate required a shorter thermal process but were too soft and poor in appearance and general acceptability (Dawson et al., 1952, Hoff and Nelson, 1965).

High moisture beans which have been held in storage absorb water somewhat faster than similar beans of low moisture content (Burr et al., 1968). Fresh, high moisture beans do not necessarily require a longer cooking time. However, after extended storage, high moisture beans may become refractory especially if stored at elevated temperatures (Morris, 1963; Morris and Wood, 1956).

Authors Quenzer, Huffman and Burns are with the Dept. of Horticultural Sciences, Texas A&M University, College Station, TX 77843.

0022-1147/78/0004-1059$02.25/0
© 1978 Institute of Food Technologists

Hardshell is a characteristic of beans impermeable to water. Two conditions exist: one involving the cotyledon (sclerma), and the other the seed coat. Hardshell beans must be steam or hot water treated prior to soaking (Morris et al., 1950). The incidence of hardshell beans can be reduced by size grading and rejection of the smallest 20% of the bean lot. Unfavorable environmental conditions along with hereditary traits play an important part in the tendency towards hardshell (Morris et al., 1950).

Cooking serves to tenderize the seed coat and cotyledon, to develop acceptable flavor and texture, and to make the bean protein nutritionally available. According to Rockland et al. (1974), the middle lamella softens during cooking, thus permitting the separation of adjacent whole cells. The separation of cells may also be related to the removal of divalent cations, particularly calcium and magnesium, from bridge positions within the pectinaceous matrix of the middle lamella.

The mineral composition of pinto beans is of importance to the food processor as well as the nutritionist. Mattson (1946) reported that the cookability of different dry pea varieties was related to their contents of phytic acid and calcium. He suggested that when the phytic acid content is low, the pectin in the middle lamella forms insoluble calcium and magnesium pectates causing poor cooking quality. Phytin, being a calcium and magnesium precipitant, prevents the hardening process by preventing the formation of insoluble pectates. Fowler (1957) found the hardening of peas can be attributed chiefly to the presence of calcium. Fluctuating levels of calcium due to variety and location have been reported to affect ease of cooking (Lantz et al., 1962).

MATERIALS & METHODS

Raw product

Three cultivars of pinto beans: Columbia, U. Idaho 111, and the U. Idaho 114 were grown at Thrall, Texas and El Paso, TX. The same cultivars were grown at Twin Falls, ID for comparison. After cleaning, the beans were stored in closed containers at 4.4°C and 80% relative humidity. Initial moisture levels of the dry beans was approximately 8.1%.

Processed product

From each cultivar, three 100-g samples were taken. The beans were soaked in distilled, deionized water for 18 hr and then boiled 90 min in stainless steel pots according to the method of Dawson et al. (1952).

Evaluations

Gardner color. A Gardner Color Difference Meter was used to determine the color of raw and cooked whole-bean samples in terms of Gardner color values of "L", "a", "b", and "a/b" ratios.

Mineral analysis. Mineral assays for calcium, magnesium, and iron were conducted on the raw bean samples. Samples were digested with nitric acid and perchloric acids following the method of Smith and Storey (1971). Quantitative measurements were determined on a Perkins-Elmer Atomic Absorption Spectrometer.

Water imbibition. The amount of water imbibed was determined by the difference in recorded weights of the beans before and after the 18 hr soak at 20°C. After 18 hr, the beans were drained and spread on paper towels to remove the excess water before reweighing.

Shear. Shear values were measured using an Allo-Kramer Shear Press equipped with an integral strip chart recorder. A 75-g cooked sample was sheared in a 13-blade multipurpose shear cell. A 3,000-lb ring with a sensitivity setting of 1,000 and a down stroke of 30 sec was employed. As the cooked samples were sheared, a sharp peak similar to that observed by Binder and Rockland (1964) for intact lima beans was

Table 1—Means and standard deviations of mineral content in raw pinto beans[a]

Cultivar description	Calcium mg/100g	Magnesium mg/100g	Iron mg/100g
Thrall 114	73.3a	149.8a	3.1a
111	84.0a	159.4a	8.4a
Columbia	65.5a	170.6a	2.9a
El Paso 114	111.1b	205.1a	6.1a
111	99.5b	193.6a	7.6a
Columbia	76.5a	173.2a	4.9a
Idaho 114	68.4a	166.7a	5.7a
111	60.7a	178.8a	7.3a
Columbia	62.6a	162.0a	7.7a
Standard deviation	18.920	20.092	1.988
Composite mean	85.060	180.200	6.073

[a] Means followed by different letters are significantly different at the 5% level according to Student-Newman-Keuls' test.

Table 2—Analysis of variance of water imbibition for raw pinto beans

Source of Variation	d.f.	Mean square	F	Prob. > F
Cultivar	2	725969.75	44.89	0.0001
Location	2	149065107.81	8599.09	0.0001
Location X Cultivar	4	874563.55	54.08	0.0001
Replication (Location)	18	16172.07		
Total	26			

Table 3—Correlation coefficients for calcium content with magnesium content and shear values

Attribute	Correlation
Shear (values)	0.55 *
Magnesium (content)	0.82 *

* $P < 0.05$

Table 4—Means and standard deviations of water imbibition in raw pinto beans, and shear in cooked pinto beans[a]

Cultivar description	Water imbibition (g)	Shear
Thrall 114	101.77a	407.4a
111	102.48a	207.0a
Columbia	102.28a	276.2a
El Paso 114	41.92c	385.0a
111	35.30b	346.3a
Columbia	26.42a	242.3a
Idaho 114	110.02a	242.0a
111	96.48a	94.0a
Columbia	108.14a	228.0a
Standard deviation	32.99	147.73
Composite mean	79.74	297.35

[a] Means followed by different letters are significantly different at the 5% level according to Student-Newman-Keul's test.

Table 5—Analysis of variance of shear for cooked pinto beans

Source of variation	d.f.	Mean square	F	Prob. > F
Cultivar	2	26088.40	1.23	0.3180
Location	2	21112.07	0.99	0.3911
Location X Cultivar	4	10747.43	0.51	0.7325
Replication (Location)	17	21271.61		
Total	25			

observed. Total peak area was measured by triangulation (width at ½ height × height).

Visual examination. Three randomly drawn trials of 100 beans were individually inspected for defective and damaged beans. The number of broken, cracked, and off-colored beans was recorded. A bean was considered broken when a portion was missing while cracked beans had hairline fissions limited to the seed coat. Discolored darks referred to beans with darkened and/or off-colored seed coats.

Sensory evaluation. A trained sensory panel composed of nine members utilized a seven-point hedonic rating scale for scoring color, flavor, texture, and appearance aside from color. The score sheet followed the style of Greenwood (1935).

Statistical interpretation

Analysis of variance was performed to determine differences in cultivar attributes. Simple correlation coefficients were calculated to indicate relationships between the measures of quality.

RESULTS & DISCUSSION

ANALYSIS OF VARIANCE indicated highly significant differences between cultivars for calcium content (Table 1) and water imbibition (Table 2). Growing location proved significant for Gardner color "a/b" ratio for cooked beans; imbibition; and calcium and magnesium contents.

Mean Gardner color "L" values on the raw bean samples was 47.20 with values ranging from 39.56 for the dark-red Thrall 114 cultivar to 58.00 for the lighter El Paso 114 cultivar. Color "a" values were higher for the Thrall-grown beans indicating a higher concentration of anthocyanin pigments. Cooking caused the lighter cultivars from El Paso and Idaho to darken and increase in redness; while the Thrall beans remained essentially unchanged. Maillard-type browning may have contributed to the development of the dark pigments since conditions were favorable from heating (Haard, 1976). Phenolase activity during initial stages of heating also results in a brown color. The presence of iron has been shown to lead to the discoloration of anthocyanins through peroxidase activity (Luh, 1977). After cooking, the beans lost yellowness, gaining blueness appreciable in the Idaho cultivars. The anthocyanin pigments have been shown to form blue or purplish insoluble salts upon contact with metals (Aristizabal, 1962).

The beans proved relatively high in magnesium and iron (Table 1) and slightly low in calcium (McCarthy et al., 1977). El Paso grown cultivars had significantly ($P = 0.05$) higher contents of calcium and magnesium (Table 1). Calcium content positively correlated with shear and magnesium (Table 3). This substantiates the work of Mattson (1946) and Fowler (1957). Calcium and magnesium contents negatively correlated with imbibition. No significant difference was found for iron content between cultivars or between locations.

The amount of water imbibed ranged from 26.42g per 100g sample for the El Paso Columbia cultivar to 110.02g for the Idaho 114. Highly significant differences were found between cultivars and between locations (Table 2). The El Paso cultivars failed to become fully imbibed; whereas the Idaho and Thrall cultivars approximately doubled in weight (Table 4). The El Paso cultivars suffered late frost damage prior to harvesting. Frost damage is believed to have caused a condition analogous to case hardening.

No significant differences in shear between cultivar or location were found (Table 5). No correlation was shown between water imbibition and shear. Maximum imbibition did not necessarily yield the most tender product (Table 4). This substantiates the studies of Powers et al. (1961). A higher water absorption capacity (less hardshell) is not necessarily correlated with a shorter cooking time (Molina et al., 1976). As stated by Burr et al. (1968), soaking and cooking are two separate processes not necessarily related.

No one location or cultivar proved superior in the production of quality beans. Separation by cultivar showed the lowest percentage of broken beans was the 114 cultivar (1.19%). The Columbia cultivars had the lowest percentage of

cracked beans (.22%) and of discolored, dark beans (1.07%). Separation by location indicated that cultivars grown at Thrall had the lowest percentage of brokens (.8%) as well as cracked beans (.27%). However, the Thrall location produced five times as many discolored dark beans as the Idaho location.

The highest sensory preference score for color was given to Thrall 111 with the Thrall Columbia, Idaho Columbia, and the El Paso 114 following in succession. The preferred color was of a medium reddish brown. The panelists rated the Idaho Columbia as the more desirable in textural characteristics. A tender bean without noticeable seed coats was preferred. The El Paso 111 was given the highest rating for flavor with El Paso Columbia, Idaho Columbia and the Thrall Columbia following closely behind. The panelists preferred a mild, distinguishable flavor. The samples of whole, plump beans received the highest hedonic scores for appearance. The El Paso cultivars: 114, Columbia, and 111 had a considerable margin over the other cultivars. Correlation was seen between flavor, texture and appearance.

SUMMARY

SHEAR VALUES were found to be the more reliable quality attribute. The expected relationship between rehydration and shear was not found. Maximum imbibition did not yield the most tender product. The amount of water imbibed varied by location. Beans from El Paso that suffered a late frost failed to imbibe water until heat treatment was applied. Firmness was found to be related to calcium and magnesium contents.

Sensory preference scores rated the Texas-grown cultivars over the Idaho-grown cultivars. Whole, reddish-brown beans of a mild distinguishable flavor were preferred.

REFERENCES

Aristizabal, L. 1962. Unpublished data. Adriance Lab, Texas A&M University, College Station, TX.

Binder, L.J. and Rockland, L.B. 1964. Use of the automatic recording shear press in cooking studies of large dry lima beans (Phaseolus lunatus). Food Technol. 18: 1071.

Burr, H.K., Kon, S. and Morris, H.J. 1968. Cooking rates of dry beans as influenced by moisture content and temperature and time of storage. Food Technol. 22: 336.

Dawson, E.H., Lamb, J.C., Toepfer, E.W. and Warren, H.W. 1952. Development of rapid methods of soaking and cooking dry beans Bull. 1051. USDA, Washington, DC.

deMoraes, R. and Angelucci, E. 1971. Chemical composition and amino acid contents of Brazilian beans (Phaseolus vulgaris). J. Food Sci. 36: 493.

Fowler, H.D. 1957. Changes in orthophosphate in relation to phytin formation in maturing peas. J. Sci. Food Agr. 8: 333.

Greenwood, M.L. 1935. Pinto beans: their preparation and palatability. Bull. 231. New Mexico Agricultural Experiment Station, Clayton, NM.

Haard, N.F. 1976. Characteristics of edible plant tissues. In "Principles of Food Science," Vol. 1, p. 677. Marcel Dekker, Inc., New York, NY.

Hoff, H.E. and Nelson, P.E. 1965. An investigation of accellerated water uptake in dry pea beans. Research Progress Report 211, Indiana Agricultural Experiment Station, Lafayette, IN.

Lantz, E.M., Gough, H. and Campbell, A. 1962. Effects of planting date on the composition and cooking quality of pinto beans. Bull. 467, New Mexico Agricultural Experiment Station, Clayton, NM.

Luh, B.S. 1977. Private communication. University of California at Davis, Davis, CA.

Mattson. 1946. The cookability of yellow peas. Acta Suecand II (2): 185.

McCarthy, M.S., Murphy, E.W., Ritchey, S.J., and Washburn, P.C. 1977. Mineral content of legumes as related to nutrition labeling. Food Technol. 31: 86.

Molina, M.R., Baten, M.A., Gonez-Brenes, R.A., King, K.W. and Bressani, R. 1976. Heat treatment: a process to control the development of the hard-to-cook phenomenon in black beans (Phaseolus vulgaris). J. Food Sci. 41: 661.

Morris, H.J. 1963. Cooking quality of dry beans. Proceedings, 6th Dry Bean Research Conference, Los Angeles, CA, Jan. 24, 1963.

Morris, H.J., Olson, R.L. and Bean, R.C. 1950. Processing quality of varieties and strains of dry beans. Food Technol. 1: 247.

Morris, H.J. and Wood, E.R. 1956. Influence of moisture content on keeping quality of dry beans. Food Technol. 10: 225.

Powers, J.J., Pratt, D.E. and Joiner, J.B. 1961. Gelation of canned peas and pinto beans as influenced by processing conditions, starch, and pectic content. Food Technol. 15: 41.

Rockland, L.B., Zaragosa, E.M. and Hahn, D.M. 1974. New information on the chemical, physical and biological properties of dry beans. Proceedings. Report of Bean Improvement Cooperative and National Dry Bean Research Assoc. Conference, Rochester, NY.

Rockland, L.B., Miller, C.F. and Hahn, D.M. 1977. Thiamine, pyridoxine, niacin, and folacin in quick cooking beans. J. Food Sci. 42: 25.

Smith, M.W. and Storey, J.B. 1971. The analysis of pecan leaves by atomic absorption spectroscopy. Soil Sci. Plant Analysis. 2(4): 249.

Snyder, E.B. 1936. Some factors affecting the cooking quality of the pea and great northern types of dry beans. Bull. 85. Nebraska Agricultural Experiment Station, Lincoln, NB.

Ms received 10/15/77; revised 1/12/78; accepted 1/18/78.

TEMPERATURES OF SPAGHETTI AFTER HEATING IN CONVECTION OVENS AT SERVICE LOCATIONS IN A COMMISARY FOODSERVICE SYSTEM

MARION L. CREMER

ABSTRACT

Effects of equipment model and position of food were examined to determine temperature variability and factors influencing temperature of spaghetti heated in convection ovens in a commissary foodservice system. Significant ($P < 0.01$) differences in temperature were found as food was heated in four models and 12 positions. Factors which appeared to have effect on temperature included oven thermostatic setting, oven power rating, location of heating elements, number of meals heated, number of fans, and the presence of an oven floor. Temperature variability existed and was related to model of oven and position of food. This appeared to be influenced by factors of equipment engineering and use which must be improved if ovens of this type are to be effectively utilized.

INTRODUCTION

CURRENTLY OPERATING commissary foodservice systems function through use of chilled or frozen centrally prepared products transported to multiple locations and heated for service in convection ovens (Unklesbay et al., 1977). Convection ovens function through forced circulation of hot air and have had particular applicability to school foodservice operations (Decareau, 1971). However, food temperature variability may be a problem inherent in use of this equipment. Cremer and Chipley (1977), in a study of a commissary foodservice system, found temperatures of food heated in a single model of convection oven to range from 34–95°C for 72 observations of chili and from 38–98°C for 72 observations of spaghetti. Variability in temperature was found in each of six trials of heating each of the two products as indicated by a standard deviation of at least 23.2. Nonuniformity of heating rates in convection ovens has previously been considered a typical characteristic (Decareau, 1971; Livingston and Chang, 1972), but data have not been presented to support this view. A major manufacturer waives claims to uniformity in temperature in various parts of convection ovens but does not indicate the extent of variability to be expected (Anonymous, 1973).

Public health significance of temperature control during cooking and reheating food for service in the foodservice industry has been recognized (FDA, 1974), but few data are available on food temperatures or heating conditions as actually occur in foodservice operations. Because of this lack of data, the potential public health hazard associated with temperature variability, and the temperature problem identified in use of a single oven, this study was undertaken. Specific objectives were (a) to determine food temperatures after heating in several models of convection ovens as used in an operating system and (b) to examine factors which might have influence on these temperatures. Data should give indication of the significance of temperature variability and the influence of equipment model on food temperature after heating in convection ovens in an operating system.

Author Cremer is with the School of Home Economics, The Ohio State University and Ohio Agricultural Research & Development Center, 1787 Neil Ave., Columbus, OH 43210.

0022-1147/78/0004-1062$02.25/0
© 1978 Institute of Food Technologists

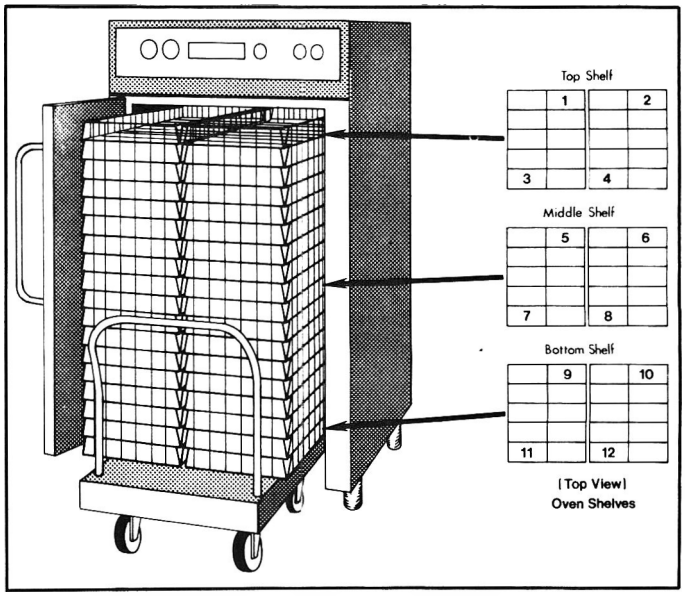


Fig. 1–Schematic representation of oven, dolly, and positions from which samples were drawn for temperature recording.

MATERIALS & METHODS

General

A commissary type school foodservice system was selected for use in conduct of study. This was the same system studied previously (Cremer and Chipley, 1977) and was chosen because of the problem in temperature variability which was identified in that study. An actual operating system was used because (a) data were unavailable on conditions in usage of convection ovens and (b) problems of temperature variability, including potential health hazard, are specifically related to operating rather than controlled laboratory conditions.

Four different models of convection ovens as utilized at four service locations within the system were selected for use. These were chosen to represent variability in ovens within the system and were identified by the director of the operating system who was an experienced and trained engineer. Choice was based on oven power rating, design, and experience in use within the system. Ovens were manufactured by four different companies and were designated as A, B, C and D.

Ovens

All four ovens were electric single cavity units designed for heating 334.38 mm wide × 646.88 mm deep × 625 mm high stacked racks of food (Fig. 1). For heating in ovens A, C and D, racks of food were stacked on dollies especially made for rolling directly into ovens. For Oven B, food was manually stacked on the base of the oven. Length and width of ovens was approximately equal to that required for two racks to be placed side by side on a dolly or the base of an oven. Oven A was different in design from all of the other ovens in that no floor was provided and heat could freely escape from the bottom. Other data on the physical characteristics of the ovens are presented in Table 1.

Product characteristics and heating conditions

Spaghetti was the food item selected through which to study temperature. This selection was based upon the frequency with which the item appeared on the menu in the operating system and because a temperature problem with this product had previously been identified (Cremer and Chipley, 1977). Formula for preparation of the product was: tomato puree, 3 cs (6 no. 10 size cans); tomato paste, 2 cs (6 no. 10 size cans); crushed tomatoes, 1 cs (6 no. 10 size cans); brown sugar, 5.7L (1½ gal); salt, 3.8L (1 gal); garlic powder, 0.47L (2 c); oregano,

Table 1—Physical description of ovens

	Oven A	Oven B	Oven C	Oven D
Type door	Vertically hung "French"	Top and bottom hinged	Vertically hung "French"	Vertically hung "French"
Interior dimensions (mm)[a]	1068.8 X 690.6 X 675	625 X 687.5 X 675	700 X 725 X 684.4	1025 X 725 X 706.3
Total power rating (kW)	30.3	15.5	24.5	22.5
Number of heating elements	4 sets of 3	3	6	2 sets of 3
Type of heating elements	Sheath	Rod	Sheath	Tubular
Location of heating elements	Sides of both top and bottom halves	Each side and bottom	3 each side	Sides of oven
Number of fans	2	1	1	2
Location of fans	Center back of top half and center back of bottom half	Center back of oven	Center back	Center back of top half and center back of bottom half
hp of fans	¾ each fan	½	¾	¾ each fan

[a] Values given are for height, width, and depth in that order.

Table 2—Temperature and other factors related to heating spaghetti in convection ovens

	Oven A							Oven B					
	Trial 1 Dolly		Trial 2 Dolly		Trial 3 Dolly			Trial 1 Dolly		Trial 2 Dolly		Trial 3 Dolly	
	1	2	1	2	1	2	Overall	1	2	1	2	1	Overall
Avg food temp after heating (°C)[a]	52	54	68	66	61	54	59	83	83	76	87	91	85
Range in food temp after heating (°C)[a]	36–77	40–74	54–82	56–80	48–84	43–77	36–84	71–94	77–93	61–92	71–93	87–94	61–94
Temp of heated food[a] s[b]	13.7	13.5	8.3	7.8	12.8	11.7	12.7	7.4	5.5	9.4	6.4	2.1	8.2
Avg food temp before heating (°C)[a]	5	4	5	5	4	4	5	10	11	5	6	4	7
Range in food temp before heating (°C)[a]	4–6	4–5	4–9	3–6	4–6	3–4	3–9	8–13	9–13	4–6	5–7	4–5	4–13
Temp of cold food[a] s	0.9	0.4	1.9	1.1	0.6	0.3	1.1	1.6	1.2	0.8	0.5	0.3	2.9
Oven thermostatic setting (°C)[c]	177	177	177[d] 190	177 190	177	177	177 avg 181 avg	218	218	218	218	218	218
Oven temp at beginning of heating (°C)[c]	177	—[e]	154	—	154	—	162 avg	221	232	213	227	271	233
Oven temp at end of heating (°C)[c]	121	121	127	138	138	—	129 avg	207	218	235	210	227	219
Time for heating food (min)[c]	25	25	25	25	25	25	25 avg	20	20	20	20	20	20
Number of meals heated[c]	173	149	170	139	167	143	157 avg	130	96	140	105	100	114
Time for preheating oven (min)[c]	25	25	31	25	30	25	29 avg	25	20	25	—	5	18

[a] Includes 12 observations for each dolly and each trial with 72 overall in Oven A and 60 overall in Oven B

[b] The symbol "s" refers to standard deviation

[c] Includes 1 observation for each dolly and each trial with 6 overall in Oven A and 5 overall in Oven B.

[d] Two numbers indicate settings for top and bottom heating elements.

[e] Indicates no value was recorded

0.24L (1 c); green bell peppers, 0.95L (1 qt); water, 22.72L (6 gal); ground beef crumbles, 68.04 kg (150 lb); and spaghetti (uncooked), 45.36 kg (100 lb). Beef patty crumbles are a commercially available frozen fully cooked product made from beef, water, textured vegetable protein, and various other ingredients including coloring and flavoring. From these ingredients, sauce was prepared, spaghetti was cooked, and these components were mechanically mixed together. The item was prepared in 684L (180 gal) batches, chilled, and mechanically dispensed at 196g (7 oz) per portion into 125 mm wide × 162.5 mm long × 32.25 mm high aluminum foil containers. Each container held a portion for one individual, and other meal components were packaged separately.

Filled containers were covered with aluminum foil and placed in 334.38 mm wide × 646.88 mm long × 65.63 mm high racks holding 10 containers each and placed in a 2 × 5 arrangement (Fig. 1). Racks were stacked on dollies and thus transported to service locations. Food was held in the chilled state until heated for service. Before heating, racks of food for heating in Ovens A, C, and D were transferred to a second dolly specially designed for individual ovens. On those dollies, racks were placed two across to form a 4 × 5 meal arrangement (Fig. 1). One dolly at a time was rolled into each oven for heating. In Oven B, which was not designed to accommodate a dolly, racks were manually stacked from the floor of the oven in the same manner and arrangement. Number of packages heated at one time and number of dollies placed for heating was dependent upon meals required (Tables 2 and 3). Oven thermostatic settings, temperatures of spaghetti prior to heating, oven preheating times, and oven temperatures at the beginning of heating were not experimentally controlled but were recorded as occurred within the operating system (Tables 2 and 3). Values for oven temperature at the beginning of heating Dolly 2 in Oven A were not recorded because placement of a thermometer was impossible in terms of oven construction and removal of the base with Dolly 1. In ovens B and D no values were shown for oven preheating time in some instances because the second dolly of food was placed in the oven directly upon removal of the first.

Temperature recording

Food temperature recordings were made from packages of food as heated in 12 positions as shown in Figure 1. These were for packages in the extreme corners of each rack in the top, middle, and bottom areas of ovens. Packages also represented the extreme corners of the total unit and, as nearly as possible, the center back and center front. These positions were selected because they appeared to provide a reasonable representation of the area within the unit, a reasonable number of samples, and because previous investigation (Cremer and Chipley, 1977) revealed considerable food temperature variability when food was sampled in this manner. Preliminary investigation indicated that reading more than 12 temperatures produced unacceptable time delay both in meal service in the operating system and in temperature recording.

Table 3—Temperature and other factors related to heating spaghetti in convection ovens

	Oven C				Oven D						
	Trial 1 Dolly	Trial 2 Dolly	Trial 3 Dolly		Trial 1 Dolly		Trial 2 Dolly		Trial 3 Dolly		
	1	1	1	Overall	1	2	1	2	1	2	Overall
Avg food temp after heating (°C)[a]	94	95	92	94	80	84	90	87	88	89	86
Range in food temp after heating (°C)[a]	89–99	88–99	87–96	87–99	74–83	71–93	83–96	77–94	83–93	82–99	71–99
Temp of heated food[a] s[b]	3.7	3.8	2.6	3.4	3.0	6.9	3.8	5.8	4.3	6.0	5.9
Avg food temp before heating (°C)[a]	5	3	5	4	3	2	5	4	6	6	4
Range in food temp before heating (°C)[a]	4–7	1–4	4–7	1–7	(–)1–9	(–)6–10	4–7	3–6	4–7	4–7	(–)6–10
Temp of cold food[a] s	1.4	1.1	1.1	1.5	3.3	4.9	1.4	1.1	1.0	1.1	2.9
Oven thermostatic setting (°C)[c]	163	177	171	170 avg	177	177	177	177	177	177	177 avg
Oven temp at beginning of heating (°C)[c]	143	182	177	167 avg	177	177	177	177	177	177	177 avg
Oven temp at end of heating (°C)[c]	182	177	177	178 avg	149	177	177	177	177	177	172 avg
Time for heating food (min)[c]	25	25	25	25 avg	20	20	20	20	20	20	20 avg
Number of meals heated[c]	164	169	146	160 avg	222	197	197	196	226	180	203 avg
Time for preheating oven (min)[c]	15	15	20	17 avg	30	–[d]	30	–	30	–	30 avg

[a] Includes 12 observations for each dolly and each trial with 36 overall in Oven C and 72 overall in Oven D.
[b] The symbol "s" refers to standard deviation.
[c] Includes 1 observation for each dolly and each trial with 3 overall in Oven C and 6 overall in Oven D.
[d] – Indicates no value was recorded.

Table 4—Overall average temperatures of spaghetti after heating and significant differences according to oven and position

Oven		A			B			C			D	
Average (°C)*[a,b]		59.3a			84.9b			93.6c			86.2b	
No. of observations		72			60			36			72	
Position	1	2	3	4	5	6	7	8	9	10	11	12
Average*[c](°C)	82.8a,b,c	87.0a	86.5a	82.8a,b	78.4c,d	86.5a	76.8d	78.3d	78.1d	80.0c,d,b	78.1d	76.9d

[a] * Indicates significant difference ($P < 0.01$).
[b] Averages across rows, followed by the same letter or letters, are not significantly different ($P < 0.05$).
[c] Each of the 12 averages is based on 20 observations.

After heating, food temperatures were read immediately after removal of each package from the oven and in the order of packages in positions 1 through 12. This was facilitated by one individual handling food and another reading temperature. All racks had to be transferred from one dolly to another to reach the respective areas. Total time for reading the total group of 12 was about 3 min, but temperature recording for each package was immediate upon removal from the oven and no individual package was held for this time period prior to temperature recording. Before heating, food temperatures were read in the same manner from the same 12 packages of food in the order of packages 9, 11, 10, 12, 5, 7, 6, 8, 1, 3, 2, and 4 as food was placed on a dolly for heating. This was to determine the influence of initial temperature on food temperature after heating. For each oven, the middle was considered to be an established number of racks from the bottom with the top position variable by one rack dependent upon number of meals heated.

Instruments used for heated food temperature recording were Weston Model 2292 dial type thermometers with 2° gradients; and for cold temperatures, instruments were Weston Model 2261 dial type thermometers with 1° temperature gradients. Both models had 125 mm (5 in) stems and were notched by the manufacturer to indicate extent of immersion required for accurate recording. Accuracy was insured by calibrating all thermometers in either boiling water or ice water dependent upon temperature range to be read. For reading both hot and cold food temperatures, thermometers were inserted through aluminum foil covers at a corner and at such angle that the hub of thermometers rested on the side of the pan. Preliminary study showed that this procedure located the center of the notched portion of the instruments in the center of the pan as determined by actual physical measurement. Angular insertion was necessary in order to immerse thermometers to the extent required for accurate recording in instruments.

Temperatures of ovens were recorded from Taylor free standing oven thermometers, U.S. Patent 2179773. Instruments had 2° temperature gradients and were checked in an accurately calibrated oven. Thermometers were placed in the approximate center of the base of ovens to record temperature at the beginning of heating and in the approximate center of the top to record temperature at the end of heating. This difference in location was because the only space available before food placement was the top rack of food. In the case of the oven where there was no base, the thermometer was placed in the center of the top of a dolly holding empty racks stacked three high.

Data collection was repeated three times at four service locations within the operating system and as spaghetti was served on three separate occasions. Differences in mean temperatures according to position in individual ovens and differences in temperature before heating in the individual ovens were determined by one-way analysis of variance. Least squares analysis of variance with two-way classification was used to determine overall differences according to position and oven. Where significant differences were found in the one-way analysis of variance, Duncan's test ($P < 0.05$) was used to determine which means were significantly different from each other. For the two-way analysis of variance, the least significant difference test ($P < 0.05$) was used to show differences. Correlation coefficients were calculated to determine relationships between food temperatures before and after heating, and standard deviations were calculated to indicate temperature variability (Snedecor and Cochran, 1967; Duncan, 1955).

RESULTS & DISCUSSION

MEAN TEMPERATURES of food as heated in 12 positions in four convection ovens and significant differences are presented in Table 4. Significant ($P < 0.01$) differences were found in overall mean temperatures both among ovens and among positions of heating. Also, significant ($P < 0.01$) differences in mean temperatures were found according to position in two, out of four of the individual ovens (Table 5).

Highest overall mean temperature of food was found in

Table 5—Average temperatures of spaghetti after heating and significant differences according to position in oven

Position	Oven A Average *a,b °C	Oven A Observations	Oven B Average °C	Oven B Observations	Oven C Average* °C	Oven C Observations	Oven D Average °C	Oven D Observations
1	61.0b,c	6	85.0a	5	96.7a	3	88.3a	6
2	74.7a	6	87.6a	5	95.0a,b,c	3	90.8a	6
3	73.2a	6	87.0a	5	96.0a	3	89.2a	6
4	64.2a,b	6	82.8a	5	96.3a	3	88.5a	6
5	54.8b,c,d	6	76.0a	5	95.7a,b	3	86.7a	6
6	74.3a	6	91.6a	5	95.0a,b,c	3	84.8a	6
7	48.2d	6	81.0a	5	92.0a,b,c,d	3	85.3a	6
8	49.2d	6	81.8a	5	95.3a,b,c	3	86.7a	6
9	50.3c,d	6	85.8a	5	92.0a,b,c,d	3	85.0a	6
10	58.5b,c,d	6	90.4a	5	90.3c,d	3	81.8a	6
11	50.7c,d	6	87.4a	5	90.7b,c,d	3	84.8a	6
12	52.7c,d	6	82.8a	5	88.7d	3	82.7a	6

[a] *indicates that averages were significantly different ($P < 0.01$); no * indicates nonsignificant difference.

[b] Averages in the same vertical column followed by the same individual letter or letters are not significantly different ($P < 0.05$).

Oven C and lowest overall mean temperature in Oven A with these means significantly ($P < 0.01$) different from each other and from those in both of the other ovens (Table 4). No significant differences were found between mean temperatures of food after heating in Ovens B and D (Table 4). As indicated by temperature ranges and standard deviations (Tables 2 and 3), the least overall temperature variability was also found in Oven C, greatest in Oven A, with B and D intermediate.

Factors which might affect temperatures of food after heating are shown in Tables 2 and 3. These, in conjunction with consideration of equipment construction and design (Table 1), may help to explain differences in heated food temperature as affected by the various ovens. Discussion in general is based on consideration of overall mean temperatures (Table 4). Except for beginning and end oven temperatures, factors affecting heating were similar for Ovens C and A (Tables 2 and 3). Power rating was higher for Oven A than C, and higher in A and C than in the other two ovens (Table 1); but Oven A was the only oven which had no floor. This feature of design may have accounted for the fact that mean food temperature in this oven was lower than for any of the others. For Oven C as compared to Oven B, oven thermostatic setting, beginning oven temperature, and ending oven temperature were lower, number of meals heated at one time was greater, but food heating time 5 min longer (Tables 2 and 3) and power rating was 9 kW higher (Table 1). Power rating and possibly heating time may account for the significant difference in overall mean temperature between the two ovens. For Oven C as compared to Oven D, mean temperatures of food before heating were similar, oven thermostatic setting and beginning oven temperature somewhat lower, but food heating time 5 min longer, end heating temperature somewhat higher, and mean number of meals heated at one time 43 fewer (Tables 2 and 3). The smaller number of meals heated at one time and a higher power rating in Oven C than D (Table 1) may account for the significant difference in overall mean temperature. Although Oven B had considerably lower power rating than Oven D (Table 1), no significant differences were found between mean temperatures of food as heated in the two ovens (Table 4). This may be attributed to higher oven thermostatic setting in B than in D, higher beginning and ending heating temperatures, and considerable differences in number of meals heated at one time (Tables 2 and 3). Temperature of food before heating did not appear to have important influence on temperature after heating as indicated by correlation coefficients of 0.1147, 0.1070, 0.1670, and −0.1036 and nonsignificant differences in mean temperatures of food before heating in individual ovens.

According to position (Fig. 1), overall significant differences are indicated in Table 4. Food from positions 1, 2, 3 and 4 in the top of the ovens and position 6 in the back right corner of the middle had highest and similar temperatures. Except for position 6, all of the food samples from the middle and the bottom of the ovens had significantly ($P < 0.05$) lower temperatures than food from the top. The right corner in the back of the ovens would appear to produce the more effective heating since mean food temperature in position 2 was hottest, food from position 6 similar to this, and that from position 10 similar to food heated in positions 1 and 4 in the top of the oven. Differences in heating in the various areas may be simply due to the tendency of heat to rise making the upper portion hotter. Relatively higher temperature in the back right corner may be due to clockwise rotation of fans forcing heat to that corner.

For individual ovens, significant ($P < 0.01$) differences, according to position, were found in Ovens A and C but not in B or D (Table 5). This was true despite the fact that Oven C had the highest overall mean temperature. Food from positions 10, 11 and 12 in the bottom of Oven C were the only ones significantly ($P < 0.05$) different from the highest mean temperature found in position 1 located in the top of the oven (Table 5). Temperature difference may have been due to inadequacy of a single fan to distribute heat.

For Oven A, means and significant differences are shown in Table 5. Reasons for precise differences are not clear. However, heating appears to be better in the top and the right rear corner of the oven. Highest mean temperatures were recorded for position 2 with no significant ($P < 0.05$) difference between this and position 6. Also, temperature in the food from the bottom right rear corner, position 10, was relatively high and not significantly different from position 4 in the top of the oven. Except for position 6, temperatures from the middle and bottom positions were not significantly different ($P < 0.05$) from each other but were all different from food temperatures in positions 2, 3 and 4 in the top of the oven (Table 5). The lack of a floor in Oven A was probably the factor producing significant differences in temperature since this oven had two fans and was provided with greater power than any of the other ovens (Table 1).

No significant differences according to position were found as food was heated in either of the other two ovens (Table 5). Lack of variability may be accounted for by the fact that a heating element was in the bottom of Oven B and two fans were incorporated in Oven D as opposed to Oven C which had only one.

In two of the four ovens, highest mean temperatures were recorded in position 2 (Table 5). In Ovens C and D, temperatures were generally hottest in the top four positions, and coldest in the bottom four, with the four in the center area

—Continued on page 1070

QUALITY CONTROL STANDARDS FOR COOKED FROZEN GREEN BEANS HELD ON A STEAM TABLE FOR VARYING HOLDING TIMES

ANNA M. BRENNER, MARY J. HITCHCOCK, R. H. LANE, GRAYCE E. GOERTZ, ADA M. CAMPBELL and J. L. COLLINS

ABSTRACT

Two varieties of whole green beans, prepared by steaming, were held on a steam table (>60°C) with portions removed at 10-min intervals up to 60 min. Dominant wavelengths and Instron measurements for force, shear peak area and work were determined for the seven holding periods. Varieties were inherently different in color ($P < 0.01$). Holding time affected dominant wavelength ($P < 0.01$), Munsell color ($P < 0.01$), force ($P < 0.05$) and work ($P < 0.05$). Texture, color and overall acceptability were evaluated by a trained sensory panel. A consumer panel also evaluated overall acceptability. Orthogonal comparisons of sensory and objective evaluations for dominant wavelengths, force and work were linear. Acceptability scores were highest at 30-min heated holding time. Statistical analysis indicated that objective quality control standards could be defined by a range of Munsell colors or dominant wavelengths and/or force and work measurements obtained at optimum holding periods.

INTRODUCTION

EXPANSION of the food service industry in recent years has dictated a re-evaluation of production, preparation and service procedures in quantity food operations to optimize the retention of nutrients and sensory attributes in food. Objective standards for attributes of food quality preferred by the consumer must be established before control of food quality can be initiated. These objective standards should be established for food preparation and service to insure maintenance of quality that the consumer desires.

Methods employed in food preparation usually include a heating operation. Both desirable and undesirable effects of heat processing may result simultaneously. Favorable alterations of product characteristics are color and textural changes leading to increased palatability. Excessive heating, however, may cause a loss of nutrients and undesirable changes in color, flavor and texture. Time-temperature parameters that produce desirable effects must be used in order to maximize both sensory and nutritional qualities of the food.

Following preparation, food is generally held at temperatures ranging from 60–100°C (Livingston et al., 1973) for a period of time prior to and during service to reduce bacterial growth. If food is to be held at these temperatures before and during service, the nutritional and sensory changes occurring need to be known. An objective standard correlated with sensory evaluation indicating food quality would be of value to the food service industry in training personnel and in maintaining quality control.

Objective measurements of texture, a food attribute, can be correlated with sensory evaluation for consumer acceptance. Szczesniak et al. (1963) developed standard rating scales for texture intensity for common food products by comparing objective measurements with judgments of nine taste panel members. One of the significant trends in instrumental texture measurement, according to Szczesniak (1972) is instrumental-sensory correlation. Choice of instrument and test cell is based on that sensory characteristic under consideration. Howard and Heinz (1970) measured shear and compression strengths of individual raw carrots with the Instron Universal Testing Machine. Compression correlated highly and shear poorly with sensory hardness as judged by compressing and flexing of carrots. Voisey and Larmond (1971) used five different test cells with the Instron to evaluate textural qualities and agronomic performance of developing varieties of beans. Objective results were highly correlated with each other and with sensory rating for hardness and cohesiveness.

Holt (1970), using a method described by Bourne et al. (1966), studied the relationship between firmness and pectic substance during ripening by obtaining force-displacement data using the Instron. More recently Breene et al. (1972, 1973) devised a procedure for determining texture profile analysis (TPA) parameters important as a quality criterion for new varieties of pickling cucumber.

The color of food provides another sensory attribute by which sensory and objective evaluations may be correlated. Walker et al. (1973) found that colors associated with fruits and vegetables were strongly linked to taste expections among children. Bowman et al. (1971) reported that sensory evaluation of fresh and frozen vegetables prepared by three different methods revealed color to be of greater importance to panelists than flavor, tenderness or texture.

Undesirable color changes that result during preparation of vegetables containing chlorophyll is a well known problem. Little and Mackinney (1972) demonstrated that weak acids present in fruits and vegetables degrade chlorophyll a and b to pheophytins a and b during preparation in aqueous solution. Degradation of chlorophyll a and b has been found to occur at different rates according to Charley (1972) and Clydesdale et al. (1971). Sweeney and Martin (1961) showed that the conversion was pH dependent with greater retention of chlorophyll occurring in cooked green beans prepared at a higher pH. Sweeney (1970) obtained percent unconverted chlorophyll and Gardner aL/bL values for beans held on a steam table. Values increased significantly when a mixture of magnesium carbonate and calcium acetate was added to the cooking water. Similarly, Clydesdale et al. (1971) measured chlorophyll retention in spinach, green peas and beans with a Hunter Lab Model D25 Difference Meter after maintaining the pH of the cooking water with a phosphate buffer and the addition of magnesium carbonate.

Clydesdale and Francis (1969a) obtained tristimulus values for spinach puree with three different instruments. These values were mathematically transferred to tristimulus values of the other two color systems and also reduced to common color functions as well as correlated with visual rankings. Good correlations of instrument and visual rank were observed.

The purpose of this study was to investigate changes in color and texture of prepared frozen green beans held heated on a steam table for different time intervals. Relationships among holding times for fully prepared frozen green beans, consumer acceptability, sensory evaluation, and objective measurements for color and texture were studied in order to

At the time the work reported in this paper was done, authors Brenner, Hitchcock, Goertz, Campbell and Collins were with the Dept. of Food Systems Administration, and Dept. of Food Technology, University of Tennessee, Knoxville, TN 37916. Author Brenner is now affiliated with the Dept. of Nutrition & Food Science, University of Kentucky, Lexington, KY 40506. Author Lane is with the Dept. of Nutrition & Food Science, University of Kentucky, Lexington, KY 40506.

0022-1147/78/0004-1066S02.25/0
© 1978 Institute of Food Technologists

recommend a range of color and texture values for objective standards of quality control.

MATERIALS & METHODS

120 lb OF PECO VARIETY and 122 lb Slender White variety grade A, frozen, whole green beans were obtained from Winter Garden, Incorporated, Bells, TN. Both varieties were blast frozen and packaged on July 25 and Aug. 2, 1973. All frozen beans were packaged in 2-lb capacity, wrapped waxed cardboard cartons. They were held in storage which did not exceed −18°C until shipped to Knoxville, TN, on June 13, 1974. The frozen green beans were stored at −18°C in a Victory two-door upright freezer from time of receiving until removal for preliminary studies and research.

Sample preparation

Six packages (12 lb) of frozen green beans were drawn at random from the freezer and emptied into a large mixing bowl. After mixing thoroughly, the following amounts were removed for objective measurements: 30g were placed in a covered dish to protect them from light and air until measurements were made with a Signature Model D-1 Kollmorgen Color Eye; two 100g portions were packaged in plastic bags and held at 3°C until analysis could be made with the Instron; 20g were held in a covered plastic container until sliced finely, weighed and placed in a vacuum oven for moisture determinations; and, 30g were used for pH measurement. The remaining green beans were divided into seven equal portions (735g) and placed in seven identical half size, 2½ inch deep stainless steel standard steam table insert pans marked with holding times of 0, 10, 20, 30, 40, 50 and 60 min.

Steam tables were three identical 4 inch deep stainless steel, standard size steam table insert pans, each placed over two small electric heating elements. For each steam table water levels were maintained at 1 inch below pans containing beans and water temperature was held at 100°C. These conditions provided an internal temperature of the green beans above 60°C (Longree, 1972).

Beans were steamed at 5 lb pressure for 30 min from the frozen state in an upright self-generating Market Forge Steamer (Model No. MF2AEWT403). Temperature of the beans after preparation was 82°C. Six of the pans were placed immediately on the three improvised steam tables and covered with stainless steel lids while one pan was covered and allowed to cool. A pan was removed from the steam table at each 10-min interval for 1 hr. Final temperature of the beans after 1 hr heated holding was 71°C. After removal the beans were stirred gently and previously stated quantities for raw beans were removed from each of the seven pans and held for objective analyses. The remaining contents of the pans were covered and cooled to room temperature for sensory evaluation.

Sensory evaluation

Sensory evaluation was performed by three volunteer panels, two of which were consumer panels, and one a trained panel. Consumer panels were composed of students, staff and faculty in the College of Home Economics. Both men and women participated. Forty consumers sampled the Peco variety and 31 sampled the Slender White variety for overall acceptability. The two varieties were presented at room temperatures on different days between 9 a.m. and 4 p.m. Seven graduate students from the Department of Food Science, Nutrition & Food Systems Administration served as members of the trained panel. Panel members were informed regarding general conditions of the study and specific holding times. Training sessions were conducted five days prior to actual testing on a distinctly different variety of bean.

A seven-point descriptive texture scale (Finney, 1971; Mackey et al., 1973) was employed with terms for firmness ranging from "very firm" to "very soft." Random numbers were assigned to each holding period and one-third cup of beans representing each holding period was placed in a glass custard cup for presentation to the panelist. Trained panel members were instructed to feel the bean, then bit through it once before chewing (Brandt et al., 1963; Sherman, 1969; Szczesniak and Bourne, 1969; Szczesniak et al., 1963). Texture was evaluated under red lights while subsequent sensory evaluations were conducted under natural white fluorescent lights.

For overall acceptability, a seven-point hedonic scale was used (Kramer and Twigg, 1970; Pilgrim, 1957). Terms for acceptability ranged from "like very much" to "dislike very much." Panelists were also requested to indicate whether they liked or disliked the color, flavor, and texture of each sample.

The color scale was a seven-point descriptive scale (Jeremiah et al., 1972) ranging from dark green to dark olive green. Panelists monitored color change with increasing holding time. Selected Munsell color charts (Anon., 1952) were placed in order around three sides of a Macbeth Color Booth, Executive Model (BBX234) for panel reference while samples were displayed randomly. Daylight fluorescent light was used to provide controlled color background and illumination for samples during the test period.

Numerical scores were assigned to descriptive terms by multiplying the descriptive term farthest to the left by a factor of seven and decreasing the multiplier by one for each column until the far right hand column was multiplied by one. Numerical values obtained were used in analyses of variance for panel scores for texture, overall acceptability and color.

Objective measurements

Objective measurements for color, force, shear peak area, and work, pH and moisture were made on the same day as the sensory evaluation for each replication.

Texture related measurements

Values of force, shear peak area and work were measured with an Instron Universal Testing Machine (TM1132) fitted with a Kramer Shear Compression Cell. Measurement of force was obtained by determining the loading rate required to shear a 100g sample of green beans (Szczesniak et al., 1970). Five hundred kg were applied to raw beans and 200 kg to cooked beans. Crosshead speed and chart speed were synchronized at 10 cm/min. Shear peak area was obtained by determining the area bounded by the maximum force curve. Work was calculated by multiplying shear peak area times force according to the method of Mackey et al. (1973).

Color related measurements

Light reflectance was measured with a Kollmorgen Color Eye (Signature Model D-1). Vitrolite standards were used for standardization before sample measurements were made. Green beans were left whole and packed tightly in a glass cuvette minimizing air bubbles and empty space before reflectance measurements were made. A twofold thickness of tightly woven black cloth was placed over both ports of the Color Eye to avoid interference by stray light. Readings for settings X, Y, Z and X^1 were converted to C.I.E. readings for X, Y, Z coordinates X and Y, yellow index, lightness, Hunter a and b, Hue Angle, and Saturation Index by standard operating procedures (Anon., 1972). Formulas given by Clydesdale and Francis (1969b) were used to convert C.I.E.; X, Y, Z readings expressed as Hg0−10C to Hunter Lab L, a and b units.

Other related measurements

pH of raw and cooked samples for the two varieties of beans was determined to observe the relationship between pH and color change. A 30g sample was blended with 50 ml distilled water and the pH determined with a Corning Model 10 pH Meter. Moisture was determined by heating 5−8g duplicate samples of finely cut beans in a vacuum oven at 68°C for 15 hr.

Experimental design and statistical analysis

A split plot design with five replication of two varieties (whole plots) was chosen. Seven holding periods (split plots) were assigned randomly within each replication for the objective measurements. Four replications of each variety were presented to the trained panel for sensory evaluation. One replication of each variety was presented to consumer panels for overall acceptability. Analysis of contingency tables using chi-square values (Snedecor and Cochran, 1967) as test statistics to test the hypothesis of no difference in preference between two varieties as a function of holding time were used.

Effects of heated holding time on means of trained panel scores for texture, color, and acceptability were partitioned into single degree of freedom contrast by use of orthogonal polynomials.

RESULTS & DISCUSSION

Sensory evaluation

Consumer panel data were arranged into contingency tables and chi-square statistics were used for testing no difference in varietal preference. Variety did not affect difference significantly for overall acceptability. Both varieties were equally acceptable to the consumer panels. The greatest difference between the two varieties of green beans for consumer acceptability with holding time was for the scale term "dislike slightly." Peco beans were disliked more during the first 20 min of holding time as a result of their greener color and firmer texture. Both attributes possibly are least acceptable to

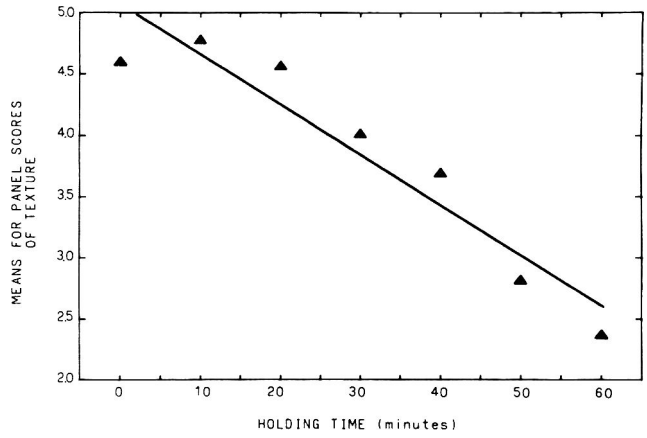


Fig. 1—Mean sensory scores (▲) averaged for texture of Peco and Slender White beans at 10-min intervals of heated holding time.

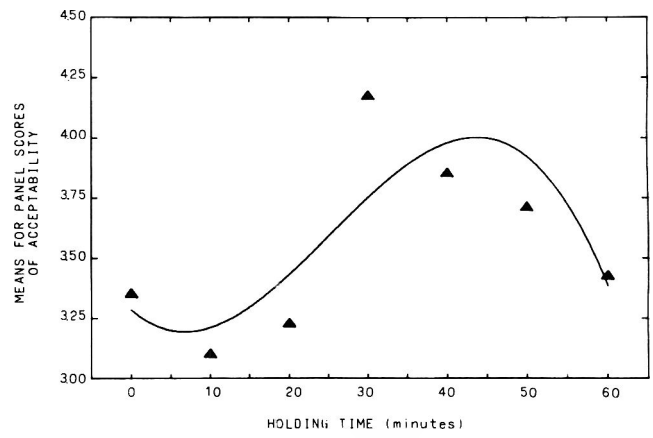


Fig. 2—Mean sensory scores (▲) averaged for overall acceptability of Peco and Slender White beans at 10-min intervals of heated holding time.

persons with southeastern regional food preferences.

Trained panel evaluation of texture showed that there was no significant difference between variety and texture or variety and heated holding time. Holding time affected sensory scores for texture significantly ($P < 0.0001$). The decrease in softness of green beans as detected by the trained panel was linear (Fig. 1). Textural changes were in agreement with objective measurement values for force and work. Trained panel evaluation of overall acceptability was not affected significantly by variety, but was affected significantly ($P < 0.05$) by holding time. There was no interaction between variety and holding time. Acceptability was highest after beans were held heated for 30 min and lowest when held 10 min as indicated by the cubic component of the orthogonal comparison (Fig. 2). The cubic component for acceptability was attributed to bimodal preference by the panel for green beans based on the attributes of texture, flavor, and color. Four of the five panelists from the southeastern region preferred the yellow-green color and soft texture that results from traditional low temperature-long time southern cookery. The other panelist agreed with those panelists reared outside the southeastern region in preferring beans that were green and barely tender after preparation.

Trained panelists' comments regarding color included that opinion that Peco green beans were too green in color at 0- and 10-min holding times. Panelists, however, considered the color of Peco beans to remain more even and acceptable over a longer range of holding times than that of Slender White beans which were non-uniform in color over the entire holding range. The most acceptable color for both varieties of beans was at 30 min heated holding time. The range of holding times within which panelists preferred the texture was narrower than that for color.

Comments regarding texture changes with holding time were directed largely toward the Slender White variety. Even though the majority of the panel members preferred a softer bean, softness became objectionable after 50- and 60-min holding time, as indicated by judges' comments. Texture of the Slender White bean generally was acceptable at an earlier holding time than the Peco bean and less acceptable at the later holding times. Texture change was uniform in all of the Peco beans but not in the Slender White beans.

Flavor was most acceptable for the 30 min holding period for both varieties of beans. The range of holding times within an acceptable flavor range was nearly as restricted as that for texture.

Variety significantly affected sensory scores for color ($P < 0.05$) indicating that trained panelists discerned the genetic difference in color of Slender White and Peco varieties. A significant linear relationship (Fig. 3) existed between color and holding time ($P < 0.001$). Color change as described by the panel was from dark green to dark olive green over the 60 min of holding time. There was no significant interaction between variety and holding time with respect to color.

Objective measurements

Variety did not significantly affect force measurements. Force decreased significantly with holding time ($P < 0.05$). A plot (Fig. 4) of force as a function of holding time demon-

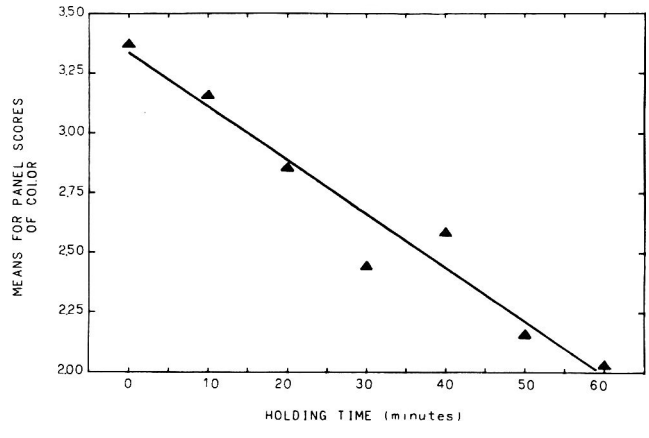


Fig. 3—Mean sensory scores (▲) averaged for color of Peco and Slender White beans at 10-min intervals of heated holding time.

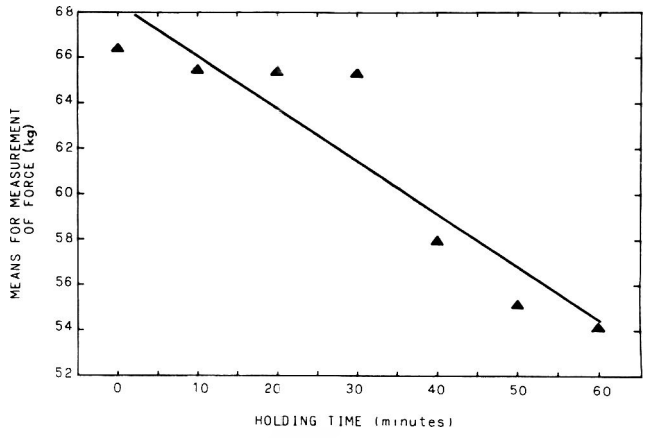


Fig. 4—Mean of force values (▲) averaged for Peco and Slender White beans at 10-min intervals of heated holding time.

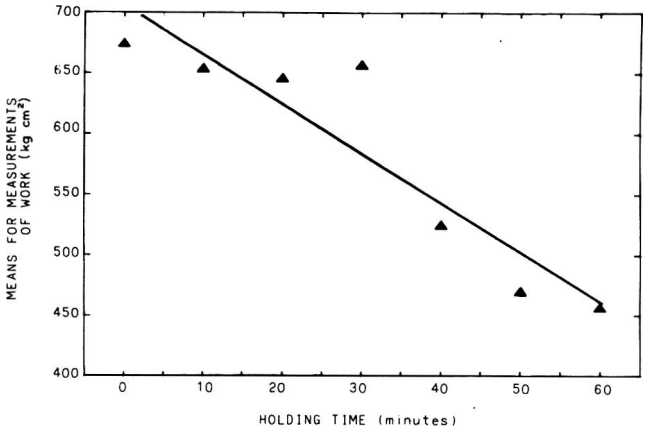


Fig. 5—Mean of work values (▲) averaged for Peco and Slender White beans at 10-min intervals of heated holding time.

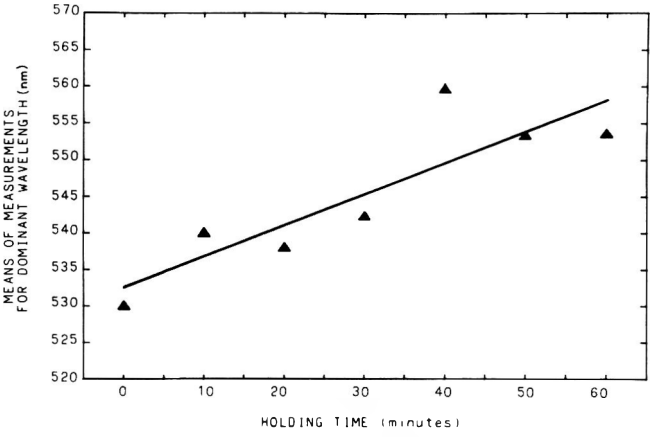


Fig. 6—Dominant wavelengths (▲) averaged for Peco and Slender White beans at 10-min intervals of heated holding time.

strated a linear effect. There was no significant interaction between variety and holding time with respect to shearing force measurement. Initial shearing of the beans was comparable to the force required to bite through the bean. Shearing force values were similar to those reported by Sweeney (1970) both as to magnitude and as to the effect of holding over steam. These values probably reflect the effects of heat on pectic substances, hemicelluloses and celluloses, all of which contribute to firmness of the green bean.

Shear peak area was not affected significantly by variety, holding time, or interaction between variety and holding time. The green beans retained nearly their same shape and moisture content throughout the 60-min holding time as reflected by shear peak area that only approached significance ($P < 0.09$).

Although variety did not affect work significantly, there was a significant linear decrease ($P < 0.05$) in work with respect to holding time (Fig. 5). There was no significant interaction between variety and holding time with respect to work.

Dominant wavelengths differed significantly ($P < 0.01$) for the two varieties of beans as a result of the characteristic color of each variety. Holding time caused a highly significant linear increase ($P < 0.01$) in dominant wavelength (Fig. 6) from green toward yellow. No significant interaction between variety and holding time was found with respect to color. Dominant wavelength means for Peco beans ranged from 528.0–551.2 nm and dominant wavelength means for Slender White beans ranged from 523.4–556.2 nm.

Munsell color values were affected significantly ($P < 0.0001$) by variety but not by holding time. There was no significant interaction between variety and holding time with respect to color. Variety did not affect Hunter a and b values significantly, nor were they affected by holding time. Although there was no significant interaction between variety and holding time with respect to Hunter a values, Hunter b values obtained by conversion from C.I.E. values (Anon., 1972) demonstrated a significant interaction ($P < 0.05$) between variety and holding time. Conversion values by procedures given by Clydesdale and Francis (1969b) were not significant.

C.I.E. values when converted to Munsell color values and Hunter a and b values were not as significant as dominant wavelength values. This may be attributed, as Clydesdale and Francis (1969c) reported, to the fact that transformation to x, y, z values from other systems result in very different absolute values than those obtained when x, y, z readings are obtained directly from a system.

The color of green beans in this study changed similarly to the description given by Charley (1972) from green to yellowish green and to an increasingly yellow green as pH values decreased with increased heated holding time. Change in color indicated breakdown of chlorophyll a and b pheophytin a and b as volatile acids accumulated in moisture within and around the green beans. Mean pH values for Peco and Slender White green beans with respect to holding time are given in Table 1. As holding time increased, a decrease in pH was observed which is consistent with reports of Clydesdale et al. (1970) and Sweeney (1970).

Moisture content varied slightly in green beans from the raw state to the end of the 60-min holding time. Peco beans varied from 91.83–91.53% moisture while Slender White varied from 89.69–89.02% moisture (Table 2). The difference in moisture content between the two varieties was attributed to the Slender White bean being larger and more fully developed.

Under the conditions of this study, objective measurements in combination with sensory evaluation resulted in objective parameters that could be predictive of acceptability to this particular group of panelists. Methodology presented here

Table 1—pH values for Slender White and Peco beans when raw and held heated for varying holding periods

Raw and holding periods (min)	pH Slender White	pH Peco
Raw	5.90	6.18
0	5.68	5.71
10	5.70	5.87
20	5.67	5.81
30	5.70	5.68
40	5.57	5.75
50	5.50	5.69
60	5.47	5.66

Table 2—Means of measurements for moisture content of Peco and Slender White beans

Raw through intervals held heated	Peco % Moisture	Slender White % Moisture
Raw	91.83	89.69
0	91.46	89.07
10	91.26	88.65
20	91.42	89.23
30	91.26	89.58
40	91.68	88.89
50	91.21	89.26
60	91.53	89.02

could be employed in other regions of the United States to indicate consumer preference and establish objective quality control standards for the food service industry.

REFERENCES

Anon. 1952. "Munsell Color Charts for Plant Tissue," 1st ed. Munsell Color Company, Inc., Baltimore, MD.

Anon. 1972. "Procedures for Operation of Food Testing Equipment and for Some Objective Tests." Dept. of Food Science, Nutrition and Food Systems Administration, The University of Tennessee, Knoxville, TN.

Bourne, M.C., Moyer, J.C. and Hand, D.B. 1966. Measurement of food texture by a universal testing machine. Food Technol. 20: 170.

Bowman, F., Page, E., Remmenga, E.E. and Trump, D. 1971. Microwave vs conventional cooking of vegetables at high altitude. J. Am. Dietet. A. 58: 427.

Brandt, M.A., Skinner, E.Z. and Coleman, J.A. 1963. Texture profile method. J. Food Sci. 28: 404.

Breene, W.M., Davis, W.D. and Chou, H. 1972. Texture profile analysis of cucumbers. J. Food Sci. 37: 113.

Breene, W.M., Davis, W.D. and Chou, H. 1973. Effects of brining on objective texture profiles of cucumber varieties. J. Food Sci. 38: 210.

Charley, H. 1972. Fruits and vegetables. In "Food Theory and Applications," Ed. Paul, P.C. and Palmer, H.H. John Wiley and Sons, New York, NY.

Clydesdale, F.M. and Francis, F.J. 1969a. Calorimetry of foods. 1. Correlation of raw, transformed and reduced data with visual rankings for spinach puree. J. Food Sci. 34: 349.

Clydesdale, F.M. and Francis, F.J. 1969b. Color measurement of foods. 13. Sample presentation/physical attributes which influence color measurement. Food Product Dev. 3: 23.

Clydesdale, F.M. and Francis, F.J. 1969c. Color measurements of foods. 14. Color scales. Food Product Dev. 3: 117.

Clydesdale, F.M., Goodman, A.W. and Francis, F.J. 1971. The effect of a phosphate buffer and magnesium carbonate on quality attributes of cooked green vegetables. J. Milk Food Technol. 34: 78.

Finney, E.E. Jr. 1971. Dynamic elastic properties and sensory quality of apple fruit. J. Texture Studies 2: 62.

Holt, C.B. 1970. Measurement of tomato firmness with a universal testing maching. J. Texture Studies. 1: 491.

Howard, P.L. and Heinz, D.E. 1970. Texture of carrots. J. Texture Studies. 1: 185.

Jeremiah, L.E., Carpenter, Z.L. and Smith, G.C. 1972. Beef color as related to consumer acceptance and palatability. J. Food Sci. 37: 476.

Kramer, A. and Twigg, B.A. 1970. "Quality Control for the Food Industry," 3rd ed, Vol. 1. p. 255. Avi Publishing Company, Inc., Westport, CT.

Little, A.C. and Mackinney, G. 1972. The coloring matters of food. World Review of Nutrition & Dietetics 14: 85.

Livingston, G.E., Ang, C.Y.W. and Chang, C.M. 1973. Effects of food service handling. Food Technol. 27: 28.

Longree, K. 1972. "Quality Food Sanitation," 2nd ed, p. 379. Wiley Interscience, New York, NY.

Mackey, A., Hard, M.M. and Zaehringer, M.V. 1973. Measuring textural characteristics of fresh fruits and vegetables, apples, carrots, cantaloupe. Bull. 123, Oregon State University Exp. Sta. Corvallis, OR.

Pilgrim, F.J. 1957. The components of food acceptance and their measurement. J. Clin. Nutr. 5: 171.

Sherman, P. 1969. A texture profile of foodstuffs based upon well-defined rheological properties. J. Food Sci. 34: 458.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," p. 543. The Iowa State University Press, Ames, IA.

Sweeney, J.P. 1970. Improved chlorophyll retention of green beans held on the steam table. Food Technol. 24: 490.

Sweeney, J.P. and Martin, M.E. 1961. Stability of chlorophyll in vegetables as affected by pH. Food Technol. 16: 263.

Szczesniak, A.S. 1972. Instrumental methods of texture measurement. Food Technol. 26: 50.

Szczesniak, A.S. and Bourne, M.C. 1969. Sensory evaluation of food firmness. J. Texture Studies 1: 52.

Szczesniak, A.S., Brandt, M.A. and Friedman, H.H. 1963. Development of standard rating scales for mechanical parameters of texture and correlation between the objective and the sensory methods of texture evaluation. J. Food Sci. 28: 397.

Szczesniak, A.S., Humbaugh, P.R. and Block, H.W. 1970. Behavior of different foods in the standard shear compression cell of the shear press and the effect of sample weight on peak area and maximum force. J. Texture Studies 1: 356.

Voisey, P.W. and Larmond, E. 1971. Texture of baked beans—A comparison of several methods of measurements. J. Texture Studies 2: 96.

Walker, M.A., Hill, M.M. and Millman, F.D. 1973. Fruit and vegetable acceptance by students. J. Amer. Dietet. A. 62: 268.

Ms received 10/28/77: revised 1/30/78; accepted 2/8/78.

CONVECTION OVENS IN COMMISSARY FOODSERVICE . . . From page 1065

intermediate (Table 5). For Ovens A and B, temperatures were generally hottest in the right rear corner (Table 5). Highest temperatures in Oven B were from positions 2, 6 and 10, and in Oven A, positions 2 and 6 with temperature of food in position 10 higher than any recorded in the other middle and bottom areas of the oven (Table 5).

Overall ranges in temperature were somewhat wide in three out of four of the ovens (Tables 2 and 3), but, except in Oven A, all temperatures recorded met public health recommended temperature of 60°C for reheating cooked food (FDA, 1974).

Data indicated that temperature variability was a significant factor in heating food in convection ovens and that equipment model had significant influence. Factors which appeared to have effect on temperature included oven thermostatic setting, oven power rating, placement of heating elements, number of meals heated at one time, number of fans, floor or base in oven, and position of food in oven. Equipment of this type may be effectively utilized in foodservice systems but must be carefully engineered, selected, and operated.

REFERENCES

Anonymous. 1973. Blodgett Zephaire forced convection ovens. 6L27J, The G.S. Blodgett Co., Inc., Burlington, VT.

Cremer, M.L. and Chipley, J.R. 1977. Satellite foodservice system assessment in terms of time and temperature conditions and microbiological and sensory quality of spaghetti and chili. J. Food Sci. 442: 225.

Decareau, R.V. 1971. Equipment used for heating pre-cooked frozen foods for foodservice. Microwave Energy Appl. Newsl. 4: 9.

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

FDA. 1974. Foodservice sanitation. Federal Register 39: 35438.

Livingston, G.E. and Chang, C.M. 1972. Second-generation reconstitution systems. Cornell Hotel Restaurant Admin. Quart. 13: 57.

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

Unklesbay, N.F., Maxcy, R.B., Knickrehm, M.E., Stevenson, K.E., Cremer, M.L. and Matthews, M.E. 1977. Foodservice systems: product flow and microbial quality and safety of foods. Res. Bull. 1018, Mo. Agric. Exp. Stn., Columbia, MO.

Ms received 10/15/77; revised 12/23/77; accepted 12/30/77.

Journal article number 164-76 of the Ohio Agricultural Research & Development Center, Wooster, OH 44691.

WATER–SOLUBLE VITAMIN CONTENT OF SOME CALIFORNIA WINES

M. N. VOIGT, R. R. EITENMILLER, J. J. POWERS and G. O. WARE

ABSTRACT

The thiamine, riboflavin, vitamin B_6, vitamin B_{12}, niacin, biotin, folate, pantothenate and vitamin C levels in several American wines were determined. Five types of white wine and eight types of red wine were examined. The levels of the eight B-vitamins were higher in the red wines than in the white. Nutritionally significant levels of riboflavin, vitamin B_6 and niacin were found in the red wines. Folate levels of the wines were lower than reported in earlier studies. Statistical evaluation of the thiamine data indicated the presence of inhibition in these assays.

INTRODUCTION

ONE OF THE FIRST studies on the vitamin content of American grape juices and wines was carried out by Morgan et al. in 1939. They reported that about two-thirds of the thiamine and riboflavin in grape juice was lost during wine making but that very little was lost during aging. Sulfite treatment of the must, which occurs before fermentation and bentonite clarification, accounted for 50% of the decrease. White wines contained more riboflavin than red wines. A dry muscat wine was found to contain nearly two-thirds the riboflavin content of fresh bovine milk. Castor (1953) studied the changes in the vitamin content of fermenting grape musts. Castor found that riboflavin increased, pantothenate usually decreased, vitamin B_6 slightly decreased, and biotin was largely lost. After 5 yr aging, 36% of the riboflavin and 45% of the pantothenate in the wines was lost. The following year, a book entitled, *Wine as Food and Medicine*, was published by Lucia (1954). Lucia reasoned that when wines are consumed as part of a balanced diet, they contribute sufficient quantities of thiamine, riboflavin, pantothenate, niacin and vitamin B_6 to be of nutritional value. Lucia indicated that certain red sweet wines contain 71% more riboflavin and 45% more folic acid than the grape juice they originated from. Hall et al. (1956) reported on the thiamine, riboflavin, niacin, pantothenic acid, folic acid, vitamin B_6 and vitamin B_{12} contents in white and red grape varieties, their musts and three vintages of wine. Red grapes and musts were richer in all of the vitamins than the corresponding white grapes and musts. The same was true for the red wines compared to white wines, except for pantothenate. The wines fermented on the skins, dry red, had vitamin contents not much greater than those not fermented in this manner, rose dry. The red sweet dessert wines had higher vitamin retentions. Thiamine and pantothenate were lost primarily during vinification, while riboflavin and folic acid contents decreased during must extraction. Niacin and vitamin B_6 decreased during both processes, especially during fermentation, except in the sweet red wines which retained nearly all of these vitamins.

Authors Voigt, Eitenmiller and Powers are with the Food Science Dept., University of Georgia, Athens, GA 30602. Author Ware is Station statistician, Georgia Agricultural Experiment Station, Athens, GA 30602.

0022-1147/78/0004-1071$02.25/0
© 1978 Institute of Food Technologists

Table 1—Unabridged names of the wines

Wine no.	Wine
1.	Italian Swiss Colony Rhineskeller
2.	Sauvignon Blanc, WAB[a] ref., bottle 1
3.	Zinfandel, WAB ref., bottle 1
4.	Cabernet Sauvignon, WAB ref., bottle 1
5.	Gallo Rhine Garten, bottle 1
6.	Inglenook Zinfandel, 1972
7.	Italian Swiss Colony Zinfandel
8.	Italian Swiss Colony Burgundy
9.	Gallo Burgundy
10.	Inglenook Burgundy, 1971
11.	Gallo Hearty Burgundy, bottle 2
12.	Gallo Hearty Burgundy, bottle 1
13.	Gallo Rhine Garten, bottle 2
14.	Italian Swiss Colony Rhineskeller, bottle 2
15.	Pau Masson Rhine Castle
16.	Cabernet Sauvignon, WAB ref., bottle 2
17.	Zinfandel, WAB ref., bottle 2
18.	Chenin Blanc, WAB ref.
19.	Sauvignon Blanc, WAB ref., bottle 2

[a] Wine Advisory Board

French and Soviet investigators have published several studies on the vitamin content of wine. Amerine (1977) recently published a study on the role of wine in human nutrition. In 1970, Durnac published an extensive study on the vitamin contents of French wine. The Soviet researcher Oprya published his findings on the changes in B-vitamin levels in grape musts during continuous fermentation in 1969. The Soviet researchers Avetisyan and Adamyan (1972) studied thiamine, riboflavin and vitamin B_6 content of 30 grape varieties and their wines. They found that grapes from high sugar and elite varieties had higher vitamin contents than low sugar and medium varieties; that varieties with intensely colored grapes had higher vitamin contents than the white skin grapes; that frost resistant high sugar and elite varieties were higher in vitamin contents than ones not resistant to frost. However, their findings indicated that wines from the white-skinned grapes had higher vitamin contents than those made form intensely colored grapes.

Since the study by Hall et al. (1956), there has been little advancement in the knowledge of the vitamin contents of American wines. The acquiring of data has not kept pace with changes in the industry itself. Modifications in cultural practices for grapes, grape handling procedures, fermentation and cellar methods, aging, bottling and distribution methods all have undergone changes in the past 20 yr. For this reason, this research was conducted to determine the B-vitamin and ascorbic acid contents of California wines.

MATERIALS & METHODS

TABLE 1 lists the complete names of the 19 wines included in this study. The wines were analyzed for nine vitamins (thiamine, biotin, vitamin B_{12}, folate, niacin, pantothenate, riboflavin, vitamin B_6 and vitamin C).

The wine samples were prepared for vitamin analysis employing the procedures described by Baker and Frank (1968) for urine vitamin analysis. Thiamine, biotin, vitamin B_{12} and folate were assayed using the corresponding Baker and Frank (1968) procedures, i.e., *Ochro-*

Table 2—Vitamin content of wine[a]

Wine[b]	Vitamin (ng/ml)								
	Thiamine-Lf[c]	Riboflavin	Vitamin B_6	Vitamin B_{12}	Biotin	Niacin	Pantothenate	Folate	Vitamin C[d]
White Wines									
Chenin Blanc (18)	6.5[e]	33[e]	210	0.068	1.3	380	260	0.68	20
Sauvignon Blanc (2)	9.5[e]	77	200	0.001[e]	0.9	530	230	9.2	0
Sauvignon Blanc (19)	0.0[e]	69	120	NV[e]	1.6	500	220	7.5	50
Rhineskeller (1)	NV[e,f]	63	120	0.001[e]	5.0	950	130	1.6	0
Rhineskeller (14)	NV[e]	79	110	0.014	6.3	900	120	1.2	30
Rhine Garten (5)	0.59[e]	16	160	NV[e]	7.4	750	250	1.2	0
Rhine Garten (13)	0.0[e]	11	90	NV[e]	5.6	650	200	1.3	0
Rhine Castle (15)	3.3[e]	61	150	0.004[e]	7.3	630	310	1.9	10
Red Wines									
Cabernet Sauvignon (4)	18[e]	250	240	0.11	26	430	310	7.9	0
Cabernet Sauvignon (16)	14[e]	210	190	0.19	25	430	240	7.4	0
Zinfandel (3)	30[e]	260	950	0.18	5.9	750	370	5.0	0
Zinfandel (17)	22[e]	260	560	0.12	5.8	730	370	7.9	20
Zinfandel (6)	2.0[e]	370	300	0.20	10	800	400	8.5	20
Zinfandel (7)	23[e]	330	170	0.14	18	930	230	2.9	10
Burgundy (12)	13[e]	340	220	0.13	14	1100	390	11	0
Burgundy (11)	13[e]	260	320	0.12	13	ND[f]	340	8.6	0
Burgundy (9)	20[e]	150	320	0.094	6.6	1100	330	11	0
Burgundy (8)	24[e]	330	190	0.09	15	950	320	6.5	40
Burgundy (10)	58[e]	330	290	0.18	13	780	440	2.0	50

[a] Pooled test and standard regressions for the calibration curves had $r^2 > 0.9$ ($P \leq 0.001$)
[b] Number in parenthesis is the wine identification number from Table 1
[c] "Lf" L. fermenti thiamine assay method
[d] Vitamin C levels calculated manually
[e] Inhibition detected at $P \leq 0.05$
[f] "NV" calculation not valid; "ND" not determined

monas danica (thiamine and biotin), *Ochromonas malhamensis* (vitamin B_{12}) and *Lactobacillus casei* (folate). Thiamine assays using *Lactobacillus fermenti* were also conducted (Difco, 1968). Niacin and pantothenate were determined using the *Lactobacillus plantarum* assays described by Difco (1968), while riboflavin and vitamin B_6 were determined using the *L. casei* and *Saccharomyces carlsbergensis* assays, respectively, as described by Difco (1968). Four aliquots of the wine extracts (0.5, 0.5, 1.0 and 1.5 ml) were analyzed. At least two experimental runs were conducted for each vitamin. The absorbance measurements, calibration curves and computer analysis methods employed were recently described (Voigt, 1977). Total vitamin C was determined by the microfluorometric procedure of Deutsch and Weeks (1965).

The computer program used to analyze the data evaluated each set of analyses (a run) for the presence of inhibition or stimulation. This was determined by comparing the slopes of the dose-response curves obtained from the analyses of increasing volumes of the wine extracts to the slope of the dose-response curves of the standard vitamin solutions. A slope from a set of wine analyses that was less than the slope of the standard curve indicated that the sample inhibited the growth of the test organism under conditions of this study. A set of wine analyses with a slope greater than the standard curve indicated that the growth of the test organism was stimulated by something in the sample extract other than the vitamin being assayed. When deviations from parallelism of the sample and standard runs were detected ($P \geq 0.05$), the sample analyses were rejected. The mean vitamin values were pooled, providing their % SEs corresponded; otherwise, only the value from the run with the smallest % SE was accepted. The statistical methods were developed by Bliss and White (1967).

RESULTS & DISCUSSION

TABLE 2 lists the vitamin content of the wines. Experimental runs showing nonparallelism were considered invalid. This stipulation invalidates the results from both the *L. fermenti* and *O. danica* assays for thiamine, although the thiamine levels indicated were in agreement with recent literature values. The wines probably contained residual sulfite which destroys thiamine (Amerine, 1977; Hammond and Carr, 1976). Nonparallelism of sample and standard slopes was also detected in the vitamin B_{12} data from white wine analyses. The vitamin B_{12} assays contained added sulfite from the extract procedure. Perhaps the added sulfite plus the residual sulfite in the white wine was sufficient in concentration to inhibit the assay culture, *O. malhamensis*.

The levels of the eight B-vitamins were lower in the white wines than in the red wines (Table 2). This characteristic is especially pronounced in the riboflavin, vitamin B_6, vitamin B_{12} and biotin data. The study by Halls et al. (1956) had comparable findings, except one of their two data sets indicated pantothenate to be higher in white than red wines. Their data are presented in Table 3. Leake and Silverman (1966) noted slightly higher levels of pantothenate in white wines. Their data is also listed in Table 3. The levels of riboflavin, vitamin B_6, folate, niacin and pantothenate found in white wine in this study were consistently lower than the levels reported earlier. Only folate levels were consistently lower in red wine. Only the riboflavin content of red wines showed a consistent increase over the levels reported earlier. The levels of folate found in this study were markedly lower than those reported by Hall et al. (1956) and Leake and Silverman (1966). The difference could be due to their use of *Streptococcus faecalis* as the assay culture. *S. faecalis* is not recommended for the assay of folate activity in foods, since it responds to many of the clinically inactive forms of folate that are present in food.

The Wine Advisory Board provided four types of wine for analysis. Duplicate bottles of three of the four types were provided. The vitamin levels determined in these duplicate bottles (Wines 2 and 19, 4 and 16, 3 and 17) did not differ significantly.

Table 4 lists the approximate percentages of the United States Recommended Daily Dietary Allowance for vitamins supplied by 250 ml of wine. The data indicate that moderate consumption of wine, especially red, can contribute nutritionally useful amounts of riboflavin, vitamin B_6 and niacin. Lucia (1954) indicated that thiamine levels in wine were sufficient to be of nutritional value. Perhaps the wines that Lucia

Table 3—Reported levels of B-vitamins in wine

	Average Levels of Vitamin (ng/ml) in Wine								
	Hall et al. (1956)				Leake & Silverman (1966)			Present study	
	Set 1		Set 2				Amerine		
Vitamin	White	Red	White	Red	White	Red	(1977)	White	Red
Thiamine	35	50	10	70	20	10	10	33	22
Riboflavin	100	130	110	180	90	130	100	51	280
Vitamin B_6	230	740	310	330	260	420	300	150	340
Folate	14	22	20	13	18	18	0.5	3.1	7.2
Niacin	730	1100	550	1420	570	690	900	660	800
Pantothenate	250	300	480	320	370	330	500	220	340
Vitamin B_{12}	–	–	–	–	0.003	0.011	0.03	0.041	0.14
Biotin	–	–	–	–	0.6 to 4.6		5.0	4.4	14

studied had not received sulfite treatment during processing. If inhibition of the test organism had not been detected in the thiamine analyses of this study, nutritionally useful levels of thiamine (3.6% of U.S. RDA per 250 ml red wine) would have been indicated. Leake and Silverman (1966) reported that most of the vitamin C in fruit juices is destroyed during fermentation. Only low levels of vitamin C were found in the wines analyzed in this study.

Table 4—Approximate percentages of the U.S. RDA supplied by 250 ml wine

	Quantity present/250 ml min-max and average		% U.S. RDA[a]	
	White wines	Red wines	White wines	Red wines
Riboflavin	2.8–20 μg, 13	38–93 μg, 70	0.76	4.1
Vitamin B_6	23–53 μg, 36	43–240 μg, 85	1.8	4.3
Vitamin B_{12}	0.24–17 ng, 4.5	20–50 ng, 35	0.075	0.58
Folate	0.17–2.3 ng, 0.77	0.5–2.8 ng, 1.8	0.19	1.45
Niacin	95–240 ng, 165	110–280 ng, 400	0.83	2.0
Biotin	0.23–3.7 ng, 1.1	1.5–6.5 ng, 3.5	0.37	1.2
Pantothenate	30–78 ng, 54	58–110 ng, 85	0.54	0.85
Vitamin C	0–13 ng, 3.5	0–13 ng, 3.2	0.0058	0.0053

[a] 1974 U.S. RDA's (riboflavin 1.7 mg, vitamin B_6 2 mg, vitamin B_{12} 6 μg, folate 0.4 mg, niacin 20 mg, biotin 0.3 mg, pantothenate 10 mg, vitamin C 60 mg).

REFERENCES

Amerine, M.A. 1977. Wine in human nutrition. Bull. Off. Internat. de la vigne et du vin (Paris). Numero special: 137–151.

Avetisyan, R.G. and Adamyan, A.K. 1972. Levels of B-vitamins in grapes of wine varieties and hybrid seedlings and in wine. Prikl. Biokhim. Mikrobid. 8: 927.

Baker, H. and Frank, O. 1968. "Clinical Vitaminology—Methods and Interpretation." Interscience Publishers, New York.

Bliss, C.J. and White, C. 1967. Statistical methods in biological assay of the vitamins. In "The Vitamins," Ed. Gyorgy, P. and Pearson, W.N. p. 23. Academic Press, New York.

Castor, J.G.B. 1953. The B-complex vitamins of musts and wines as microbial growth factors. Appl. Microbiol. 1: 97.

Deutsch, M.J. and Weeks, C.E. 1965. Microfluorometric assay for vitamin C. Assoc. Offic. Anal. Chem. J. 48: 1248.

Difco. 1968. "Difco Supplementary Literature." Difco Laboratories, Detroit.

Durnac, A. 1970. The vitamins of wine. Annal. Nutr. et De L'-Alimentation. 24: 333.

Hall, A.P., Brinner, L., Amerine, M.A. and Morgan, A.F. 1956. The B-vitamin content of grapes, musts and wine. Food Research 21: 362.

Hammond, S.M. and Carr, J.G. 1976. The anti-microbial activity of SO_2—with particular reference to fermented and nonfermented fruit juices. In "The Society for Applied Microbiology Symposium Series No. 5. Inhibition and Inactivation of Vegetative Microbes," Ed. Skinner, F.A. and Hugo, W.B., p. 89. Academic Press, New York.

Leake, C.D. and Silverman, M. 1966. "Alcoholic Beverages in Clinical Medicine," The World Publishing Co., Cleveland.

Lucia, S.P. 1954. "Wines as Food and Medicine," Country Life Press, Garden City, New York.

Morgan, A.F., Nobels, A.L., Wiens, A., Margh, G.L. and Winkler, A.J. 1939. The B-vitamins of California grape juices and wines. Food Research 4: 217.

Oprya, A.P. 1969. Change in the level of group B-vitamins in grape must during continuous fermentation. Sadovid. Vinograd. Mold. (SVVMAC)24: 26.

Voigt, M.N. 1977. A comparative study of protozoan and conventional vitamin analysis methods. Ph.D. dissertation, University of Georgia, Athens, GA.

Ms received 11/4/77; revised 12/23/77; accepted 12/30/77.

INFLUENCE OF ORANGE JUICE COMPOSITION ON THE THERMAL RESISTANCE OF SPOILAGE YEASTS

B. J. JUVEN, J. KANNER and H. WEISSLOWICZ

ABSTRACT

The increased heat resistance of yeasts in orange concentrates as compared with single-strength juices cannot be accounted for on the basis of sugar contents only. Citric acid is demonstrated to play a role in the heat resistance of spoilage yeasts in concentrated orange juices. Other major chemical constituents of citrus juices, namely, ascorbic acid and pectin, did not exhibit any significant effect. A model system, based on sucrose, glucose, fructose, citric acid and tripotassium citrate, is proposed for the evaluation of the thermal resistance of yeasts in citrus concentrates.

INTRODUCTION

YEASTS AND LACTIC ACID BACTERIA are the microbial groups involved in the spoilage of citrus single-strength juices (10–12° Brix) and semi-concentrates (20–30° Brix) (Berry et al, 1956; Juven, 1976); higher concentrated juices (40–60° Brix) are usually spoiled by yeasts (Berry et al., 1956; Murdock and Brokaw, 1965). From the microbiological standpoint, the aseptic packaging of "commercially sterile" citrus concentrates has opened up the possibility of shipping and marketing these products unfrozen, therefore considerably reducing storage costs.

The heat resistance of yeasts (Gibson, 1973; Corry, 1976) and bacteria (Goepfert et al., 1970; Gibson, 1973) was shown to be enhanced in sugar solutions at reduced a_w. Osmophilic yeast cells, heated in aqueous solutions of sucrose or in sucrose/glucose mixtures (made to give a_w values from 0.99 to 0.85), showed higher resistance at the lower a_w values (Gibson, 1973).

Murdock et al. (1953) tested the heat resistance of a composite suspension of 13 yeast strains in a 9.8° Brix orange juice (pH 3.7) and a 42° Brix orange concentrate (pH 3.45) and obtained an F value for the concentrate at 65.5°C about 20 times higher than that found for the juice. The authors raised the possibility that this was due to differences in sugar content, and suggested that pasteurization—in the production of frozen orange concentrates—would be more efficient if applied to the single-strength juice rather than to the concentrate.

However, in the manufacture of aseptic-packaged concentrates, before the packaging stage itself, the concentrate might have to be heat processed in order to destroy any contaminant present, able to grow and cause spoilage (Anon., 1975). This creates, therefore, a need for a better understanding of the factors which might influence the thermal resistance of potential spoilage organisms in the citrus concentrates.

The present study dealt with the effect of major chemical constituents of orange juice on the thermal resistance of several spoilage yeasts.

Authors Juven, Kanner and Weisslowicz are with the Division of Food Technology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50-200, Israel.

0022-1147/78/0004-1074$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Test organisms

Three yeast strains, isolated from a 60° Brix orange concentrate, were used in this study: *Candida lambica* OY 13, *Saccharomyces chevalieri* OY 26 and *Torulopsis magnoliae* OY 14. Stock cultures of these organisms were maintained in sterile distilled water at room temperature according to the procedure described by McGinnis et al. (1974).

The sugar tolerance of the test organisms was determined by their ability to grow in sugar media at low water activities (a_w). The basal medium employed in this determination consisted of 0.5g yeast extract and 2.0g agar dissolved in an amount of distilled water calculated to give, after addition of the glucose solution supplement, a final weight of 100g. The basal medium was sterilized at 121°C and then supplemented by adding previously boiled solution containing 40, 50, 55 or 60g of D-glucose in 20g of distilled water. The final medium was designated YG.

Determination of a_w

Equilibrium relative humidity measurements of both agar media and heating menstrua were made at 20°C with Hygrosensor elements (nos. 4-4836 and 4-4837; Hygrodynamics, Inc., Silver Spring, MD) mounted in lids of 8-oz jars. Hygrometer sensors were attached to a Hygrometer indicator (model no. 15-3030 E; Hygrodynamics, Inc.) and an equilibrium time of not less than 24 hr was allowed before measurements were made from 90-ml portions of each tested material. Sensor calibrations were made against saturated KNO_3, K_2CrO_4 and $(NH_4)_2SO_4$ solutions.

Chemical analyses

Sucrose, glucose and fructose were determined by the procedure described by Ting (1956). Ascorbic acid was analyzed by the 2,6-dichlorophenolindophenol method described by the Association of Official Analytical Chemists (1975). The pH was measured with a Radiometer PHM26 pH-meter (Radiometer A/S, Copenhagen, Denmark). The titratable acidity (TA) was determined by titrating a 10-g sample to pH 8.1 with 0.1N NaOH. It is expressed as milliequivalent (meq) acid per kg. Total pectin was determined by the procedure described by Rouse and Atkins (1955).

Orange preparations

A 61° Brix orange var. Shamouti frozen concentrate was used throughout this study. It had the following composition: sucrose, 23.0% (wt/wt); glucose, 13.1%; fructose, 12.6%; ascorbic acid, 0.2%; TA, 785 meq acid (ca 95% citric)/kg; total pectin content, 0.41%; pH 3.10. It contained less than 1 microorganism per gram enumerated on plates of orange serum agar (OSA, pH 5.4) (Murdock et al., 1952). Dilutions of the 61° Brix concentrate were done with sterile distilled water to yield 50°, 30° and 12° Brix juices; their pH was readjusted to 3.10 ± 0.05 with a filter-sterilized solution (2.5 M) of citric acid.

Model systems

Model systems were prepared for evaluating the role of the main chemical constituents of citrus juices, namely, sugars, citric acid, ascorbic acid and pectin, in the thermal resistance of spoilage yeasts. The composition of these model systems is presented in Table 1. Sucrose, D-glucose and D(-)-fructose of analytical grade were used; ascorbic acid was obtained from E. Merck, AG, Darmstadt, W. Germany, and pectin from citrus fruits, grade I, was obtained from Sigma Chemical Co., Saint Louis, MO.

Inoculum preparation

Inocula were prepared from cells grown in potato dextrose agar (Difco) slants, incubated for 7 days at 30°C. They were washed once with sterile distilled water, centrifuged at 3,000g (10 min, 10°C) and resuspended in distilled water at a concentration of ca 10^7 viable organisms per ml, enumerated on OSA. In the preparation of the inocula, media, diluents and incubation conditions were chosen that gave minimal cell clumping and budding. Twenty-four-hour slant-cul-

—Text continued on page 1076

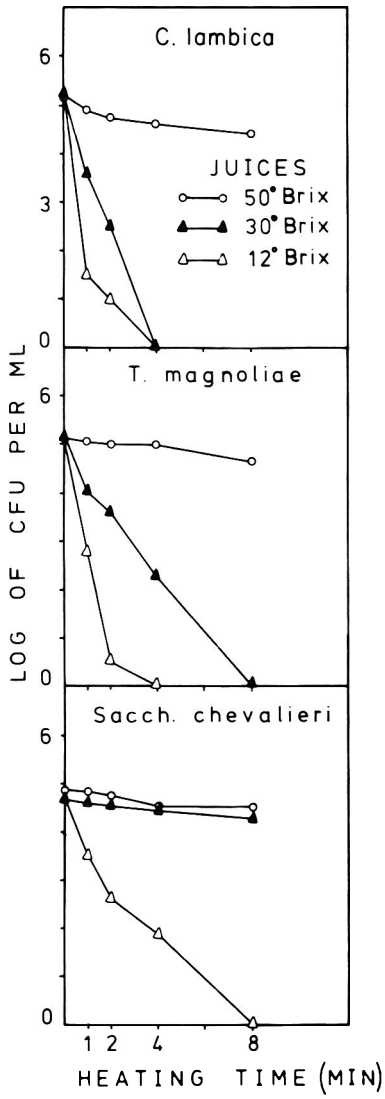


Fig. 1—Survival of three yeast strains in orange juices (pH 3.10) at 60° C.

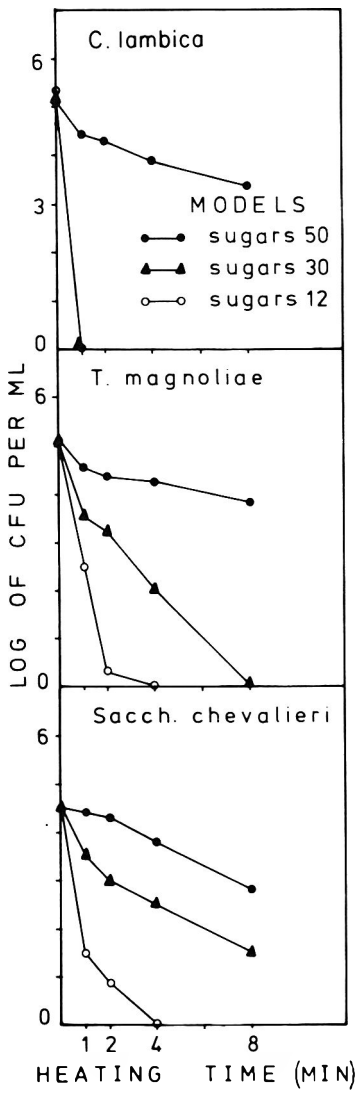


Fig. 2—Survival of three yeast strains in sugars model systems (pH 3.10) at 60° C. For composition of heating menstrua, see Table 1.

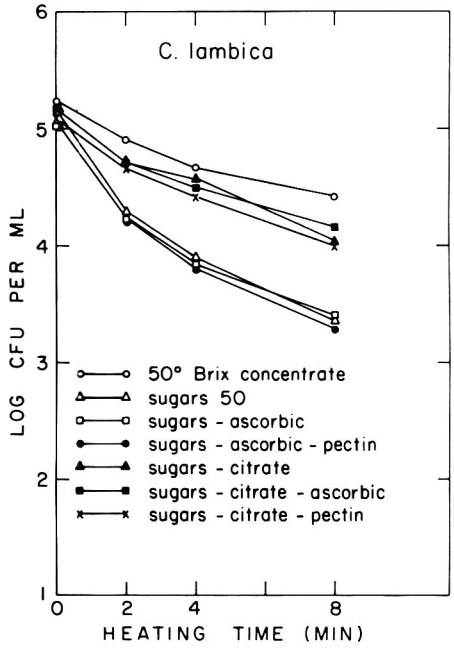


Fig. 3— Survival of Candida lambica in 50° Brix orange concentrate and in several model systems (pH 3.10; a_w 0.92—0.93) at 60° C. For composition of heating menstrua, see Table 1.

Table 1—Composition of the heating menstrua tested (pH 3.10 ± 0.05)

		g component per 100g final weight						
Definition	a_w	Orange conc 61° Brix	Sucrose	Glucose	Fructose	Water	Citric acid · H_2O	tri-Potassium citrate · H_2O
Juices								
Concentrate 50	0.92	80				20		
Concentrate 30	0.96	50				50		
Juice 12	0.99	20				80		
Model systems[a]								
Sugars 50	0.93		18.40	10.48	10.08	61.03	*ca.* 0.01	
Sugars 30	0.97		11.50	6.55	6.30	75.64	*ca.* 0.01	
Sugars 12	0.99		4.60	2.62	2.52	90.25	*ca.* 0.01	
Sugars-citrate[b]	0.92		18.40	10.48	10.08	54.45	4.38	2.21
Sugars-modified I	0.91		18.62	15.64	10.20	55.54	*ca.* 0.01	
Sugars-modified II	0.91		26.97	10.15	9.76	53.12	*ca.* 0.01	

[a] Effects of ascorbic acid and pectin were tested by adding 200 mg of L-ascorbic acid or 400 mg of pectin per 100g of the sugars-citrate model.
[b] Sugars-citrate model system had a titratable acidity of 608 meq/kg as compared with 628 meq/kg measured in the 50° Brix concentrate. The pH of the system (3.10) was achieved through a balance between citric acid and tri-potassium citrate, the concentration of the latter being controlled to give a potassium concentration (7,960 ppm) within the limits found in 50° Brix orange concentrates.

tures were used in some of the experiments, in which the influence of the age of the culture was considered.

Heating procedures

The juices or model systems were held in a water bath in 100-ml quantities in 250-ml Erlenmeyer flasks and stirred magnetically; the temperature of the water was controlled within ± 0.1°C by circulation through a Thermomix II Braun Thermostat (B. Braun, Melsungen, Germany). After equilibration of the sample (100 ml) to the desired temperature (equilibration times previously determined with thermocouples), it was inoculated with 1.0 ml of the inoculum suspension. Unheated controls were kept under stirring and inoculated simultaneously. At suitable time intervals, 5-ml aliquots were withdrawn to glass tubes (128 × 9 mm) cooled to ca 10°C in a 7°C water-bath. The time required to cool the samples down to 30°C was not longer than 50 sec. With this method, heating and cooling lags were negligible. The samples were stored at 10°C and plated within less than 3 hr. The number of replicates for a given treatment was 3–6.

Recovery procedures

Appropriate decimal dilutions of the juices or model systems were made in 0.3 M sucrose, and 1.0-ml aliquots plated on OSA. The plates were incubated at 30°C for 5 days and the number of colony-forming units (CFU) was determined. Since the survivor curves obtained were not straight exponential, D values were not calculated, and the data obtained are presented as the logs of CFU 2, 4 and 8 min after inoculation.

RESULTS & DISCUSSION

THREE YEAST STRAINS, isolated from a 60° Brix orange concentrate, were used in this study. *T. magnoliae* OY 14 was defined as a "sugar-tolerant" strain because of its ability to grow on plates of YG containing 60% glucose (0.83 a_w). *Sacch. chevalieri* OY 26 was able to grow on YG with up to 50% glucose (0.89 a_w) and *C. lambica* OY 13 grew in the presence of 40% (0.93 a_w) but not with 50% glucose; they were therefore defined as non-tolerant strains. *T. magnoliae* was able to grow and cause spoilage of a 60° Brix orange concentrate (pH 3.1; a_w 0.86) at 30°C, whereas *Sacch. chevalieri* and *C. lambica* did not grow in 60° C Brix concentrates but did grow and cause spoilage of a 50° Brix concentrate (pH 3.1; a_w 0.92).

Effect of degree of concentration of juice

Figure 1 shows the heat resistance curves obtained in orange juices of three different concentrations for one strain of sugar-tolerant yeast (*T. magnoliae*) and two non-tolerant strains (*Sacch. chevalieri* and *C. lambica*). In the 50° Brix concentrate, the heat resistance of all the three strains was higher than in the 30° or 12° Brix juices. After 4–8 min at 60°C, inocula of ca 10^5 organisms per ml were completely destroyed in 12° Brix juices, whereas the reduction in counts was never higher than 0.8 log in the 50° Brix concentrates. The small differences which exist among the strains tested do not seem to be related to their sugar tolerance.

The number of viable yeast cells in the juices as well as in model systems (Fig. 2) was not found to decrease exponentially with the time of exposure to heat (in the range 55°–60°C). Several authors reported the fact that the survivor curves for yeasts after heat treatment in buffer or sugar solutions were not straight semilogarithmic curves, exhibiting an initial sharp drop followed by a less steep slope (Gibson, 1973; Corry, 1976). This type of survivor curve could be explained by the existence of cells of different heat sensitivity in the population (Hansen and Riemann, 1963; Stumbo, 1973) or by clumping of cells occurring during the early part of heating (Stumbo, 1973).

Effect of orange juice chemical constituents

The hypothesis that sugars are responsible for the increased heat resistance of yeasts in orange concentrates, was tested. Sucrose, glucose and fructose constitute practically the total sugars in orange juices (Kefford and Chandler, 1970). The heat resistance curves for yeasts in model systems, containing sucrose, glucose and fructose in amounts corresponding to those found in 50°, 30° and 12° Brix orange juices are presented in Figure 2.

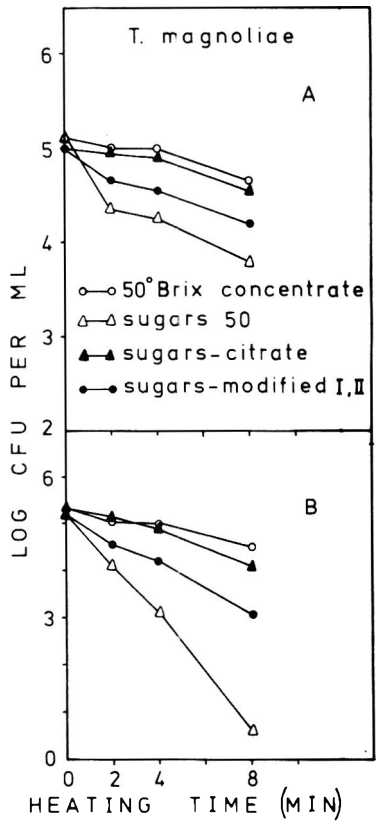


Fig. 4—Survival of Torulopsis magnoliae in 50° Brix orange concentrate and in several model systems (pH 3.10; a_w 0.91–0.93) at 60°C. (A) 7-day culture; (B) 1-day culture. For composition of heating menstrua, see Table 1.

In sugars-model systems the heat resistance of the yeasts was higher as the sugars concentration was increased. However, comparison of the results presented in Figure 1 with those in Figure 2 indicate that yeasts are more resistant in the juices than in the corresponding model systems containing sugars only. Therefore, the possibility that juice constituents—other than sugars—do play some role in the thermal resistance of yeasts, was considered.

Figure 3 shows the survival at 60°C of *C. lambica* in model systems containing sugars, citrate, ascorbic acid and pectin in amounts designed to give a chemical composition similar to that of the orange concentrate.

The addition of citrate enhanced the protection given by the Sugars 50 model practically to the level of that afforded by the 50° Brix orange concentrate. On the other hand, the addition of ascorbic acid or pectin did not effect any significant change in the degree of protection afforded either by the sugars or by the sugars-citrate model.

The possibility that the effect of citric acid and potassium citrate is not specific but acts through a reduction in a_w, was considered. However, in model systems, in which citric acid and citrate have been replaced by equimolal amounts of glucose or sucrose (Sugars-modified I and Sugars-modified II, Table 1), the heat resistance of yeasts was significantly lower than that obtained with the sugars-citrate model (Fig. 4).

The lower heat resistance of *T. magnoliae* in the Sugars-50 model as compared with the 50° Brix concentrate was more apparent when a one-day culture of the yeast was used rather than 7-day culture (Fig. 4). With both cultures, the Sugars-citrate model gave results almost identical to those obtained with the orange concentrate.

—Continued on page 1080

EFFECT OF CONTROLLED ATMOSPHERE ON FLAVOR STABILITY OF ALMONDS

D. G. GUADAGNI, E. L. SODERSTROM and C. L. STOREY

ABSTRACT

A low oxygen atmosphere, known to control insect infestations in stored almonds, was studied to determine its effect on flavor changes in stored inshell almonds and almond meats at 18.5 and 27°C. Sensory comparisons of the products stored in low oxygen and normal atmosphere at these temperatures after 1, 3, 6, 9 and 12 months of storage indicate the following: (1) In normal atmosphere at both 18.5 and 27°C, inshell almonds were much more stable than almond meats; (2) In the low oxygen atmosphere, no significant differences were observed between storage as meats or inshell; (3) Temperature had a highly significant effect on off-flavor development in meats stored in normal atmosphere but no significant effect on those stored in the low oxygen atmosphere; (4) Storage in the low oxygen atmosphere caused less off-flavor development than in normal atmosphere for both meats and inshell almonds.

INTRODUCTION

THE ANNUAL FARM VALUE of the almond crop in California averages 134 million dollars. Since the total crop cannot be processed as fast as it is harvested, large quantities are stored at the processing plants until needed. The stored almonds are infested with the navel orangeworm, *Paramyelois transitella* (Walker), which initiates its infestation in the orchard. During storage, various stored product insects may infest the nuts.

Insect control is now accomplished by spraying with malathion or fumigating with methyl bromide or phosphine. A proposed method for controlling these insect infestations without the use of toxic chemicals is with a low oxygen atmosphere. This method has shown promise in controlling insects in stored grain (Storey, 1975a, 1975b, 1976c). The low oxygen atmosphere has been shown to control the navel orangeworm (Storey and Soderstrom, 1977). This research is in accordance with the USDA, ARS, policy for developing safer and more effective ways of controlling insects in agricultural commodities.

The effects of controlled atmosphere storage on almond quality and stability has not been studied. This paper deals with the effect of storage atmosphere and temperature on flavor and acceptability of almonds stored inshell and with shells removed (meats).

MATERIALS & METHODS

Controlled atmosphere generator

The storage tests were conducted using a laboratory scale exothermic controlled atmosphere generator built by Gas Atmospheres, Inc. of Port Washington, WI. The generator produces 2.83 m^3/hr of controlled atmosphere composed of less than 1% O_2, 9–9.5% CO_2, 86–89% N_2, 1% Ar, and 1.5% or less of H_2 and CO. During operation, natural gas and air are ignited under controlled pressure at a ratio of about 1 part gas to 10 parts air. Combustion takes place in a water-cooled, refractory-lined combusion chamber. The exhaust gas from the chamber passes through a water-cooled platecoil and into a condensate separator. Water removed from the gas leaves the separator through a seal leg located beneath the separator. Relative humidities of the inert and normal atmospheres were held at 50 ± 5% RH by passing moisture saturated atmosphere through carboys placed in incubators or refrigerated water baths maintained at selected temperatures below the dew point of the atmosphere, then reheating the atmosphere to the treatment temperature prior to release through the almond samples. Oxygen levels in the controlled atmosphere were measured periodically throughout each test using a paramagnetic oxygen analyzer. Although some minor variations occurred during day-to-day operations, the concentration of O_2 in the inert atmosphere flowing through the almond samples was within a range of 0.25–0.5%.

Small jar treatment

Nonpareil inshell almonds from the 1974 crop were thoroughly mixed, subdivided into 2,000-g lots, and stored in 3.8 L glass jars under controlled (CA) and normal flowing atmosphere (FA) at 18.5 ± 1°C or 27 ± 1°C. Almond meats from the 1974 crop were treated in the same manner except they were stored in 1.9 L jars. Each jar was sealed with a lid fitted with a rubber stopper. Plastic lines placed through the stopper served as inlet and outlet tubes for the atmospheres; the inlet line ran to the bottom of each jar whereas the outlet line extended only into the overhead air space. Flow rates (50 cc/min) were controlled with the aid of purge meters. One jar from each lot was removed for quality evaluation after storage periods of 1, 3, 6, 9 and 12 months.

Intermediate test

Nonpareil inshell almonds from the 1975 crop were thoroughly mixed, subdivided into 223 kg lots and stored in metal silos (45 cm in diameter by 4.27m high) under controlled atmosphere (CA), flowing air (FA), and static air (SA) at 50 ± 5% RH and 27 ± 1°C. Each silo was equipped with a perforated tube (ca 2 cm diameter) placed crosswise through the base of the silo. The (CA) and (FA) were released through purge meters into the perforated tube and passed upward through the almonds. Air exhausting from the top of the silos was vented to the outside. The rate of flow of the atmospheres through the silos was 500 cc/min. Samples (33 kg) for quality evaluation were drawn from the silos through a 10 cm diameter gate valve located at the base of the silos after storage for 1, 3, 6, 9 and 12 months. Untreated samples (CSSA) were held in closed fiber drums stored at 4 ± 1°C. After 1, 3, 6, 9 and 12 months storage, samples from each storage atmosphere and temperature were shipped to the Western Regional Research Center, Berkeley, CA for flavor evaluation.

Moisture content of the almond meats varied from about 4–6% during storage for 1 yr.

Sensory evaluation

On arrival at the laboratory, all samples were frozen at −23.3°C and kept at this temperature until the morning of the day they were evaluated by the sensory panel. All samples were equilibrated to tasting room temperature (23.3 ± 1°C).

The sensory panel was selected from laboratory personnel whose ages ranged from 24–59 yr and were about equally divided between male and female. A group of 20 subjects was selected on the basis of willingness to participate and demonstrated ability to detect flavor differences in almonds of varying quality.

All sensory tests were conducted in a room held under slight positive pressure and containing individual booths supplied with air passed through a bank of granular charcoal cannisters and maintained at a constant 23.3 ± 1°C and 50% RH. Each booth was equipped with running water to rinse between samples and with subdued lighting (7.5 watt green bulbs) to prevent possible color or appearance differences from influencing taste judgments.

The shelled almonds were placed in coded paper cups and presented to the judges in groups of 4 samples. Treatment position in each block of 4 samples was randomized, and the samples were ranked from 1 to 4 in order of least off-flavor with 1 representing least and 4 most off

Author Guadagni is with the USDA Western Regional Research Center, ARS, Berkeley, CA 94710. Author Soderstrom is with the USDA, Stored-Product Insects Laboratory, ARS, Fresno, CA 93727. Author Storey is with the USDA U.S. Grain Marketing Research Laboratory, ARS, Manhattan, KS 66502.

flavor. The samples in each block were arranged in various treatment combinations to evaluate the effect of interactions among variables. Separate tests were conducted to determine the relative likeability or acceptability of the samples from different treatments. The judges were presented with 4 coded samples and asked to rate each on a 9 to 1 like-dislike hedonic scale (9= like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). They were also asked to indicate whether the sample was acceptable or not acceptable.

All 4 sample comparisons were replicated twice—once in mid-morning and once in mid-afternoon. The hedonic data were analyzed by analysis of variance and Duncans multiple range test. The rank data are presented as the average rank obtained by dividing total rank sums for each sample by total judges x replications. Significance of the rank results was determined from tables developed by Kahan et al. (1973). The dotted lines in Figures 1–5 represent the nonsignificant range ($P < 0.05$) for all 4 sample blocks with an N of 40. For any storage period in which one or more points fall outside the dotted lines, significant differences were established and the rank order in terms of least to most off-flavor is from the lowest to the highest average rank.

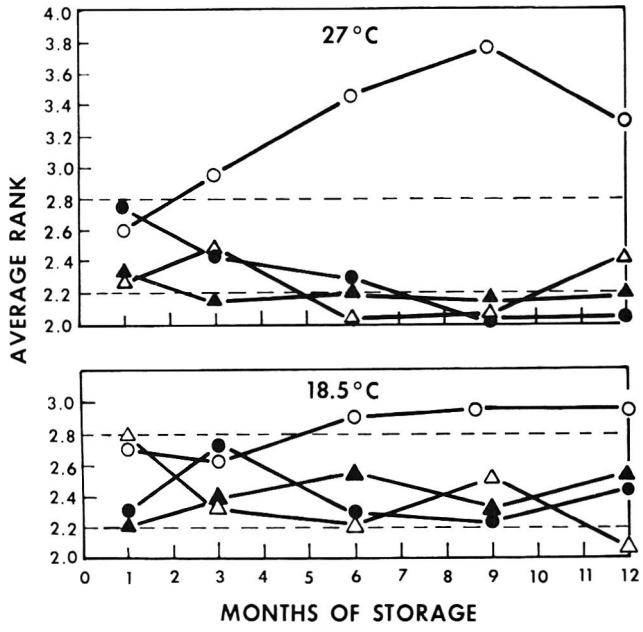


Fig. 1—Effect of storing almonds as meats or inshell at 18.5 and 27°C. Average rank based on N = 40, 1 = least off-flavor, 4 = most off-flavor: ●—FA inshell, ○—FA meats, ▲—CA inshell, △—CA meats.

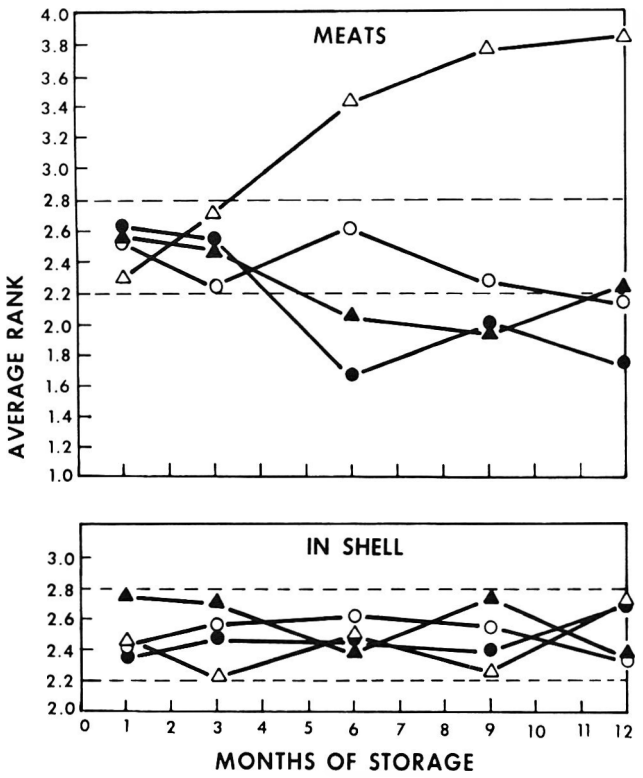


Fig. 2—Effect of temperature and atmosphere on average flavor rank of inshell and almond meats: ●—CA, 18.5°C, ○—FA, 18.5°C, ▲—CA 27°C, △—FA 27°C.

RESULTS AND DISCUSSION

Jar tests

The effect of atmosphere on flavor changes during storage at 18.5 and 27°C is shown in Figure 1. It is apparent that the most significant flavor changes during storage occurred in almond meats stored in FA. The rank data showed that these samples had significantly more off-flavor than the others after 3 months at 27°C. At 18.5°C off-flavor development in the FA samples was not significant prior to 6 months storage. There were no significant differences among the other 3 samples during the 12-month storage period at either 18.5 or 27°C. While the difference in stability between meats and inshell almonds was very marked in a FA atmosphere, the meats and inshell samples stored in CA were not significantly different from one another during the entire 12-month storage period. Firstly, this suggests that the almond shell is an effective package against oxidative changes in a normal atmosphere environment, and secondly, CA is effective in preventing the oxidative changes which occur in meats stored in FA.

The skin of the meats was fractured or broken in many of the samples during shelling, packaging and handling. These fractures allowed the relatively high oxygen content of normal atmosphere to initiate oxidative changes in the oil of the kernels, whereas the CA was apparently too low in oxygen to initiate similar oxidative changes at these temperatures. Even though the inshell samples were stored in a relatively high oxygen environment, the shell effectively protected the kernel from physical damage and the entrance of a fresh oxygen supply from the FA. This seems to be a plausible explanation for the results shown in Figure 1.

The effect of temperature on both meats and inshell almonds is more clearly shown in Figure 2. The curve for the meats in FA at 27°C shows a trend of rapid increase in average rank, clearly indicating off-flavor development while the same meats stored at 18.5°C show little or no change during the 12-month storage period. After 6 months there was a highly significant flavor difference between 18.5 and 27°C. On the other hand, temperature (18.5 and 27°C) did not cause any significant difference in the meats stored in CA for the entire 12-month period. After 6 and 9 months the meats stored in CA at 27°C were ranked as having less off-flavor than meats stored in FA at 18.5°C. Again, this indicates that the CA produced by this generator was particularly effective in preventing oxidative flavor changes at temperatures up to 27°C.

The data for the inshell samples shows no significant differences due to temperature or atmosphere for the entire 12-month period. Again these data emphasized the value of the shell in protecting against oxidative flavor changes, but it also shows that CA storage did not cause any deleterious effects at either temperature.

Figure 3 shows results of the hedonic ratings for meats and inshell almonds stored at 18.5 and 27°C. After 1 month, hedonic ratings for all samples ranged from 6.3–7.4. At 27°C all of the samples except those stored in FA were not significantly different from one another at each of the storage periods. The average hedonic rating of the FA meats was significantly lower than the others after 3 months, and rapidly

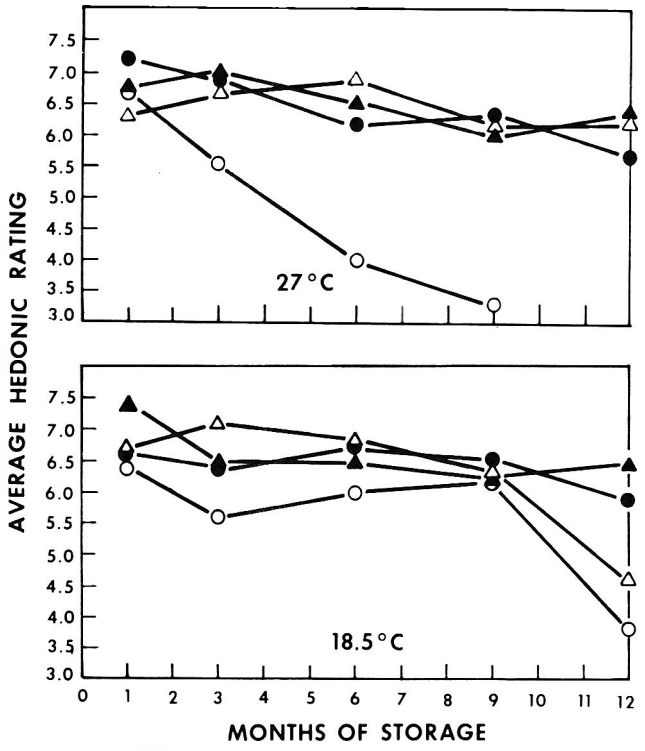


Fig. 3—Effect of temperature and atmosphere on average hedonic rating of inshell and almond meats: ●—FA inshell, ○—FA meats, ▲—CA inshell, △—CA meats.

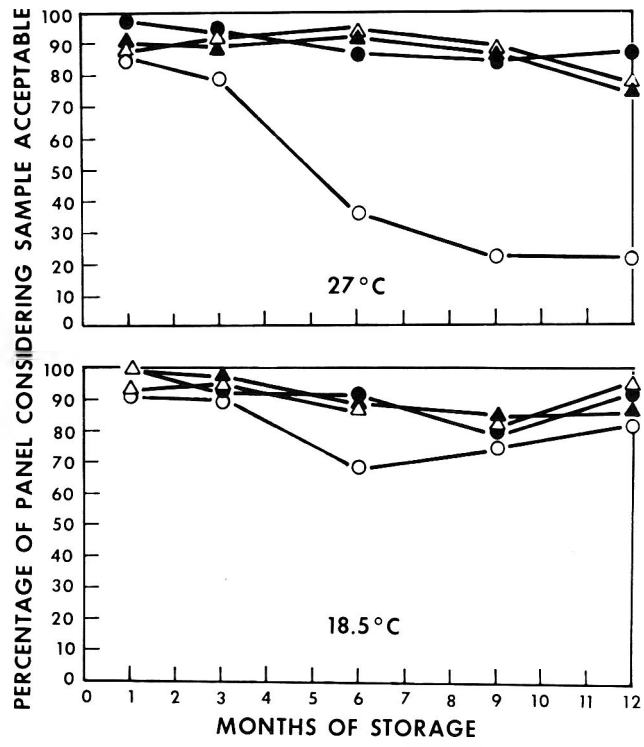


Fig. 4—Effect of temperature and atmosphere on percentage acceptability of inshell and almond meats: ●—FA inshell, ○—FA meats, ▲—CA inshell, △—CA meats.

declined to a value of 4.0 after 6 months indicating a slight dislike for these samples. The meats and inshell samples stored in Ca were not below 6.0 indicating "satisfactory acceptability" retention during the entire period. At 18.5°C the results were more variable, but again, the meats in FA tended to receive the lowest hedonic ratings, particularly after 12 months storage.

Figure 4 shows relative acceptability of the different samples in terms of percentage of the panel considering each sample acceptable or not acceptable. These results are similar to the hedonic ratings in that acceptability of all samples except meats in FA was very similar throughout the storage period. This was true at both 27 and 18.5°C. Up to 9 months of storage at both temperatures, 80% or more of the panel considered these samples acceptable, but only about 20–35% of the panel considered the meats in FA acceptable after 6–12 months at 27°C. These data clearly show that CA storage was effective in maintaining acceptability of almond meats at 18.5 and 27°C., but FA was not. They also show that inshell samples were more stable than meats in an FA atmosphere.

Intermediate test

In this test all of the samples were stored inshell. The CA and FA samples were stored in silos to simulate large scale practical storage conditions at a temperature of 27°C. The other two samples consisted of static air storage in sealed fiber drums at 27°C (SA) and 4°C (CSSA). The rank and hedonic data obtained for these samples during a 12-month storage period are shown in Figure 5. The rank data indicate that significant differences were observed after 1 month with the SA sample having the least and FA having the most off-flavor. During subsequent storage, however, the average rank of the CSSA sample (cold storage control) gradually decreased in relation to the other samples as would be expected on the basis of temperature. After 6, 9 and 12 months the CSSA sample had significantly less off-flavor than the FA sample. After 9 and 12 months it had significantly less off-flavor than

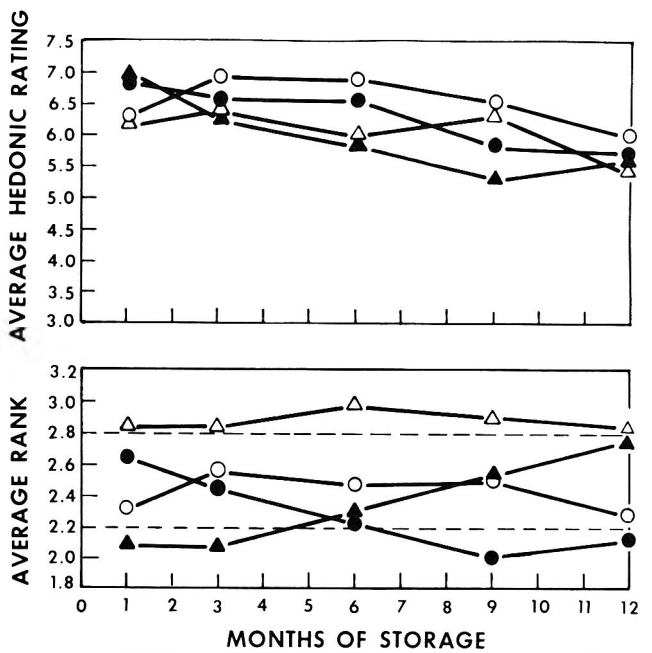


Fig. 5—Effect of temperature and atmosphere on average flavor rank and hedonic rating of inshell almonds (intermediate test): ●—CSSA, 4°C, ○—CA 27°C, ▲—SA 27°C, △—FA 27°C.

the SA sample indicating the effect of temperature alone since both samples were stored in static air. The average ranks of the FA and CA samples remained fairly constant during the 12-month period, but the FA sample was consistently higher in rank indicating more off-flavor. It is interesting to note that after 12 months, the CA sample at 27°C was ranked very similar to the cold storage control at 4°C, while SA and FA samples were ranked similarly but considerably higher, indicating less off-flavor in the CA sample.

—Text continued on page 1080

CONCLUSIONS

WHILE THE MAIN PURPOSE of this study was to determine whether the specific CA known to destroy insects would cause any abnormal taste or enhance deterioration of almonds during storage, other significant information on storage behavior of almonds was obtained. In both small scale jar tests and the larger intermediate tests, the CA atmosphere was shown to be equal or superior to normal atmosphere (FA or SA) for storage of almonds inshell or as meats (18.5 and 27°C).

No evidence was obtained indicating the CA atmosphere used in these studies caused any off-taste initially, or during storage for 12 months. Storage of almond meats in CA was definitely superior to storage in normal atmosphere.

The almond shell itself proved to be an effective package in preventing oxidative deterioration during storage at 27°C. Almond meats stored in normal atmosphere were much less stable than similar almonds stored inshell. Even when all treatments were applied to inshell almonds in the intermediate test, samples from the CA atmosphere were consistently ranked superior to those from FA. Hedonic ratings for the CA samples were generally higher than those for FA samples, but the differences were not statistically significant. Therefore, considering related research on insect infestation, the CA atmosphere would appear to be beneficial for almond storage in two main respects: (1) control of insects; and (2) better maintenance of original flavor quality.

REFERENCES

ASTM. 1968. "Manual on Sensory Testing Methods." ASTM Special Technical Publication No. 434.

Kahan, G., Cooper, D., Papavasilious, A. and Kramer, A. 1973. Expanded tables for determining significance of differences for ranked data. Food. Technol. 27(5): 61.

Storey, C.L. 1975a. Mortality of adult stored-product insects in an atmosphere produced by an exothermic inert atmosphere generator. J. Econ. Entomol. 68: 316.

Story, C.L. 1975b. Mortality of three stored product moths in atmospheres produced by an exothermic inert atmosphere generator. J. Econ. Entomol. 68: 736.

Story, C.L. 1976c. Mortality of Sitophilus oryzae (L.) and S. granarius (L.) in atmospheres produced by an exothermic inert atmosphere generator. J. Stored Prod. Res. 11: 217.

Storey, C.L. and Soderstrom, E.L. 1977. Mortality of navel orangeworm in low oxygen atmosphere. J. Econ. Entomol. 70: 95.

Ms received 11/16/77; revised 2/6/78; accepted 2/15/78.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

HEAT RESISTANCE OF YEASTS IN ORANGE CONC . . . From page 1076

On the basis of these data, the Sugars-citrate model system described herein seems to us as a suitable substitute for concentrated orange juices, as far as the heat resistance of yeasts in concerned. Such a model could enable prediction of the processing conditions for citrus concentrates without the practical difficulties caused by the high viscosity of the concentrates.

REFERENCES

Anonymous. 1975. Orange concentrate in aseptic package builds business for Swedish dairy. Food Engng. 47(8): 21.

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Inc., Washington, DC.

Berry, J.M., Witter, L.D. and Folinazzo, J.F. 1956. Growth characteristics of spoilage organisms in orange juice and concentrate. Food Technol. 10: 553.

Corry, J.E.L. 1976. The effect of sugars and polyols on the heat resistance and morphology of osmophilic yeasts. J. Appl. Bact. 40: 269.

Gibson, B. 1973. The effect of high sugar concentrations on the heat resistance of vegetative microorganisms. J. Appl. Bact. 36: 365.

Goepfert, J.M., Iskander, I.K. and Amundsen, C.H. 1970. Relation of the heat resistance of Salmonella to the water activity of the environment. Appl. Microbiol. 19: 429.

Hansen, N.H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bact. 26: 314.

Juven, B.J. 1976. Bacterial spoilage of citrus products at pH lower than 3.5. J. Milk Food Technol. 39: 819.

Kefford, J.F. and Chandler, B.V. 1970. "The Chemical Constituents of Citrus Fruits," p. 24. Academic Press, New York.

McGinnis, M.R., Padhye, A.A. and Ajello, L. 1974. Storage of stock cultures of filamentous fungi, yeasts, and some aerobic actinomycetes in sterile distilled water. Appl. Microbiol. 28: 218.

Murdock, D.I. and Brokaw, C.H. 1965. Spoilage developing in 6-oz cans of frozen concentrated orange juice during storage at room temperature and 40° F, with particular reference to microflora present. Food Technol. 19: 666.

Murdock, D.I., Folinazzo, J.F. and Troy, V.S. 1952. Evaluation of plating media for citrus concentrates. Food Technol. 6: 181.

Murdock, D.I., Troy, V.S. and Folinazzo, J.F. 1953. Thermal resistance of lactic acid bacteria and yeast in orange juice and concentrate. Food Res. 18: 85.

Rouse, A.H. and Atkins, C.D. 1955. Pectinesterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experimental Station. Florida Agr. Expt. Sta. Bull. no. 570.

Stumbo, C.R. 1973. "Thermobacteriology in Food Processing." 2nd ed, p. 78. Academic Press Inc., New York.

Ting, S.V. 1956. Rapid colorimetric methods for simultaneous determination of total reducing sugars and fructose in citrus juices. J. Agr. Food Chem. 4: 263.

Ms received 9/7/77; revised 12/9/77; accepted 12/14/77.

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. 1977 series, no. 220-E.

FUNCTIONAL PROPERTIES OF HYDROLYZED LACTOSE: SOLUBILITY, VISCOSITY AND HUMECTANT PROPERTIES

N. O. SHAH and T. A. NICKERSON

ABSTRACT

Three sugar syrups were studied that were equivalent to 70, 90 and 100% hydrolysis of lactose. The viscosity and solubility of the sugars, both individually and as mixtures, were determined within the range 10–60°C. At every temperature, lactose was least soluble, galactose next, and glucose most soluble. Added sugars of the types present in hydrolyzed lactose syrups lowered the solubility (40°C) of the individual sugars. Even so, the maximum soluble sugar solids (TS) content was about the same in solutions representing partially (62.96% TS from 3 sugars) and completely (61.95% TS from 2 sugars) hydrolyzed lactose. Hydrolysis of lactose permits the production of stable syrups with higher TS. The viscosity of hydrolyzed lactose syrup was very similar to that of its individual constituent sugars and to sucrose when the percent TS was 50% or less; at higher percent TS the viscosity tended to be less than that of sucrose. Humectant properties were studied at 20°C. They showed that the water-retention capacity of hydrolyzed mixtures at 20°C and 15–93% relative humidity was very similar to that of sucrose syrups of comparable TS.

INTRODUCTION

WHEY, a by-product of the cheese industry, has become a severe disposal problem. Cheese whey contains 70% lactose on a dry-weight basis, so better utilization of lactose would be one way to reduce the whey problem. Considerable interest has therefore been directed toward: using cheese whey as a base for manufacture of a variety of beverages, both alcoholic and nonalcoholic (Holsinger et al., 1974); and hydrolysis of whey or lactose solutions and utilizing the products (Bouvy, 1975; Holsinger and Roberts, 1976).

Lactose can be hydrolyzed by the enzyme lactase (β-galactosidase). Lactases have been isolated from a number of microbial sources, including *Escherichia coli* (Hu et al., 1959), thermophilic fungi (Borglum and Steinbert, 1972), and lactose-fermenting yeasts such as *Kluyveromyces fragilis* (Mahoney et al., 1975). Alternatively, acid hydrolysis of aqueous solutions of lactose appears possible without excessive destruction of the sugars if carried out near 60°C (Coughlin and Nickerson, 1975).

Now that hydrolysis on a commercial basis appears feasible, some of the functional properties of the resulting syrups must be known. Although much work has been done on the use of lactose and glucose in foods, few data are available on the properties of galactose and of syrup mixtures representing partially hydrolyzed lactose syrups that would be useful to food processors.

The sugar syrups studied were equivalent to 70, 90 and 100% hydrolysis of lactose. This range was chosen because 100% hydrolysis represents the maximum effect of hydrolysis; 90% hydrolysis indicates a practical level of hydrolysis; and 70% hydrolysis is a level easily achieved by either acids or enzymes.

Author Nickerson is with the Dept. of Food Science & Technology, University of California, Davis, CA 95616. Author Shah, formerly with the University of California, is currently with the Dept. of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01002.

0022-1147/78/0004-1081$02.25/0
© 1978 Institute of Food Technologists

Table 1–Mixtures equivalent to hydrolyzed lactose

Hydrolysis equivalence	Sugars present	Weight ratio
100%	Glucose and Galactose	50:50
90%	$Lactose_{hyd}$, Glucose and Galactose	10:45:45
70%	$Lactose_{hyd}$, Glucose and Galactose	30:35:35

This study was done to measure some of the functional properties of the three sugars and of sugar-syrup mixtures with a view to obtaining information useful for increased use of hydrolyzed lactose syrups in food.

The data presented concern the solubility, viscosity and humectant properties of the three sugars and of mixtures representing hydrolyzed lactose syrups.

MATERIALS & METHODS

ALL CHEMICALS were reagent-grade. Alpha-lactose hydrate powder (U.S.P.) was obtained from the Foremost Food Company, San Francisco; D-glucose anhydrous (analytical reagent) from Mallinckrodt; and D-galactose (anhydrous) from Nutritional Biochemical Corporation.

Preparation of syrups

Lactose hydrate, upon hydrolysis, gives equal quantities of glucose and galactose on a weight basis. To simplify standardization and eliminate variables introduced by hydrolysis itself, the syrups were prepared from pure sugars rather than from hydrolyzed syrups. Table 1 shows the composition of the mixtures studied, equivalent to 70, 90 and 100% hydrolysis.

Solubility

To determine the effect of concentration (2–10%, g/100 ml) on specific optical rotation of lactose, glucose and galactose, a known weight of sugar was dissolved in distilled water and made to volume. It was held overnight in a water-bath (25°C) and the optical rotation was measured the following day with a thermostatically controlled polarimeter at 25°C (Perkin-Elmer 141). The data were used to construct a standard curve relating specific optical rotation to sugar concentration, which was later used to calculate the solubility of each sugar at the specific temperatures used in this study.

For the determination of solubility, solutions of known sugar concentration were prepared in a 500-ml wide-mouthed bottle. Then sufficient sugar was added to exceed maximum solubility. By this method, solubility was reached from the direction of undersaturation rather than supersaturation (Peters, 1928). Ten μl alkyl benzyl dimethylammonium chloride per 100 ml solution was added to prevent microbial growth. The bottle, carefully sealed with temperature-resistant tape and with "Scotch" tape to prevent entry of water, was rotated at 21 revolutions per minute submerged in a Magni-Whirl water-bath. The solubility of the individual sugars was determined at 10, 25, 35, 40 and 50°C. Also used for 10 ± 0.1°C was a Bath-Cool (Neslab Instrument Bath Cool PBC-2).

An initial sample was taken the day after the solution was prepared. Thereafter, a sample was taken every third day until the concentration was essentially constant as determined polarimetrically. Then, samples were taken for five successive days. Samples from the last 2 days were also kept frozen at −20°C for enzymatic analysis of sugar concentrations (glucostat and galactostat) as described by Worthington Biochemical Corp., Freehold, NJ. A similar procedure was used to determine solubility of the three sugars at all test temperatures.

To measure the concentration of sugar, duplicate samples of about 20 ml were removed and filtered rapidly (to minimize temperature change) with a Gelman pressurized filter funnel and 25.0 U Millipore

Table 2—Concentration range of sugar syrups used in the viscosity tests

	Total solids (%)
Sugars	
Sucrose	10–80
Lactose	10–60
Glucose	10–80
Galactose	10–60
Equivalent % hydrolysis:	
100%	10–70
90%	10–80
70%	10–70

Table 3—Composition of the sugar syrups used for humectant study

	Total solids (%)
Sugars	
Sucrose	40, 60, 80
Equivalent % hydrolysis:	
100%	50, 60, 70
90%	60, 70, 80
70%	40, 50, 60, 70

filter (metrical G-A series). Five ml of the filtrate were transferred to a weighed 50-ml volumetric flask, which was then reweighed to establish the weight of the solution. Water was added to bring the solution to volume, and the flask contents were well mixed. The optical rotations of the solutions were measured the next day.

The concentration data (g/100g) were examined with an unpaired *t*-test, and an average and a standard deviation were found, using only the values which did not differ significantly from each other at the 5% level of probability.

Determination of the solubility of partially and completely hydrolyzed lactose at 40°C

Since one sugar influences the solubility of another (Talley and Hunter, 1952) the sugars were mixed in the ratio of their individual solubilities at 40°C. Sugar mixtures were added daily in proportion to their individual solubility for 11 days, after which the solution was saturated. Solutions representing partial hydrolysis of lactose required mixing on a weight-ratio basis of La:Glu:Ga, whereas complete hydrolysis required only Glu:Ga, as shown in Table 1. This gradual adding of sugar mixtures prevented adding excess of one sugar and too little of another; it also prevented decreased solubility of one sugar due to excess of another. Preservative was added, and the sugar solutions were held at constant temperature as described above.

The initial optical rotation was taken after 1 day in the water-bath, with subsequent measurements taken every 3 days. Unlike in prior experiments, however, no specific optical rotation, α, can be used here to calculate the concentration, so the solution was assumed to be at equilibrium when optical rotation remained constant.

Samples of the final 5 days were stored at refrigerator temperature (5°C) for enzymatic (glucostat and galactostat) analyses. For completely hydrolyzed mixtures, containing only glucose and galactose, the concentration of the sugars was determined enzymatically. To check the results, the optical rotation equivalent to these determined concentrations was calculated and compared with the measured optical rotation. For partially hydrolyzed mixtures, glucose and galactose were measured enzymatically and the optical rotation due to them was calculated. The concentration of lactose was obtained from the difference between the observed optical rotation and that calculated on the basis of glucose and galactose.

Viscosity

Viscosity was determined with a Brookfield Synchrolectric viscometer, Model LTV, fitted with adapter UL for low-viscosity samples and adapter LV 1 for higher-viscosity samples. Brookfield standard oils were used ranging from 5–99 rps. The temperatures used were 10, 25, 40, 50 and 60°C. The temperatures 10 ± 0.1°C and 25 ± 0.1°C were controlled with Bath-Cool. Julabo-Eka therm PC5 was used for the whole temperature range from 10–60°C. Sorbic acid was added to the prepared solution at 0.200 g/100g to inhibit microbial growth. Table 2 shows the range of concentration (percent total solids; TS) for mixtures equivalent to 70–100% hydrolysis and for pure sugar syrups prepared in this study.

Humectant property

The compositions of the sugar syrups used in the humectant study are shown in Table 3. The relative humidity ranged from 15–93% at 20°C. Seven glass desiccators were used, each at a specific relative humidity controlled by reagent-grade inorganic salts (*Handbook of Chemistry & Physics*, 1951). The equivalent of twice its solubility of an inorganic salt was added to a desiccator, along with 250 ml of distilled water. The mixture was well stirred and the lid was tightly sealed with Dow Corning C-150 compound. The desiccator was then transferred to a 20°C room and left for 3 days to attain equilibrium before the experimental samples were added to the desiccator.

Sugar syrups shown in Table 3 were prepared, and about 15 ml of each sugar syrup was weighed into an aluminum dish. Each desiccator held 13 aluminum dishes, each containing a specific sugar syrup. The desiccators were visually checked every week to ensure that they were saturated with their respective salts.

After 30 days, aluminum dishes from each desiccator were weighed to ±0.01 mg. The difference between the final and initial weights of the sugar syrup indicated whether the sugar syrup gained or lost moisture at a specific relative humidity. From these weight data, equilibrium relative humidity (ERH) was calculated for each syrup.

RESULTS & DISCUSSION

Solubility

Table 4 presents the solubilities of individual sugars (lactose, glucose, and galactose) from 10–50°C. The tabulated percentages (g anhydrous sugar/100g of solution) were calculated, and the concentrations obtained by the polarimeter method were compared with those from enzymatic analyses.

The value 17.66 ± 0.12 g/100g for the solubility of lactose at 25°C is in agreement with values in the literature (Whittier, 1944). The solubility of glucose at 25°C (50.50 ± 0.09 g/100g) agrees with the value 50.81 g/100g interpolated from data of Jackson and Silsbee (1922). The value for galactose (32.19 ± 0.19 g/100g at 25°C) agrees very well with 32.09 g/100g as reported previously (Gould, 1940). Table 5 shows the solubility of the individual sugars as a function of temperature. Glucose increased in solubility with increases in temperature more rapidly than did lactose or galactose, which increased at similar rates. The enzymatic method gave slightly higher values than the polarimetric method in measuring galactose.

The data show that although lactose has a relatively low solubility, upon hydrolysis the resulting sugars have a much greater solubility. Therefore, it should be possible to produce

Table 4—Solubility of lactose, glucose and galactose at 10–50°C (g anhydrous sugar/100g of solution)

	Lactose	Glucose		Galactose	
Temp (°C)	Polarimeter	Polarimeter	Enzymatic	Polarimeter	Enzymatic
10	13.32 ± 0.05	40.70 ± 0.06	40.98	27.86 ± 0.20	28.50
25	17.66 ± 0.12	50.50 ± 0.09	52.06	32.19 ± 0.19	34.04
30	19.82 ± 0.02	54.03 ± 0.03	54.66	35.90 ± 0.01	37.02
40	24.35 ± 0.02	63.47 ± 0.01	62.40	41.01 ± 0.03	42.97
50	30.06 ± 0.02	69.94 ± 0.05	70.60	46.54 ± 0.01	47.00

Table 5—Equilibrium solubilities of glucose and galactose in solutions representing complete hydrolysis of lactose and of lactose, glucose, galactose in solutions representing partially hydrolyzed lactose at 40°C (g anhydrous sugar/100g of solution)

Extent of hydrolysis	Lactose	Glucose	Galactose	Total solids
Complete	–	40.47 ± 0.82	21.48 ± 0.53	61.95
Partial	9.41 ± 0.66	33.47 ± 0.30	20.08 ± 0.39	62.96

Table 6—Viscosity in centipoise of sugar syrups from 10–80% total solids at 10°C

% Total solids	Sucrose	Lactose	Glucose	Galactose	Hydrolysis (sugar syrup) 100%	90%	70%
10.0	1.85	1.86	1.81	1.80	1.76	1.89	1.96
18.7	2.48	2.59	2.46	2.35	2.41		
20.0						2.57	2.76
27.0	3.81	4.16	3.61	3.63	3.60		
30.0		5.27				4.34	4.66
35.0	6.41		5.79	5.70	5.70		
40.0		11.40		11.38		8.56	9.18
42.4	11.12		9.72		9.54		
49.4			17.75				
50.0	22.00			22.80	19.80	21.30	22.85
56.0	52.50				38.40		
60.0	114.80		71.60		72.00	77.00	93.00
62.0							
62.5							
67.7							
70.0	1370.00				505.00		730.00
80.0	87300.00					19600.00	

Table 7—Viscosity in centipoise of sugar syrups from 10–80% total solids at 25°C

% Total solids	Sucrose	Lactose	Glucose	Galactose	Hydrolysis (sugar syrup) 100%	90%	70%
10.0	1.26	1.26	1.31	1.26	1.30	1.34	1.38
18.7	1.62	1.62	1.61	1.70	1.64		
20.0						1.81	1.85
27.0	2.35	2.45	2.21	2.21	2.20		
30.0						2.57	2.84
35.0	3.76	4.07	3.37	3.31	3.40		
40.0						4.81	5.16
42.4	5.96	6.76	5.41	5.08	5.39		
49.4			9.20	9.28			
50.0	11.50	17.00			9.74	10.50	11.26
56.0	23.90		19.00		16.67		
60.0					30.30	31.60	35.40
62.0	60.00						
62.5			42.50				
67.7	194.40						
70.0			156.80		141.20	185.00	199.00
80.0	70000.00					2548.00	

higher-solids syrups without crystallization. On the basis of our measured solubility of galactose (32.19 ± 0.19 g/100g) at 25°C, 100% hydrolysis should make it possible to increase the total solids to 64.38g before the galactose would be expected to crystallize. Talley and Hunter (1952), however, found that the solubility of each of these sugars is decreased by the presence of the others. Table 5 shows that in a completely hydrolyzed mixture at 40°C the solubility was 40.47 ± 0.82 g/100g for glucose and 21.48 ± 0.53 g/100g for galactose. Thus, individual solubilities were decreased considerably by the presence of another sugar (Table 4). Surprisingly the total solubility of glucose and galactose combined (representing total lactose hydrolysis) was slightly less (61.95%) than the total solubility of all three sugars (62.96%), which simulated a partially hydrolyzed lactose syrup (Table 5). Even so, hydrolysis increased total soluble sugars to a much higher level than is possible with lactose alone.

Viscosity

Viscosity data for the sugar syrups are given in Tables 6, 7, 8, 9 and 10. The viscosity of all the sugar syrups increased with the increase in percent TS. The relationships of percent

Table 8—Viscosity in centipoise of sugar syrups from 10–80% total solids at 40°C

% Total solids	Sucrose	Lactose	Glucose	Galactose	Hydrolysis (sugar syrup) 100%	90%	70%
10.0	1.02	1.02	1.00	1.00	1.03	1.04	1.05
18.7	1.25	1.36	1.30	1.25	1.21		
20.0						1.37	1.39
27.0	1.81	1.81	1.60	1.57	1.59		
30.0						2.07	1.97
35.0	2.38	2.53	2.20	2.16	2.23		
40.0						3.16	3.23
42.4	4.18	4.19	3.51	3.40	3.55		
49.4			5.61	5.78			
50.0	6.50	8.48			5.98	6.31	6.46
56.0			10.15		9.30		
60.0		29.00		13.98	14.85	17.00	18.40
62.0	29.70						
62.5			29.35				
67.7	77.20						
70.0			56.50		61.00	66.00	80.80
80.0	10900.00		679.00			663.00	

Table 9—Viscosity in centipoise of sugar syrups from 10–80% total solids at 50°C

% Total solids	Sucrose	Lactose	Glucose	Galactose	Hydrolysis (sugar syrup) 100%	90%	70%
10.0	0.88	0.84	0.82	0.89	0.82	0.87	0.90
18.7	1.07	1.10	1.10	1.07	1.05		
20.0						1.01	1.11
27.0	1.46	1.50	1.36	1.36	1.35		
30.0						1.81	1.85
35.0	1.90	2.06	1.84	1.79	1.81		
40.0						2.44	2.57
42.4	3.05	3.76	2.56	2.66	2.55		
49.4			4.11	3.96			
50.0	4.70	6.08			4.38	4.67	4.86
56.0	9.20		6.96		6.72		
60.0		20.75		9.82	10.10	10.78	11.16
62.0	18.80						
62.5			13.66				
67.7	48.60		33.00				
70.0				32.60	36.50	38.00	50.00
80.0	1028.00		282.00			325.00	

Table 10—Viscosity in centipoise of sugar syrups from 10–80% total solids at 60°C

% Total solids	Sucrose	Lactose	Glucose	Galactose	Hydrolysis (sugar syrup) 100%	90%	70%
10.0	0.81	0.80	0.79	0.82	0.81	0.80	0.83
18.7	0.91	1.06	0.85	0.91	0.88		
20.0						0.92	1.01
27.0	1.20	1.22	1.16	1.07	1.15		
30.0						1.64	1.61
35.0	1.60	1.75	1.50	1.46	1.51		
40.0						2.16	2.16
42.4	2.26	3.12	2.02	2.10	2.02		
49.4			3.12	3.08			
50.0	3.96	6.20			3.44	3.57	3.66
56.0			5.23		4.82		
60.0		17.75		8.58	7.08	7.80	8.28
62.0	13.20						
62.5			9.40				
67.7	30.70						
70.0			30.00		30.00	24.10	32.50
80.0	835.00		228.18			174.00	

Table 11—Changes in weight and equilibrium relative humidity (erh) of sugar syrups when held at 15%—93% relative humidity and 20°C for 30 days

% Total solids	Relative humidity[a] 15%	32.3%	52%	65%	70%	84%	93%	Calc erh
100% hydrolysis								
50% T.S.	−7.24777	−6.77117	−6.22310	−5.61413	−3.58104	−2.19231	+1.11202	90%
60% T.S.	−6.09946	−5.81384	−4.22305	−1.89789	−1.29928	+0.94905	+3.40481	81%
70% T.S.	−3.82391	−2.88716	−1.34296	+0.77411	+1.75205	+1.35082	+4.63495	68%
90% hydrolysis								
60% T.S.	−5.66402	−5.37623	−3.64070	−1.78917	−1.22538	−0.92273	+2.37136	86%
70% T.S.	−3.52619	−3.14994	−0.38575	−0.45480	+0.75213	+1.80909	+4.83339	69%
80% T.S.	−1.62520	−0.99092	+0.89679	+2.35910	+3.11931	+3.71959	+7.63118	43%
70% hydrolysis								
40% T.S.	−8.75707	−7.55751	−7.42690	−6.14649	−5.82193	−5.13189	−3.06825	>93%
50% T.S.	−7.27755	−6.98719	−6.04889	−4.30085	−3.10719	−1.11951	−0.88826	>93%
60% T.S.	−5.63314	−5.75956	−4.33457	−2.62907	−0.89741	−0.59735	+1.18144	87%
70% T.S.	−3.50048	−2.69428	−1.53089	+0.49063	+1.60893	+1.48575	+4.16530	62%
Sucrose								
40% T.S.	−8.14031	−7.75735	−6.95066	−5.82685	−5.27618	−5.02900	−3.2983	>93%
60% T.S.	−4.82003	−5.40433	−5.86123	−4.95524	−0.30626	+0.24097	+1.9169	80%
80% T.S.	−1.00543	−0.49575	+0.97820	+1.83043	+3.90264	+4.19785	+5.42828	39%

[a] − loss in moisture; + gain in moisture

TS vs viscosity for all sugar syrups were curvilinear (parabolic relationship). Viscosity increased gradually, up to 50% TS, and rapidly thereafter. All sugar syrups were similar in viscosity with 50% TS or less. At 60% TS and above, sucrose syrups were higher in viscosity than the hydrolyzed lactose syrups. In general, 90% and 70% hydrolyzed syrups had slightly higher viscosities than syrups 100% hydrolyzed. Viscosity decreased substantially with increasing temperature, especially in higher-solids syrups. That is an advantage in handling and transporting such products.

Relative humidity

Table 11 shows the ability of the sugar syrups to lose or gain moisture during 30 days in relative humidities from 15−93% at 20°C. The ERH of each syrup was calculated from those data. Overall, each was similar in ERH to sucrose syrups of the same TS content.

REFERENCES

Borglum, G.B. and Steinbert, M.Z. 1972. Properties of fungal lactase. J. Food Sci. 37: 169.

Bouvy, F.A.M. 1975. Applications for lactase-treated whey. Food Product Dev. 9(3): 10.

Coughlin, J.R. and Nickerson, T.A. 1975. Acid catalyzed hydrolysis of lactose in whey and aqueous solution. J. Dairy Sci. 58: 169.

Gould, S.P. 1940. Final solubility of D-galactose in water. J. Dairy Sci. 23: 227.

Handbook of Chemistry and Physics. 1951. 33rd ed Chemical Rubber Publ. Co., Cleveland, OH.

Holsinger, V.H., Posati, L.P. and DeVilbiss, E.D. 1974. Whey beverage: A review. J. Dairy Sci. 57: 849.

Holsinger, V.H. and Roberts, N.E. 1976. New products from lactose-hydrolyzed milk. Dairy and I.C. Field 159(3): 30.

Hu, A.S.L., Wolfe, R.G. and Reithal, F.J. 1959. The preparation and purification of β-galactosidase from Escherichia coli ML 308. Arch. Biochem. Biophys. 81: 500.

Hudson, C.S. 1908. Further studies on the forms of milk-sugar. J. Am. Chem. Soc. 30: 1767.

Jackson, R.F. and Silsbee, C.G. 1922. Solubility of dextrose in water. Bu. Standard Sci. Papers 17:715 No. 437.

Mahoney, R.R., Nickerson, T.A. and Whitaker, J.R. 1975. Selection of strain, growth conditions and extraction procedures for the optimum production of lactase from Kluyveromyces fragilis. J. Dairy Sci. 58: 1620.

Peters, P.N. 1928. Solubility relationship of lactose-sucrose solution at low temperature. J. Phy. Chem. 32: 1856.

Ramsdell, G.A. and Webb, B.H. 1945. The acid hydrolysis of lactose and the preparation of hydrolyzed lactose syrup. J. Dairy Sci. 28: 677.

Taley, E.A. and Hunter. A.S. 1952. Solubility of lactose and its hydrolytic products. J. Am. Chem. Soc. 74: 2789.

Whittier, E.O. 1944. Lactose and its utilization: A review. J. Dairy Sci. 27: 505.

Ms received 10/3/77; revised 12/22/77; accepted 12/30/77.

Presented at the 36th Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 6—9, 1976.

The authors thank Mr. Edwin E. Moore for excellent technical assistance.

This research was supported in part by a grant from Whey Products Institute, Chicago, IL.

FUNCTIONAL PROPERTIES OF HYDROLYZED LACTOSE: CRYSTALLIZATION

N. O. SHAH and T. A. NICKERSON

ABSTRACT

The crystallization habit of simulated hydrolyzed lactose syrups containing 40–80% total solids was studied at 25°C and 40°C. At 25°C, crystallization did not become a problem until total solids were 70% or above, thereby greatly exceeding the solubility of the constituent sugars. Hydrolyzed products reduced crystal growth as did elevation of temperature. In syrups hydrolyzed 100%, galactose was the predominant crystalline sugar, whereas lactose predominated in the crystals recovered from syrups hydrolyzed 70% and 90%. Syrups were still stable after storage for 6 months.

INTRODUCTION

SINCE LACTOSE FORMS over 50% of the nonfat solids in milk, its low solubility can result in crystallization which is responsible for a gritty (sandy) texture in some concentrated or frozen dairy products. Hydrolysis of lactose yields a mixture of sugars whose solubility is greater than that of lactose.

Crystallization would be undesirable during storage and transport of such syrups. Therefore data were needed on the maximum level of total solids (TS) that can be used while avoiding the crystallization. The presence of one sugar can influence the crystallization of another (Nickerson and Patel, 1972). That can affect the texture of candies, confections, frostings, or other toppings. Crystallization is dependent also on the degree of supersaturation (Herrington, 1934), temperature (Wittier and Gould, 1931), presence of salts (Jelen and Coulter, 1973), and viscosity (Peters, 1928). This crystallization study was undertaken to determine at what percent of TS that crystallization occurred and which sugars crystallized from syrups simulating hydrolyzed lactose. It forms part of a study of the functional properties of hydrolyzed lactose (Shah and Nickerson, 1978).

MATERIALS & METHODS

SUGAR SYRUPS equivalent to hydrolyzed lactose syrups were prepared from pure sugars (lactose, glucose, and galactose) as described by Shah and Nickerson (1978). The solids contents of the sugar syrups are shown in Table 1.

All sugar syrups were weighed into flasks in lots of 500 ± 0.1g. Sorbic acid (0.2 g/100g solution) was added, and the sugars were dissolved by placing the flasks of known weight in a steam-bath. Any loss in weight during heating was adjusted after cooling by the addition of distilled water. Sugar syrups were then filtered, using a Gelman pressurized filtration funnel with a 5.0-μm Millipore filter to remove foreign particles which might initiate crystallization.

One hundred ml of the sugar syrup was then transferred to each of the two weighed Nalgene wide-mouth screw-top containers. The containers were reweighed, sealed with temperature-resistant tape, and transferred immediately to a constant-temperature water-bath. One set was transferred to a static water-bath, and another to a shaking water-bath (30 times per minute). The syrups were held at two temperatures (25 ± 0.1°C and 40 ± 0.1°C). Each water-bath was covered with foil to ensure constant temperature of the contents.

Refractometer readings were used to determine the percent TS. The relative amounts and appearance of crystals formed were recorded from visual observation of the syrups every other day for 25 days. At the end of 25 days the bottles were photographed and the crystals were recovered by filtering and washing with a minimum amount of ice-cold distilled water. Recovered crystals were dried to constant weight in a vacuum oven at 70°C (24 hr). The crystals were finely ground, well mixed, and transferred to a 25-ml plastic container and stored in a desiccator until required for final identification.

The TS content of the remaining solution was analyzed refractometrically, and the solutions were left standing in their respective water-baths for an additional 6 months. Any new crystals were then noted.

Crystals were identified by thin-layer chromatography (Pifferi, 1965), and also by the phenyl hydrazone method (Mulliken, 1905) when the quantities were sufficient.

Table 1—Sugar syrups used in the crystallization study.

Equivalent % hydrolysis	% total solids
100% hydrolysis	50%, 60%, 70%
90% hydrolysis	60%, 70%, 80%
70% hydrolysis	40%, 50%, 60%, 70%

Table 2—Weight of crystals recovered at the end of the crystallization study (25 days)

% Total solids	At 25°C Static water-bath (g)	At 25°C Shaking water-bath (g)	At 40°C Static water-bath (g)	At 40°C Shaking water-bath (g)
100% Hydrolysis				
50% T.S.	0.006	0.056	0.002	0.003
60% T.S.	0.082	0.057	0.014	0.02
70% T.S.	14.1	21.1	10.5	10.7
90% Hydrolysis				
60% T.S.	0.099	0.082	0.003	0.028
70% T.S.	2.51	2.91	1.63	2.03
80% T.S.	19.7	20.5	10.2	16.9
70% Hydrolysis				
40% T.S.	0.007	0.002	0.0002	0.0005
50% T.S.	0.008	0.008	0.0009	0.033
60% T.S.	4.54	6.07	0.008	0.033
70% T.S.	15.3	18.1	15.6	14.4

RESULTS & DISCUSSION

TALLEY AND HUNTER (1952) derived equations representing the solubility relationships of lactose, glucose, and galactose mixtures at 25°C. Those equations were used to determine the percent TS at which sugar syrups would become saturated with one of the constituent sugars. The calculations show the syrup would become saturated with respect to galactose at 46.18% TS in syrups simulating 100%-hydrolyzed lactose, at 54.38% TS in 90%-hydrolyzed sytup, and at 37.03% TS in 70%-hydrolyzed syrup. Therefore, in our study, sugar syrups were prepared above calculated saturation as shown in Table 1.

Author Nickerson is with the Dept. of Food Science & Technology, University of California, Davis, CA 95616. Author Shah, formerly with the University of California–Davis is now affiliated with the Dept. of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01002.

0022-1147/78/0004-1085$02.25/0
© 1978 Institute of Food Technologists

Table 3—Percent total solids in the 25° and 40° C sugar syrups before and after the crystallization study.

% Total solids	Sugar syrups held at 25° C			Sugar syrups held at 40° C		
	Before crystallization	After crystallization		Before crystallization	After crystallization	
		Static water-bath	Shaking water-bath		Static water-bath	Shaking water-bath
100% Hydrolysis						
50% T.S.	49.85	49.80	49.70	50.00	50.00	50.00
60% T.S.	59.35	59.30	59.30	59.30	59.20	59.25
70% T.S.	68.80	65.70	63.70	68.85	66.70	66.00
90%Hydrolysis						
60% T.S.	59.55	59.60	59.40	59.65	59.60	59.60
70% T.S.	69.25	68.20	68.90	69.30	69.00	69.00
80% T.S.	79.15	76.00	73.90	79.20	76.40	76.25
70% Hydrolysis						
40% T.S.	40.25	40.20	40.20	40.05	40.00	40.00
50% T.S.	50.20	50.20	50.20	49.95	49.90	50.00
60% T.S.	59.95	58.85	58.00	60.00	60.00	59.20
70% T.S.	69.85	65.50	65.20	69.90	66.90	66.80

Crystal formation

Visual observation of the syrups at both 25°C and 40°C showed all syrups on the initial day to have a similar crystal pattern: fine needle-like feathery crystals floating on the surface of the syrup, probably held there by the surface tension. This pattern remained unchanged for lower-percent TS (40% to 60%), but for higher-percent TS (70% to 80%) the crystalline pattern changed by the 8th day, and a smooth compact layer formed on the bottom of the container. By the 16th day, granular crystals were evident on the surface of the bottom layer, and by the 25th day granular crystals were observed also on the side of the container. In samples held in the shaking water-bath, crystals were observed to distribute throughout the syrup. The amount of crystals formed was less when syrups were held static than when shaking.

Weight of crystals formed

Table 2 shows the amount of crystals collected on the 25th day at each temperature. Crystallization does not appear to become a problem for syrups that are 100% and 90% hydrolyzed until TS reached 70%, where several grams of crystals were formed, compared with only milligram quantities at lower concentrations. For 70%-hydrolyzed syrups, larger quantities of crystals were found when the percent TS was 60% or above.

The amount of crystals collected at 40°C decreased because of increased solubility of the sugars in the mixture. Even so, crystallization was still evident in the higher-TS syrups, indicating that the increase in solubility of sugars in mixture was not sufficient to alter crystallization drastically. In high-TS syrups, increasing the temperature of storage did not reduce crystallization substantially. An exception noted was that lactose crystallization from the 70%-hydrolyzed syrups with 60% TS was reduced appreciably by increasing the temperature to 40°C.

Table 3 shows refractometric determination of the TS content in each sugar syrup initially and after the crystals were recovered. The TS did not change appreciably even in syrups of higher percent TS. The weight of the crystals recovered (Table 2) with higher-solids syrups was greater than the decrease in the percent TS (Table 3), indicating that the sugars crystallized in hydrate form (lactose and glucose often crystallize in hydrate form).

Crystal identification

Identification of crystals recovered from all syrups showed, at both temperatures, that galactose was the predominant sugar crystallizing in syrups equivalent to 100% hydrolysis, and that lactose was the major sugar crystallizing out from syrups equivalent to 90 and 70% hydrolysis.

This study shows that crystallization does not become a problem until TS reached 70%, indicating that the constituent sugars are more stable as a mixture. This may be because one sugar interferes with the development of nuclei in another sugar. This stability was also noted by Ramsdell and Webb (1945), who attributed it to retardation of crystal growth by the hydrolytic products. It was noted that elevating the temperature to 40°C reduced the amount of crystals formed in almost all cases. Storage of sugar syrups for 6 months after the crystals were recovered, again, showed stabilities similar to those of the original syrups. Crystallization would not be expected to cause difficulties in commercial practice except in high-TS syrups (70% or above).

REFERENCES

Herrington, B.L. 1934. Some physico-chemical properties of lactose. 1. The spontaneous crystallization of supersaturated solutions of lactose. J. Dairy Sci. 17: 501.

Jelen, P. and Coulter, S.T. 1973. Effect of certain salts and other whey substances on the growth of lactose crystals. J. Food Sci. 38: 1186.

Mulliken, S.P. 1905. "A Method for Identification of Pure Organic Compounds". Vol. 1. John Wiley and Sons, London, New York.

Nickerson, T.A. and Patel, N.K. 1972. Crystallization in solution supersaturated with sucrose and lactose. J. Food Sci. 37: 693.

Peters, P.N. 1928. Solubility relationship of lactose-sucrose solution at low temperature. J. Phy. Chem. 32: 1856.

Pifferi, P.G. 1965. Improved thin layer chromatographic separation of hexoses and pentoses using Kiesel gel G. J. Chromatography 37: 925.

Ramsdell, G.A. and Webb, B.H. 1945. The acid hydrolysis of lactose and the preparation of hydrolyzed lactose syrup. J. Dairy Sci. 28: 677.

Shah, N.O. and Nickerson, T.A. 1978. Functional properties of hydrolyzed lactose. (Submitted to J. Food Sci.)

Talley, E. A. and Hunter, A. S. 1952. Solubility of lactose and its hydrolytic products. J. Am. Chem. Soc. 74: 2789.

Whittier, E. O. and Gould, S.P. 1931. Speed of crystallization of lactose, galactose, glucose and sucrose from pure solution. Ind. Eng. Chem. 23: 670.

Ms received 10/20/77; revised 1/21/78; accepted 1/30/78.

Presented at the 36th Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 6—9, 1976.

The authors thank Mr. Edwin E. Moore for excellent technical assistance.

This research was supported in part by a grant from Whey Products Institute, Chicago, IL.

MECHANICAL INTERPRETATION OF COMPRESSIVE STRESS-STRAIN RELATIONSHIPS OF SOLID FOODS

J. F. CALZADA and M. PELEG

ABSTRACT

True compressive stress-strain relationships of various solid foods have been demonstrated in a three-dimensional display that has included the tests true strain histories. The shapes of the stress-strain curves and complementary relaxation and compressiblity data indicated the existence of two antagonistic mechanisms which regulated the stress levels. These were internal fractures which tended to decrease the mechanical strength of the deformed specimens and structural compaction that had the tendency to increase it. It has also been demonstrated that the true stresses could be influenced by the specimen dimensions as well as by the strain rate.

INTRODUCTION

MOST OBJECTIVE textural evaluation techniques are based on the interpretation of force-deformation relationships. These are usually obtained under some kind of standardized conditions with regard to the geometry of the system and the deformation rate. Because most solid foods exhibit rheological properties which are far from being simple, and because the physical arrays of the tests are sometimes rather arbitrary and not absolutely defined, results may depend to a significant extent on the test procedure. In such cases it is difficult to separate the effects which are contributed by the mechanical properties of the material and those produced by the operational conditions. The latter includes the deformation rate, the friction at the contact surfaces (Culioli and Sherman, 1976) and the physical dimensions of the specimen (Szczesniak et al., 1970; Culioli and Sherman, 1976; Peleg et al., 1976). In many cases, the size dependency has some severe theoretical and practical implications which could hardly be remedied by the establishment of standard test conditions only. Since the ratio between various mechanical parameters of different materials or samples can depend on the test conditions, the selection of the latter will affect any comparison between samples.

In order to understand some of the problems involved in texture evaluation, the behavior of some selected solid foods was studied under uniaxial compressive deformation. This particular test has been chosen on the basis that it forms the essential part of the commonly used instrumental textural profiling method (Szczesniak et al., 1963) performed with a Universal testing machine (Bourne, 1968; Breene, 1975). An additional reason has been the fact that compressive deformation, though in much less defined ways, also contributes to results of other instrumental techniques such as the shear-press (Voisey, 1976). Understanding the compression component or stage may reveal at least part of the necessary information for the comprehensive interpretation of experimental results.

Authors Calzada and Peleg are with the Dept. of Food & Agricultural Engineering, University of Massachusetts, Amherst, MA 01003. All correspondence should be addressed to Dr. Peleg.

0022-1147/78/0004-1087$02.25/0
© 1978 Institute of Food Technologists

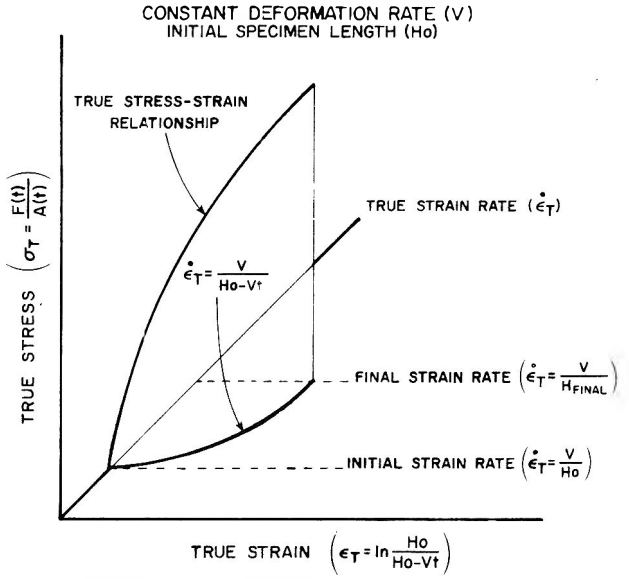


Fig. 1—Schematic view of a true stress-strain-strain rate relationship of a specimen in uniaxial compression at a constant deformation rate.

Apparent and true stress strain relationships in compressive tests

The relevant range of compressive testing of foods covers the region termed as large deformation and many of the assumptions which are justified for small deformations are no longer valid.

These have the following aspects:

(a) The cross-sectional area of the specimen is considerably expanded and therefore cannot even be approximated by the original area.

(b) The true strain is significantly different from the apparent strain and the difference increases with the strain.

From the fundamental mechanics (Marin, 1962) the true compressive strain (ϵ_T) (also called Hencky or natural strain) is:

$$\epsilon_T = \ln \frac{H_o}{H_o - \Delta H} = \ln (1 - \epsilon_{AP}) \qquad (1)$$

where H_o is the original length of the specimen, ΔH is the absolute deformation and ϵ_{AP} the apparent strain, (i.e. $\Delta H/H_o$).

(c) At a constant deformation rate (V) the true strain rate ($\dot{\epsilon}_T$) is progressively increasing with the strain thus producing a strain history which is given by (Dieter, 1976):

$$\dot{\epsilon}_T = \frac{V}{H_o - \Delta H} = \frac{V}{H_o - Vt} \qquad (2)$$

Schematic representation of the true stress-strain-strain rate relationship in a three dimensional display is given in Figure 1. Eq (1) implies that even an ideal elastic material would show a

nonlinear curve in apparent coordinates. This also means that even in such an ideal situation the recorded force-deformation curve and the apparent stress-strain relationship would depend on the operational conditions. Theoretical evaluation of other rheological models under such rate histories indicated that the response pattern is rather complicated (Peleg, 1977a). The analysis showed, however, that regardless of whether the model is elastic or Maxwellian, the force deformation and the apparent stress strain relationships would appear as a concave upward curve with an ever increasing slope. Only when internal fracture overwhelmingly outweighs the combined effects of the increasing area, strain and strain rate, would there appear a convex upward curve in apparent coordinates. Because the apparent stress level of real materials represents the total effect of a variety of physical processes, it tells very little about their relative contributions. Some insight into these mechanisms can be achieved by transforming the apparent curves into true curves and by the addition of relaxation data.

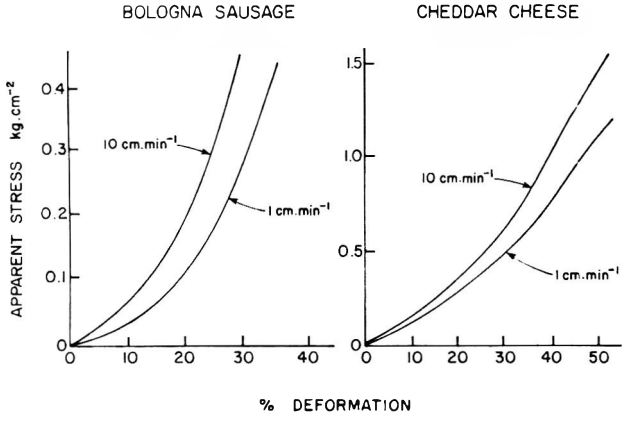


Fig. 2—Typical apparent compressive stress-strain relationships of solid foods. (Same shape as the force deformation curves).

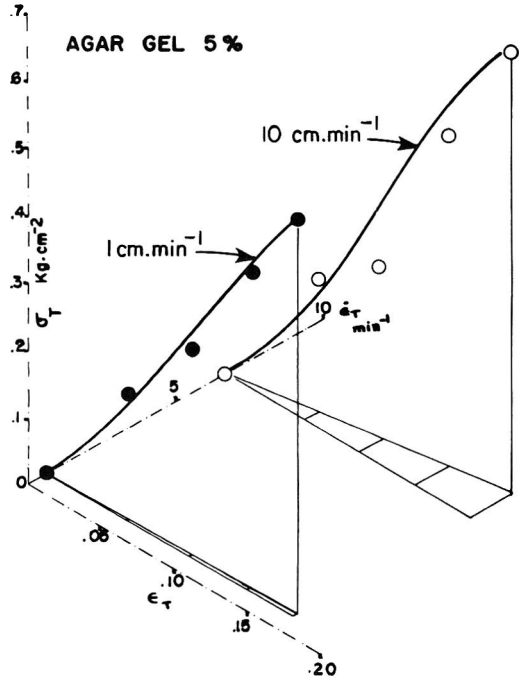


Fig. 3—True stress-strain relationship of 5% Agar gel deformed at two constant deformation rates. (σ_T—true stress, ϵ_T—the true strain, $\dot{\epsilon}_T$—the true strain rate.)

Generally speaking, if the stresses of deformed specimens decay rapidly to zero or very low levels the material is referred to as having rheological properties resembling those of liquid. If in contrast most of the stress remains unrelaxed the material is said to show more solid properties. Residual stresses after long enough time at relaxation may serve, therefore, as an index of the "solid" contribution to the deformation behavior. Relaxation data obtained after different straining histories may also indicate structural changes that resulted from the deformation (Peleg and Calzada, 1976).

EXPERIMENTAL

BOLOGNA SAUSAGES, bread loaves, cheddar cheese, potatoes, squash and turnips were purchased locally. Agar gels (5%) were prepared from Agar (Difco CO10-Q48) and analyzed after being left overnight at room temperature.

Cylindrical specimens were cut with cork borers and trimmed to the desirable length by parallel blades. Unless other wise stated, the initial dimensions of the specimens were: length, 1.5 cm and diameter, 1.5 cm. The specimens were compressed to various selected predetermined strains by an Instron Universal Testing Machine at rates of 1 and 10 cm/min. (The applied crosshead speeds did not exceed 10 cm/min to minimize errors due to the recorder pen response). Cross-sectional area changes were measured by monitoring the specimen diameter with a caliper.

Deformation specimens were also allowed to relax in a procedure previously described (Peleg and Calzada, 1976).

The force-deformation curves and the residual relaxed forces after 6 min at relaxation were used to calculate the true stress-strain curves and the residual relaxed stresses. These were calculated by dividing the force by the actual cross-sectional area at any given strain or:

$$\sigma_T = \frac{F(\epsilon)}{A(\epsilon)} \qquad (3)$$

where F is the force and A the cross-sectional area. Though there is a way of direct calculation of $A(\epsilon)$ from the strain (Marin, 1962) the experimental method was selected because compressibility of the food materials could not be ruled out.

The true strains and strain rates were calculated from Eq (1) and (2), respectively.

RESULTS & DISCUSSION

MOST OF THE REPORTED DATA on the mechnical behavior of solid foods has been presented in the form of force-deformation curves or apparent stress-strain relationships. These have been obtained at various deformation rates which could show to what degree the apparent values were rate dependent. As mentioned, such an approach has some innate difficulties. This is mainly due to the fact that force-deformation curves do not provide any indication about the contribution of the cross-sectional area increment to the total recorded force. This will result in the commonly observed curves that have a typical concave upwards shape (Fig. 2) in apparent coordinates. Furthermore, if only the true stresses are calculated and plotted versus the apparent strains, the shape of the curve will still appear distorted because the effective strains are different from the apparent ones (Eq 1). The true shape of different stress-strain relationships in a three-dimensional representation are shown in Figures 3 to 9. The figures demonstrate the difficulties involved in a quantitative evaluation and comparison of rheological behavior of foods under tests performed at different deformation rates. Similar problems would arise when specimens varying in length are compared because a distinct length also implies a different true strain history as shown in Eq (2).

Shapes of the true stress-strain curves

All the tested materials showed apparent force-deformation (and apparent stress-strain) curves that had concave upwards shape as shown in Figure 2. They also showed rate dependent

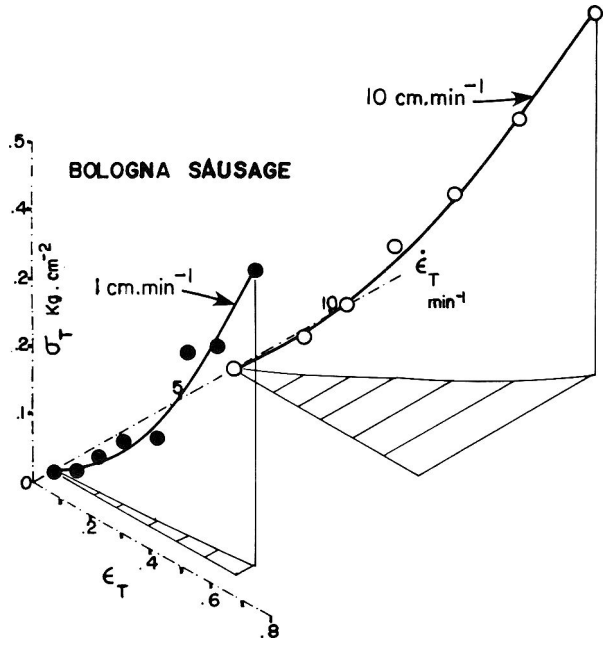


Fig. 4—True stress-strain relationships of bologna sausage deformed at two constant deformation rates (For legends see Fig. 1).

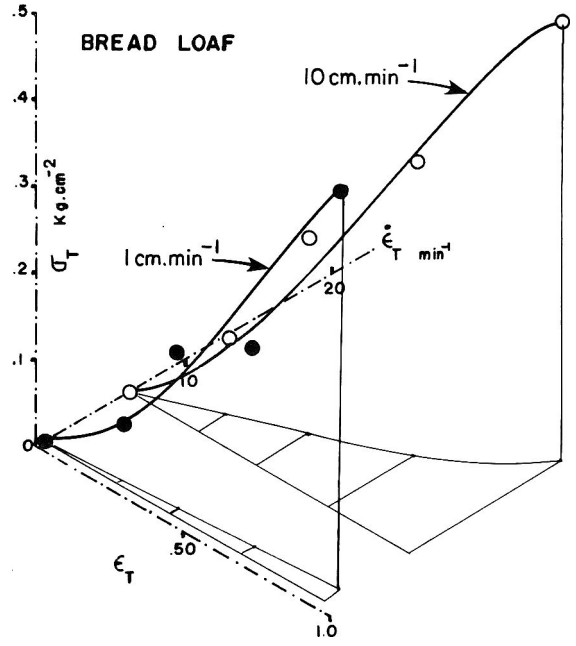


Fig. 5—True stress-strain relationships of bread deformed at two constant deformation rates. (For legends see Fig. 1.)

mechanical response as could be expected from their viscoelastic nature, (Sherman, 1970; Mohsenin, 1970). However, they not only differed by their absolute strength and the overall magnitude of the rate effect but also by the general shape of their true stress-strain curves.

These were composed of three basic parts which included (not necessarily all or in a consequent order) an apparent linear, a concave upward and a convex upward stage. These stages were probably the result of two antagonist mechanisms which participate in the compressive large deformation process. These are:

(a) Fracture of structural elements which tends to reduce the overall strength.
(b) Compaction of original and fractured elements to produce a denser structure which tends to increase the overall strength.

At each strain level, these mechanisms could balance one another (linear region) or be dominated by one of them. In a case of dominant fracture mechanism the slope of the stress-strain curve decreases with the strain and an upward convex region appears. If compaction dominates the situation the slope of the curve increases progressively and an upward concave curve will develop.

Testing the double mechanism hypothesis

From Figures 3 to 9 it is realized that only two materials, i.e. bologna sausage and bread (Figures 4 and 5) had a continu-

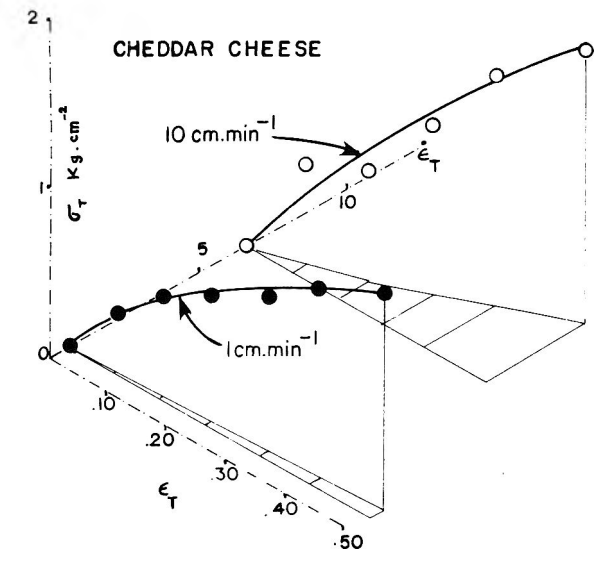


Fig. 6—True stress-strain relationships of cheddar cheese deformed at two constant deformation rates. (For legend see Fig. 1.)

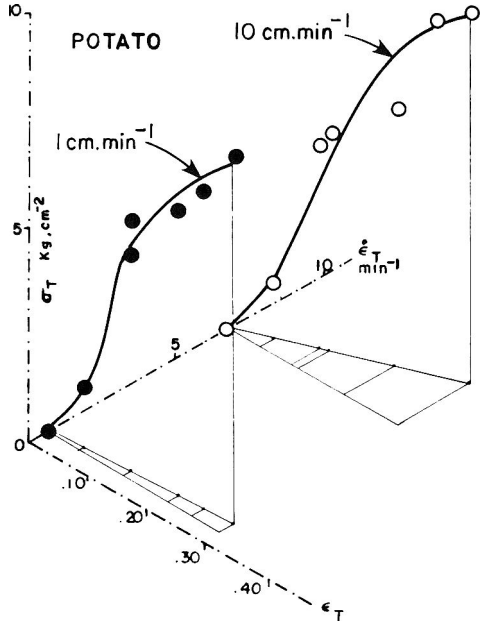


Fig. 7—True stress-strain relationship of potatoes deformed at two constant deformation rates. (For legends see Fig. 1.)

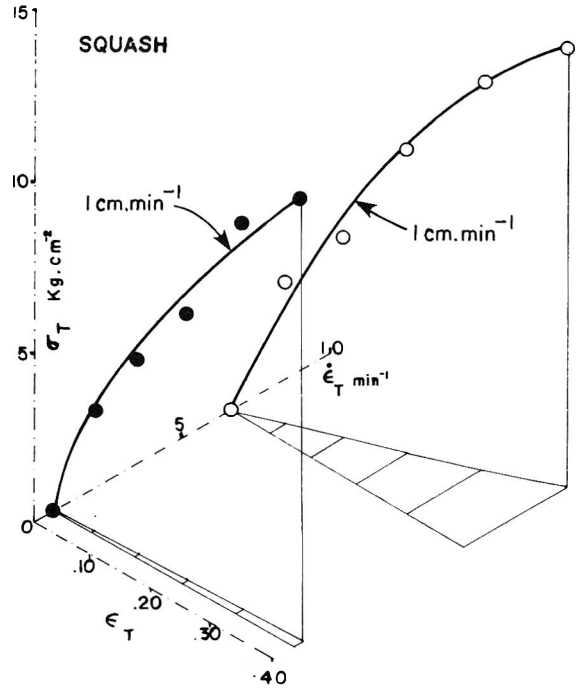


Fig. 8—True stress-strain relationships of squash deformed at two constant deformation rates. (For legends see Fig. 1).

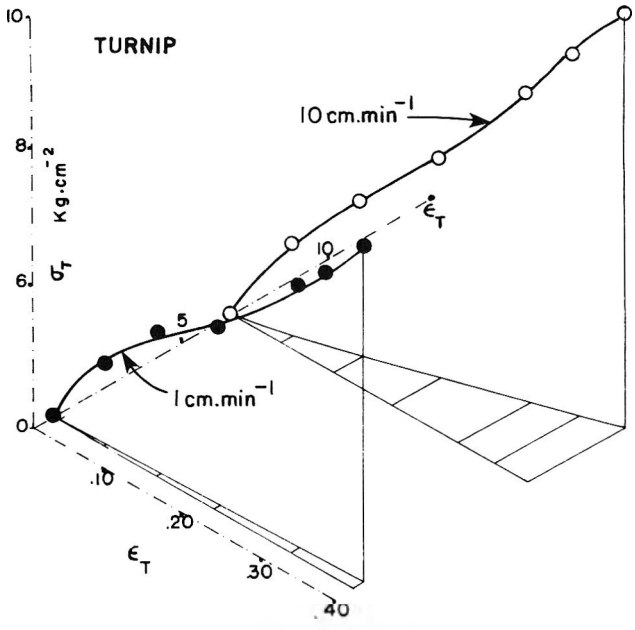


Fig. 9—True stress-strain relationships of turnip deformed at two constant deformation rates. (For legends see Fig. 1).

ous concave upward curve. If the suggested mechanisms really represent the situation, considerable compressibility is expected in the applicable deformation range. Table 1 demonstrates that this, in fact, has been the case.

The occurrence of fracture would imply that the curve of residual relaxed stresses plotted vs the strain would have a convex upward shape and that its values would depend on the strain history (Peleg and Calzada, 1976).

The relationships between residual true-stresses (after relaxation) and the true strains of the various materials are shown in Figures 10 to 16. These indicated that in all the materials where the true stress-strain relationships showed clear dominancy of fracture mechanism, the latter could also be detected in the relaxation pattern (e.g. cheese and squash.) It should also be mentioned that in the commodities where alternating dominances have been found, fracture progressed at a faster rate as the strains approached the gross failure level (e.g. agar gel, potato, turnip). A visual demonstration of the latter was offered by the translucent agar gels in which minature cracks could be detected prior to the development of a gross failure and the drop in the recorded force.

Effect of the specimen dimensions on the true stress-strain relationships

Theoretical evaluation of the role of the dimentions in uniaxial compression indicated that short specimens are expected to show higher apparent strength than long specimens (Peleg, 1977a, b). The models that have been applied also predict that the true stresses would differ too, though to a lesser extent. To test the hypothesis it was attempted to deform specimens of the same diameter with varying lengths but technical difficulties of two kinds arose:

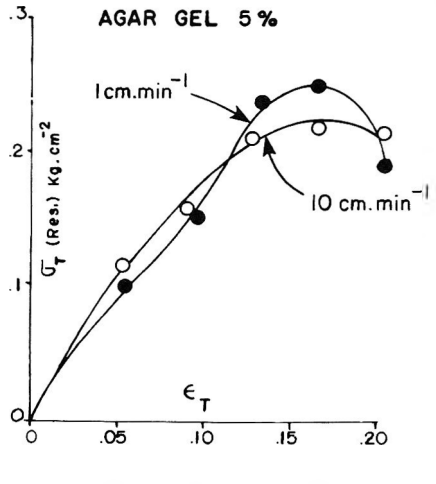


Fig. 10—Residual relaxed stress (after 6 min. of relaxation time) as a function of true strain of 5% Agar gel. (σ_T(Res) is the residual true stress and ϵ_T is the true strain.)

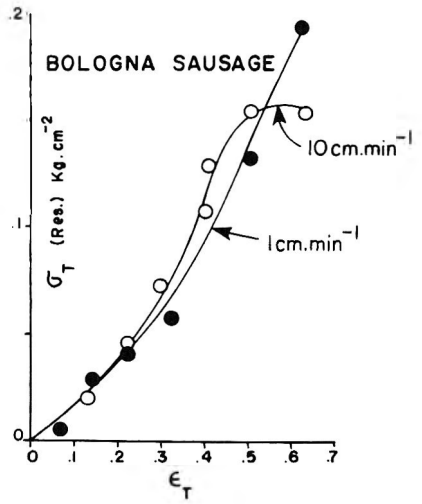


Fig. 11—Residual relaxed stress (after 6 min.) as a function of the true strain of bologna sausage. (For legends see Fig. 10).

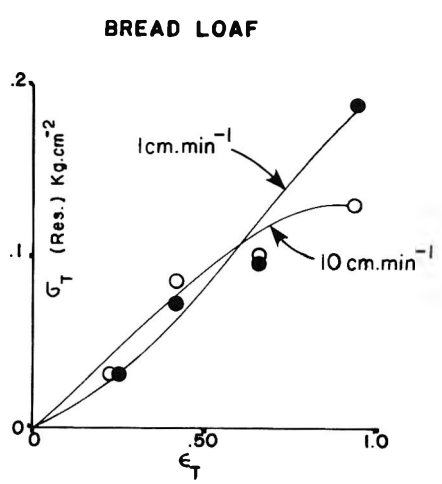


Fig. 12—Residual relaxed stress (after 6 min.) as a function of the true strain of bread. (For legends see Fig. 10).

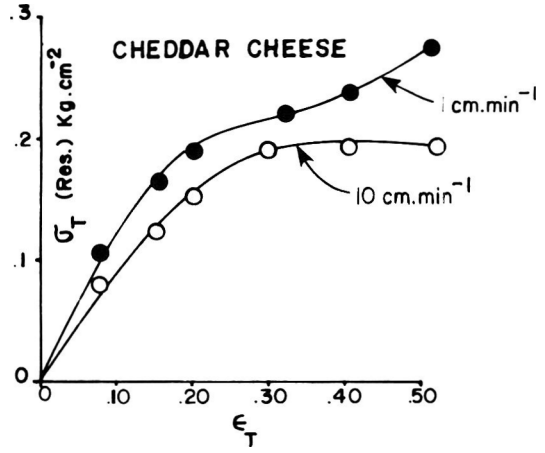


Fig. 13—Residual relaxed stress (after 6 min.) as a function of the true strain of cheddar cheese. (For legends see Fig. 10).

(a) A very long specimen could buckle.
(b) A very long specimen could have undergone a considerable compaction. Comparisons of different specimens at the same strains could therefore be meaningless because of significant differences in density.

A further theoretical difficulty is produced by the fact that the cross-sectional area expansion itself may have features and dependencies that complicate any meaningful comparison where very distinct dimensional differences are involved. If however, specimens with relatively close dimensions are compared, the natural variations can in many cases mask the significance of the trend, especially when a small number of specimens can be tested. (It would, obviously, be desirable to test a large number of specimens but this can sometimes be technically impossible due to the physical size of the natural units of the commodities.)

In order to demonstrate the dimensions effect, therefore, specimens of distinctly different shape were tested. The results (Table 2) show clearly that "flat" specimens develop higher stresses than "long" specimens even when the latter is compared in true stress terms. The magnitude of the differences is obviously a result of the combined effects of the strain rate history and the fracture-compaction balance and therefore may be characteristic of both the material structure and the test operational conditions.

Table 1—Compressiblity of various solid food material

Material	True strain[a]	Volume reduction %
Agar gel 5%	0.25	less than 3
Bologna sausage	0.62	12
Bread	0.94	51
Cheddar cheese	0.40	9
Potato	0.25	less than 3
Squash	0.26	less than 3
Turnip	0.40	6

[a] Prefailure level

Table 2—Effect of the specimen dimensions on the true stress level[a]

Material	True strain	Mean stress (kg/cm²) "Flat"	Mean stress (kg/cm²) "Long"	Difference[b] (%)
Agar gel 5%	0.22	1.1	1.0	11*
Cheese	0.23	0.36	0.28	29*
Potato	0.31	7.8	6.9	13*
Turnip	0.40	16.2	15.6	4*

[a] The "flat" specimens had 2 cm in diameter and 1.5 cm in length while the "long" specimens had 1.5 cm in diameter and 2.5 cm in length. They were deformed at crosshead speeds of 0.5 and 1 cm/min respectively.
* Significant at $p \leq 0.05$

Effect of the specimen wall

It is quite probable that the preparation of the specimens by cork borers affected their mechanical response. Because it is difficult to change the wall without the overall dimensions it is difficult to evaluate its effects separately. With Agar gels however, it was possible to produce gels of the same composition and dimensions but with different wall properties. This

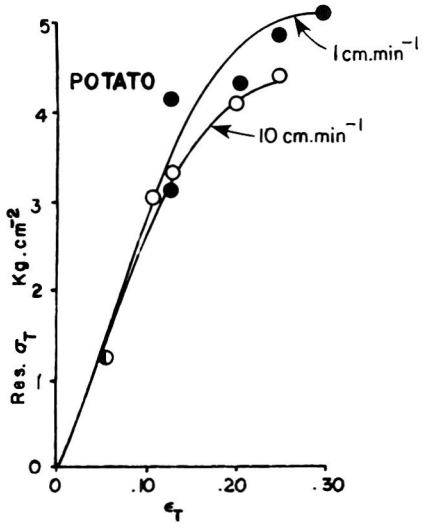


Fig. 14—Residual relaxed stress (after 6 min.) as a function of the true strain of potatoes. (For legends see Fig. 10).

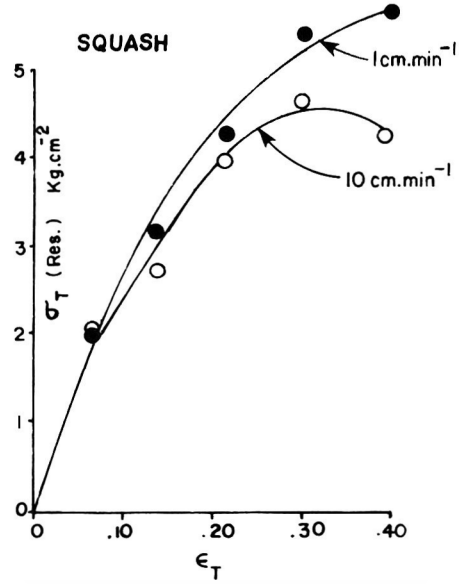


Fig. 15—Residual relaxed stress (after 6 min.) as a function of the true strain of squash. (For legends see Fig. 10).

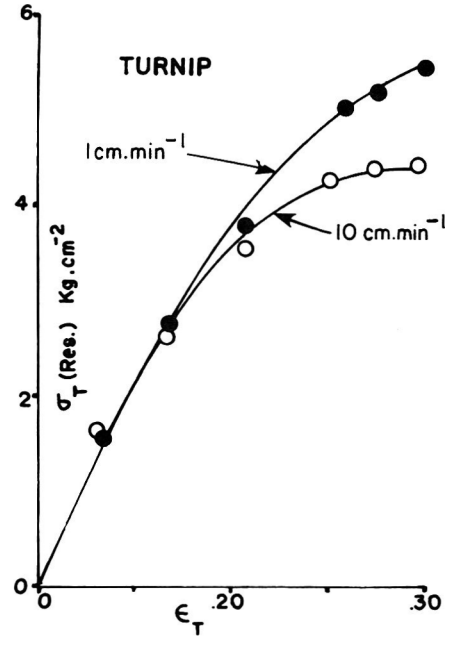


Fig. 16—Residual relaxed stress (after 6 min.) as a function of the true strain of turnip. (For legends see Fig. 10).

was done by forming gels in a tube and in a container. This provided specimens with identical dimensions in which only one kind was obtained by a borer. Comparison of results indicated that the differences observed (order of magnitude of 2%) were smaller than the natural variations and could not be directly related to the wall properties only. This is by no means a final proof of the insignificance of the wall effects. It does strengthen the point of view, though, that the specimen wall, if considered a factor, is playing only a minor role in the stress-strain relationships.

CONCLUSIONS

IT HAS BEEN SHOWN that the true stress-strain curves of solid foods can have a variety of distinct shapes. These, although affected to a certain degree by the test conditions, are mainly determined by the deformation mechanism. The latter, however, is an objective characteristic of the material textural properties and is closely associated with its specific physical structure. This means that true stress-strain curves provide a better indication of the true nature of the material mechanical properties than apparent curves. The reason is that much of the characteristic information associated with the curves shape is lost in apparent coordinates. Probably with the exception of very few cases, all the apparent curves have a similar general shape (Fig. 1), at least at their initial stage. Meaningful evaluation of textural differences or the effects of textural modifications, under such circumstances becomes an extremely difficult task. If, however, we not only translate the force deformation curves into true stress-strain relationships, but also add relaxation data, then a much better insight into the material objective properties can be gained.

The relaxation data enables us to separate the total stress into two components, i.e. the residual stress and the decayed stress. The relative contribution of these parameters and its variation with the strain are objective textural characteristics by themselves. (Generally speaking, the higher the contribution of the residual stresses, the more "solid" the material is from a rheological point of view). They also help us reveal internal changes occurring during deformation which cannot always be detected from stress-strain relationships alone.

In comparison with the apparent stress-strain or the force-deformation curves therefore, all the mentioned true parameters are better representatives of the fundamental rheological properties of the tested material. This also includes the mode in which they are affected by the strain history. If sensory parameters could be associated with certain types of fundamental rheological properties (and obviously with other physical and chemical features as well), then the correct interpretation of the stress-strain relationships would become an essential step in their specification and eventually their control.

REFERENCES

Bourne, M.C. 1968. Textural profile of ripening pears. J. Food Sci. 33: 223.

Breene, W.M. 1975. Application of texture profile analysis to instrumental texture evaluation. J. Texture Stud. 6: 53.

Culioli, J. and Sherman, P. 1976. Evaluation of Gouda cheese firmness by compression tests. J. Texture Stud. 7: 353.

Dieter, G.E. 1976. "Mechanical Metalurgy," 2nd ed. McGraw-Hill Book Co., N.Y.

Marin, J. 1962.8 "Mechanical Behavior of Engineering Materials." Prentice Hall, Inc., New Jersey.

Mohsenin, N.N. 1970. "Physical Properties of Plant and Animal Materials," Vol. 1. Gordon Breach Science Publisher, N.Y.

Peleg, M. 1977a. Operational conditions and the stress-strain relationships of solid foods. J. Texture Stud. 8: In press.

Peleg, M. 1977b. The role of the specimen dimensions in uniaxial compression of food materials. J. Food Sci. 42: 643.

Peleg, M. and Calzada, J.F. 1976. Stress relaxation of deformed fruits and vegetables. J. Food Sci. 41: 1325.

Peleg, M., Gomez Brito, L. and Malevski, Y. 1976. Compressive failure patterns of some juicy fruits. J. Food Sci. 41: 1320.

Sherman, P. 1970. "Industrial Rheology." Academic Press, London and N.Y.

Szczesniak, A.S., Brandt, M.A. and Friedman, H.H. 1963. Development of standard rating scales for mechanical parameters of texture and correlation between the objective and sensory methods of texture evaluation. J. Food Sci. 28: 397.

Szczesniak, H.S., Humbaugh, P.R. and Block, H.W. 1970. Behavior of different foods in the standard shear-compression cell and the effect of sample weight on peak area and maximum force. J. Texture Stud. 1: 356.

Voisey, P. 1976. The influence of mechanical properties on the interpretation of results from food texture tests in research and quality control. First International Congress on Engineering and Food, Boston, USA.

Ms received 9/19/77; revised 12/10/77; accepted 12/14/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

Paper No. 2080 of the Massachusetts Agricultural Experiment Station, Univ. of Massachusetts, Amherst. Supported (in part) from Experiment Station Project No. 334.

The authors express their thanks to Mr. R.J. Grant for his graphical aid.

SOME MATHEMATICAL ASPECTS OF MASTICATION AND ITS SIMULATION BY MACHINES

M. PELEG

ABSTRACT

If the deformation process of a specimen satisfies the condition: $H(t) = g(H_o) \cdot f(t)$ (Eq. 1), where H(t) is the deformed specimen length at time (t), $g(H_o)$ is a function of the initial length (H_o) only and f(t) a function of time only, then the true strain $\epsilon(t)$ and the strain rate $\dot{\epsilon}(t)$ are: $\epsilon(t) = \ln f(t)$ (Eq. 2), and $\dot{\epsilon}(t) = [d \ln f(t)]/dt$ (Eq. 3). These imply that the stress-strain (or force-time) relationship of a homogeneous material is independent of the specimen length and is only determined by the rheological properties of the material and the selected function f(t). If the deformation histories produced in mastication satisfy the condition set by Eq (1) it would provide a partial mathematical explanation to why sensory textural-rheological properties are not affected by the specimen dimensions in contrast to instrumental parameters obtained by existing testing machines. The construction of machines that provide such straining histories is physically possible (they would not, however, be operated at a constant deformation rate), and therefore it is theoretically possible to obtain instrumental parameters that are dimension independent. The concept is demonstrated with some simplified rheological models and its possible application to food testing evaluated.

INTRODUCTION

MOST OF THE instrumental methods for textural evaluation of solid foods are essentially deformation tests (Voisey, 1976). There is, however, a great variety with respect to both the geometry of the instruments, the geometry of the specimens which are deformed, the nature and level of deformation and the deformation rate history.

It has long been recognized that solid foods are viscoelastic materials (Sherman, 1970) and therefore show a mechanical response that significantly depend on the deformation history. The latter implies that the specimen's dimensions can effect the stress-strain-time relationship. This dimensional dependency has been observed experimentally (Szczesniak et al., 1970; Culioli and Sherman, 1976; Peleg et al., 1976; Calzada and Peleg, 1978) in both the shear press and in uniaxial compression tests.

It should be mentioned that the actual character of the stress-time relationship depends on:

a. The rheological characteristic of the tested material (which in the case of foods may be quite complex).
b. The effective strain and strain rate history imposed on the material.
c. The geometry of testing system.
d. Instrumental sensitivity and artifacts (Voisey and Kloek, 1975; Voisey, 1976; Bourne, 1976; Culioli and Sherman, 1976).

The last aspects will not be discussed in this work and reference to machines implies theoretical instruments free from artifacts of any kind.

Author Peleg is with the Food & Agricultural Engineering Dept., University of Massachusetts, Amherst, MA 01003.

0022-1147/78/0004-1093$02.25/0
© 1978 Institute of Food Technologists

The second and third factors pose a crucial problem. The information existing machines (of any geometry) provide us is in the form of force-time relationship which depends by definition on the specimen dimensions. (The dependency cannot be eliminated by transformation of the force into stresses true or apparent). Textural properties, however, are inherent characteristics of the material and therefore ought to be independent of the specimen dimensions. (It would not make much sense to claim that a piece of food longer than another piece of the same food must also be softer although this would probably be the result of a compressive test). Nevertheless, in real mastication a small number of bites would, in many cases, be sufficient to identify the material textural nature in an unambiguous way and regardless of the piece dimensions. It is self-evident that in establishing the textural properties we are aided by a highly sophisticated physical and chemical sensing and feedback systems that cannot be imitated by any kind of existing testing machines. Some of the complexities involved in real mastication have been demonstrated by Ledley (1971) who discussed the geometry and the stress situation in the mouth. Though it seems hopeless to try to imitate all the physical aspects of mastication by machines it does make sense to inquire whether testing machines can be operated at conditions that would provide mechanical information that is both unambiguous and independent of the specimen dimensions. In this study some of the mathematical aspects of this problem are discussed and their possibilities demonstrated.

Deformation rate of existing machines

Most universal machines (regardless of their attachments) are operated at a constant deformation rate, i.e. constant crosshead speed–(V). The choice of the constant speed has been made primarily for the reason of construction simplicity (Voisey, 1976). From a rheological point of view, such machines do not provide any advantage because both the uniaxial strain and the strain rate that they produce are length dependent (Dieter, 1976). Interpretation of stress-strain relationships under such conditions is difficult especially when rheologically complex materials are tested at large deformation levels (Peleg, 1978; Calzada and Peleg, 1978).

The GF Texturometer (Friedman et al., 1963) is based on sinusoidal deformation. Because the relative orientation of the specimen and the plunger vary during the deformation process, the geometry is not as straightforward as in machines based on uniaxial movement (Bourne, 1976). The geometrical aspects of the GF texturometer motion have recently been analyzed by Brennan et al. (1975). Their study implies that the recorded force level does depend on the specimen dimensions as well as other geometrical characteristics of the instrument and its deforming device.

The latter is applicable to most other instruments or attachments to universal machines in which the geometry of the deformation may be even less defined. Under such circumstances evaluation of the experimental results by analytical mathematical methods would become an extremely difficult task.

Length independent strain history

From a mathematical point of view, a uniaxial compressive strain history that is length independent must satisfy the con-

dition that the functions that represent the strain and the strain rate do not have a length term as a variable.

Let us now assume a deformation process in which the length of a specimen at time t is H(t) and is defined as the product of two functions. One $g(H_o)$ is a function of the initial length H_o only and the other, f(t) is a function of time only, or:

$$H(t) = g(H_o) \cdot f(t) \tag{1}$$

In such a case the true strain $\epsilon(t)$ is:

$$\epsilon(t) = \int_{H_o}^{H} \frac{dH}{H} = \int_{o}^{t} \frac{1}{f(t)} \cdot \frac{df(t)}{dt} \cdot dt = \ln f(t) \tag{2}$$

and the true strain rate $\dot{\epsilon}(t)$ is:

$$\dot{\epsilon}(t) = \frac{d\epsilon(t)}{dt} = \frac{d \ln f(t)}{dt} \tag{3}$$

As can be seen from Eq (2) and (3), the term $g(H_o)$ is cancelled out and therefore the strain and the strain rate are only affected by the selected function f(t).

This implies that any uniaxial straining (deformation) process that can be described in the form of Eq (1) will also produce a strain history that is independent of the specimen length.

It is self evident that the common procedure of straining at a constant deformation rate or:

$$H(t) = H_o - Vt \tag{4}$$

(where V is a constant crosshead speed) cannot be transformed into the form of Eq (1) and both the strain and the strain rate are functions of the initial length.

It is also easy to show that a deformation process characterized by a constant strain rate history (Laun and Munstedt, 1976) can be written in the form of Eq (1).

If the true strain rate ($\dot{\epsilon}$):

$$\dot{\epsilon} = \frac{dH}{Hdt} = C \tag{5}$$

where C is a constant, then the true strain (ϵ):

$$\epsilon = Ct \tag{6}$$

and the length H(t):

$$H(t) = H_o \exp(-Ct) \tag{7}$$

in this case, therefore $g(H_o) = H_o$ and $f(t) = \exp(-Ct)$.

GENERAL APPLICATION

LET US ASSUME a specimen of a theoretical material that obeys a rheological state equation:

$$\sigma = \sigma(\epsilon, \dot{\epsilon}, t) \tag{8}$$

where σ is the stress.

If we subject such a specimen to a straining history which satisfies Eq (1), the stress which would develop would be independent of the specimen length by definition. In other words, the stress level at any time would only be a function of the rheological properties of the material and the function f(t). This implies that for any selected function f(t) the information in a form of stress-time relationship is a unique representation of the material rheological properties. It should be added, that this mathematical property of Eq (1) does not impose any restrictions on the functions $g(H_o)$ and f(t) themselves. The latter can be selected in a way that would satisfy requirements of a completely different nature as would be shown in the course of the discussion.

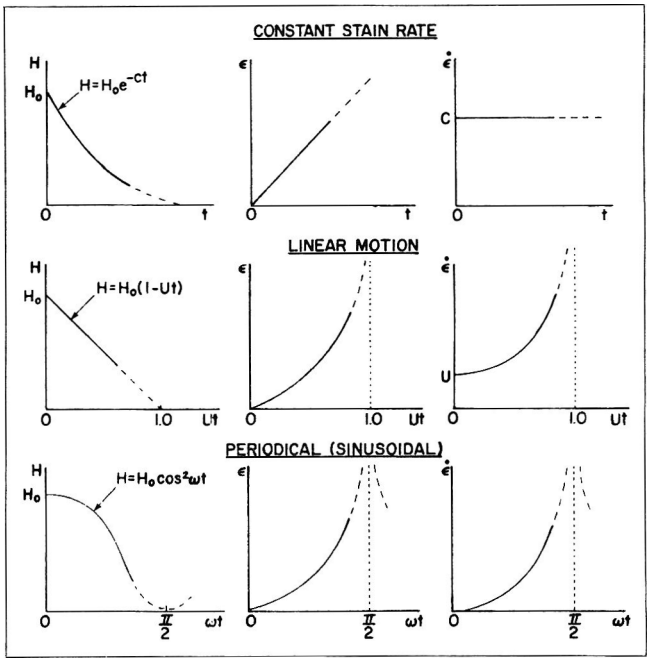


Fig. 1—Schematic representation of three straining procedures that provide length independent stress-strain (or stress-time) relationships: (H_o initial specimen length, H specimen length, ϵ true strain, $\dot{\epsilon}$ true strain rate).

Table 1—An example of three theoretical deformation procedures that proveide length ındependent strain histiry

Procedure	Specimen length vs time[a] $H(t) = g(H_o) \cdot f(t)$	True strain ϵ (t)	Strain Rate $\dot{\epsilon}$ (t)
Constant strain rate	$H(t) = H_o \exp(-Ct)$	Ct	C
Linear motion	$H(t) = H_o(1-Ut)$	$\ln(1-Ut)$	$\frac{U}{1-Ut}$
Periodical[b] (sinusoidal)	$H(t) = H_o\cos^2\omega t$	$2\ln\cos\omega t$	$2\omega tg\ \omega t$

[a] Eq (1) where $g(H_o) = H_o$.
[b] The second power in order to avoid negative values.

Simulation by rheological models

Let us describe three different straining procedures that satisfy Eq (1). The first one, the already mentioned constant strain rate history, the second a linear motion that probably can be achieved at relative ease from technical point of view and the third a periodical function that may probably represent repeated bites. The three procedures are represented schematically in Figure 1. Their mathematical definitions are given in Table 1. It is clear that all three procedures cannot be considered as a perfect simulation of the deformation history in a real mastication process. The reason from a purely mathematical point of view is that these equations imply unrealistic conditions around H(t) = 0. In reality there must be a deceleration mechanism to prevent such conditions developing in the mouth. Let us therefore consider these procedures only at the range at which the extreme situations are excluded. Now we can simulate the response of any rheological model simply by the substitution of Eq (8) by its characteristic equation. For the demonstration we shall assume three kinds of rheological behavior patterns. The first an ideal elasticity, the second a nonlinear elasticity, and the third a Maxwellian behavior. The stress-time relationship of these three models (or elements) in the different straining procedures is shown in Table 2.

Table 2—Stress-time relationships of three rheological models (elements) deformed by three length independent straining procedures[a]

Model	Rheological equation	Stress-time relationship: Constrant strain rate	Stress-time relationship: Linear motion	Stress-time relationship: Periodical (sinusoidal)
Linear elastic	$\sigma = E\epsilon$	$\sigma = Ect$	$\sigma = E\ln(1-Ut)$	$\sigma = 2E\ln \cos \omega t$
Non-linear elastic	$\sigma = K\epsilon^n$	$\sigma = KC^n t^n$	$\sigma = K[\ln(1-Ut)]^n$	$\sigma = 2^n K(\ln\cos \omega t)^n$
Maxwell element	$K\dot{\epsilon} = \dot{\sigma} + a\sigma$	$\sigma = \frac{KC}{a}[1-\exp(-at)]$	$\sigma = K\exp\left[\frac{a}{U}(1-Ut)\right]\cdot\left[\ln(1-Ut)+at-\sum_{2}^{\infty}\frac{(-a)^n[1-(Ut)^n]}{U^n \cdot n \cdot n}\right]$	$\sigma = 2K\omega \exp(-at)\cdot \int_0^t \exp(-at)\,tg\omega t\, dt$

[a] See details in Table 1.

The latter shows that regardless of the selected procedure, the stress-time relationship can be quite complex. In the examples presented in the table the simplified models were characterized by a very small number of constants (a, E, K and n). If more realistic models are applied, the stress response would become even more elaborate from a mathematical point of view. Similarly, application of realistic deformation histories, (that may include various stages with alternating deformation rates and directions) would also produce responses that would need an elaborate mathematical representation. In both cases, however, and as long as Eq (1) is satisfied, the relationship between the stress level and the real time would be mathematically unambiguous and characteristic of the models rheological properties only.

Application to instrumental methods for texture evaluation

As previously mentioned, satisfying Eq (1) is sufficient to provide length independent stress-strain relationships. The latter may therefore provide a possible partial explanation to why, in mastication, the sensory response in real time is similarly uneffected by the specimen dimensions. Such an explanation, however, ought to be verified from a mathematical point of view i.e. that the condition set by Eq (1) is not only sufficient but necessary too, and from a mechanistic point of view, i.e. that the pertinent information obtained from mastication is in fact (or at least partly) in the form of force-real time relationships. Determination of the significance of these aspects is crucial to proper understanding and evaluation of the mastication process and their importance should not be overlooked.

However, even if these questions concerning mastication cannot satisfactorily be answered at the present time, useful application of the mathematical concept in testing can be achieved nevertheless. Producing deformation histories that satisfy Eq (1) is by no means a technical impossibility from a mechanical point of view (Laun and Munstedt, 1976). The technology of building programmable testing machines is also developed and rapidly improving. It is quite probable therefore that future standard universal testing instruments would enable the selection of a variety of deformation histories rather than the variety of constant speeds which are common in today's machines. (When such testing machines are built for food testing, their main technical problem would most probably be the sophistication needed for accurately deforming small specimens at high speeds.) When, however, such instruments are available they would facilitate consistent evaluation of rheological properties of solid food. The parameters obtained from such analysis would be independent of the specimen length and therefore their correlation with textural properties would become more meaningful than in existing methods.

REFERENCES

Bourne, M.C. 1976. Interpretation of force curves from instrumental texture measurements. In "Rheology and Texture in Food Quality," Ed. deMan, J.M., Voisey, P.W., Rasper, V.F. and Stanley, D.W. Avi Publishing Co., Inc., Westport, CT.

Brennan, J.G., Jowitt, R. and Williams, A. 1975. An analysis of the action of the General Foods texturometer. J. Texture Studies 6: 83.

Calzada, J.F. and Peleg, M. 1978. Mechanical interpretation of compressive stress-strain relationships of solid foods. J. Food Sci. 43: (In press).

Culioli, J. and Sherman, P. 1976. Evaluation of Gouda cheese firmness by compression tests. J. Texture Stuides 7: 353.

Dieter, G.E. 1976. "Mechanical Metallurgy," (2nd ed). McGraw-Hill Book Co., N.Y.

Friedman, H.H., Whitney, J.E. and Szczesniak, A.S. 1963. The texturometer—A new instrument for objective texture measurements. J. Food Sci. 28: 390.

Ledley, R.S. 1971. Dental forces and mastication. J. Texture Studies 2: 3.

Laun, H.M. and Munstedt, 1976. Comparison of the elongation behavior of polyethylene melt at constant stress and constant strain rate. Rheol. Acta 15: 517.

Peleg, M. 1978. Operational conditions and the stress-strain relationship of solid foods. Theoretical evaluation. J. Texture Studies 8: 291.

Peleg, M., Gomez Brito, L. and Malevski, Y. 1976. Compressive failure patterns of some juicy fruits. J. Food Sci. 41: 1320.

Sherman, P. 1970. "Industrial Rheology." Academic Press, London and New York.

Szczesniak, A.S., Humbaugh, P.R. and Block, H.W. 1970. Behavior of different foods in the standard shear-compression cell and the effect of the sample weight on peak area and maximum force. J. Texture Studies 1: 356.

Voisey, P.W. 1976. Instrumental measurement of food texture. In "Rheology and Texture in Food Quality," Ed. deMan, J.M., Voisey, P.W., Rasper, V.F. and Stanley, D.W. Avi Publishing Co., Inc., Westport, CT.

Voisey, P.W. and Kloek, M. 1975. Instron recorder pen response. J. Texture Studies 6: 379.

Ms received 11/4/77; revised 1/31/78; accepted 2/8/78.

Paper No. 2185 of the Massachusetts Agricultural Experiment Station, University of Massachusetts, Amherst. This research has been supported in part by Experiment Station Project No. 417.

Submitted for presentation at the 38th Annual Meeting of IFT, Dallas, Texas.

SHORT-CUT METHOD FOR THE CALCULATION OF STERILIZATION CONDITIONS YIELDING OPTIMUM QUALITY RETENTION FOR CONDUCTION-TYPE HEATING OF PACKAGED FOODS

H. A. C. THIJSSEN, P. J. A. M. KERKHOF and A. A. A. LIEFKENS

ABSTRACT

Based on analytical equations for the temperature distribution history in a container, the relation between the reductions of the concentrations of heat labile food components, including microorganisms, nutrients and sensory factors, and the kinetic parameters of the reactions causing these reductions, has been calculated numerically. For a constant temperature of the heating medium with time it is concluded that the loss of quality is almost minimal at one Fourier value of the heating time. This optimal Fourier value is a function of only the Biot number and the relationship between initial product temperature, cooling water temperature and retort temperature. At an infinite value of Biot and a cooling water temperature equal to the initial product temperature the optimum Fourier value amounts to 0.5. On the basis of this conclusion a short-cut calculation method is developed. The method is valid for Biot numbers larger than 10, initial homogeneous product temperatures equal to or larger than the temperature of the cooling medium and for the main container geometries including spheres, cylinders and rectangular bodies. The method does not require tedious interpolations of tables. The calculation of the optimum process conditions to obtain the desired sterility and the calculation of the retention of the quality factor of interest require only a few minutes on a pocket calculator. For routine calculations the procedure can conveniently be programmed.

INTRODUCTION

THE MICROBIAL SAFETY of food is the prime criterion for determining the thermal process conditions for packed foods. The required lethal effect, however, can be obtained over a wide range of combinations of sterilization times and retort temperatures (Teixeira et al., 1969). With a decrease in sterilization time the retort temperature has to be increased and vice versa. The HTST, or the even more extreme UHT method, which is based on the fact that the effect of temperature on destruction rate of microorganisms is much higher than its effect on chemical quality degrading reactions, cannot be used for in-package sterilization. Very high temperatures will have caused severe thermal degradation of the food near the container wall, long before the food at the center of the container has risen in temperature. A relatively low retort temperature, however, will also cause great quality losses by adverse chemical reactions because of the long time it will take to obtain sterility. Consequently there is an optimum in the temperature-time relationship.

Various calculation procedures have been developed to aid in the prediction of nutrient retention in thermal processing (Ball and Olson, 1957; Hayakawa, 1969; Teixeira et al., 1969; Jen et al., 1971). The method of Ball and Olson requires major computational efforts. The finite difference method of Teixeira requires computer calculations for each process condition. The dimensionless group method of Hayakawa also requires computational efforts and the interpolation of values for five dimensionless groups. The calculation procedure of Jen is an extension of the method of Stumbo (1973). Although it may be readily computer programmed, all calculations involved in its use can be carried out manually. All the same, the method is still rather time-consuming and requires interpolations of the Stumbo tables. The consequence is that none of the methods mentioned lends themselves readily to application in the daily practice of the canning industry.

It is believed that the procedure to be described obviates to a great extent the disadvantages of the methods referred to. The calculations can be carried out manually or very quickly on a calculator and can be used without any interpolations of tabulated data. They produce the process conditions that give the required degree of sterilization at maximum retention of the quality for the various geometries and sizes of the containers, including cans and pouches.

THEORY

THE DESTRUCTION RATE of microorganisms is first order in the concentration of living microorganisms and can be described by the rate equation

$$\frac{d \ln C_i}{dt} = - k_i \qquad (1)$$

in which k_i is the reaction rate coefficient, C_i is the concentration and t the time. Although at the onset of a thermal treatment at constant product temperature the value of k_i may be variable, it is generally taken constant. The subscript i refers to microorganisms of the same strain and age. For chemical reactions in foods, including quality degrading reactions such as vitamin destruction and discoloration reactions, a first order dependence is also assumed in general. After integration Eq (1) becomes

$$\frac{C_{i,t}}{C_{i,o}} = \exp \left(- \int_0^t k_i \, dt\right) \qquad (2)$$

The temperature dependence of k can be represented by an exponential function (Fennema, 1975). For the inactivation of enzymes and microorganisms Eq (3) holds in general

$$k_i = k_{i,o} \exp (a_i T) \qquad (3)$$

in which T is the absolute temperature in degrees Kelvin, the constant a_i represents the dependence of k on temperature and $k_{i,o}$ is the value of k_i at T = O and slightly modified

$$k_i = k_{i,T_r} \exp \left[a_i (T - T_r)\right] \qquad (4)$$

in which k_{i,T_r} is the value of k_i at the reference temperature T_r.

Microbiologists use the value z to indicate the temperature dependence and the decimal reduction time D instead of k. D is the time required to reduce the concentration of the reactant, or the number of spores, by one order of magnitude; z is the increase in temperature expressed in degrees Fahrenheit that is required to reduce the value of D by one order of magnitude. It can be derived from the definitions that:

$$D_i = \frac{2.303}{k_i} \qquad (5)$$

and

$$z_i = \frac{4.145}{a_i} \qquad (6)$$

Authors Thijssen, Kerkhof and Liefkens are with the Dept. of Chemical Engineering, Eindhoven University of Technology, The Netherlands.

0022-1147/78/0004-1096$02.25/0
© 1978 Institute of Food Technologists

For *chemical* reactions the temperature effect is generally described by the Arrhenius equation:

$$k_i = A_i \exp(-E_i/RT) \tag{7}$$

in which E is the activation energy, R the gas-law constant and A is the frequency factor. For the relationship between a_i and E it follows,

$$E_i = a_i \cdot T \cdot T_r \cdot R \tag{8}$$

If the temperature range of relevance to the process is not too large, the difference between Eq (3) and (7) is small. Below Eq (3) and its variant (4) will be used for both the destruction of microorganisms and quality degrading reactions.

The reduction of the concentration of heat-labile components averaged over the total volume V of the pack after heating time t becomes

$$\frac{C_{i,t}}{C_{i,o}} = \int_o^V \exp\left[-\int_o^t k_i\, dt\right] dV \tag{9}$$

and after substitution of k_i by k_{i,T_r}

$$\frac{C_{i,t}}{C_{i,o}} = \int_o^V \exp\left[-k_{i,T_r}\int_o^t \exp\left[(a_i(T-T_r)\right] dt\right] dV \tag{10}$$

which becomes in dimensionless groups

$$\frac{C_{i,Fo}}{C_{i,o}} = \int_o^V \exp\left[-N_{i,1}\int_o^{Fo} \exp(N_{i,2}\phi)\, d\,Fo\right] dV \tag{11}$$

in which

$$Fo \equiv \frac{D't}{R_s^2} \tag{12}$$

$$N_{i,1} \equiv \frac{R_s^2 k_{i,T_r}}{D'} \tag{13}$$

$$N_{i,2} \equiv a_i(T_h - T_r) \tag{14}$$

$$\phi \equiv \frac{T - T_r}{T_h - T_r} \tag{15}$$

and $\bar{C}_{i,Fo}$ = mean concentration of component i (microorganism or nutrient) after a dimensionless time Fo; D′ = effective thermal diffusivity of the food in the pack (in case of pure conductive heating D′ = $\lambda/\rho c_p$); R_s = the characteristic radius of the pack, see Eq (19a)–(21).

As the temperature-time relationship at any place in the pack is also a function of the initial temperature T_o of the product, the retort temperature T_h and the cooling water temperature T_c, it will be obvious that the reduction of the heat-labile constituents after heating and subsequent cooling, is a function of four key variables.

$$\ln\frac{C_{i,o}}{\bar{C}_i} = f(Foh, N_{i,1}, N_{i,2}, \beta) \tag{16}$$

in which

$$N_{i,1} = \frac{R_s^2 \cdot k_{T_c}}{D'} \tag{13a}$$

$$N_{i,2} = a_i(T_h - T_c) \tag{14a}$$

$$Foh = \frac{D't_h}{R_s^2} \tag{12a}$$

and

$$\beta = \frac{T_o - T_c}{T_h - T_c} \tag{17}$$

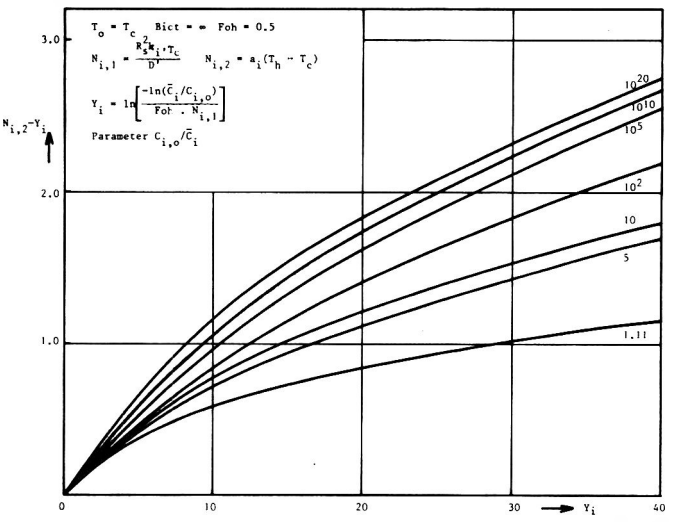


Fig. 1—Numerically calculated relationship between $N_{i,2}$ and Y_i with the reduction of the heat labile component as parameter. Bi = ∞, β = 0 and Foh = 0.5.

where t_h is the time elapsed between the onset of the heating of the container and the onset of cooling. For convenience sake the cooling water temperature is taken as reference temperature, thus $T_r = T_c$.

For the main geometries, including spheres, cylinders and retangular bodies, the temperature profiles in the bodies as a function of time can be calculated analytically. Solutions for constant temperatures of the heating medium and constant initial temperature are given by Carslaw and Jaeger (1959) and Luikov (1968). For the main geometries these analytical solutions are used to solve Eq (11) numerically. In the program, the container is subdivided into 17 elements in the axial direction and 18 in the radial direction. The time steps are variable with a minimum value of 0.002 in Fourier. The time steps are chosen so that the temperature change per step never exceeds a dimensionless temperature change in ϕ of 0.005. The computing time required to perform the solution of Eq (11) for a homogeneous initial temperature of the product T_o, a constant retort temperature T_h, a dimensionless heating time Foh and a subsequent cooling of the pack with cooling water of temperature T_c at Bi = ∞ (outside temperature of the pack equal to the retort or cooling water temperature) was about 200 sec on a Burroughs B7700 digital computer. The results were correlated with Foh, $N_{i,1}$ $N_{i,2}$ and β.

COMPUTER RESULTS

MOST OF THE CALCULATIONS were performed for a standard cylinder (cylinder of equal diameter and height) at $\beta = 0$. For the standard cylinder the characteristic radius R_s is equal to the radius of the can. In the graphical presentation of the results the dimensionless parameters Foh and $N_{i,1}$ have been grouped together with the reduction of the heat labile component i in a new quantity Y:

$$Y_i = \ln\left(\frac{-\ln(\bar{C}_i/C_{i,o})}{Foh \cdot N_{i,1}}\right) \tag{18}$$

For the standard cylinder, Bi = ∞, $\beta = 0$ and Foh = 0,5. Figure 1 shows the numerically calculated relationship between $N_{i,2} - Y_i$ and Y_i with the reduction $C_{i,o}/\bar{C}_i$ as parameter. In the calculations the cooling water temperature T_c has again been taken as reference temperature, thus $T_r = T_c$.

The a_i values have been varied between 0.05 and 0.3 corresponding to z values of between 12 and 85 and $k_{i,20°C}$ values of between 7×10^{-4} and 5×10^{-18}. Consequently the D_{250} values have been varied between 2.2×10^{-1} and 2.3×10^{5} sec.

Figure 2 gives for Bi = ∞, and $\beta = 0$ the calculated relationship between

$$\frac{(N_{i,2} - Y_i)_{Foh}}{(N_{i,2} - Y_i)_{Foh = 0.5}} \quad \text{and Foh}$$

with the reduction as parameter. With the aid of Figures 1 and 2 the relationship between $N_{i,2} - Y$ and Y can be calculated for any Foh number between 0.3 and 1.0. This range of Fourier number covers the whole field of practical sterilization conditions.

Effect of the geometry of the pack

From numerical solutions of the function (16) for other geometries it has been concluded that for a given geometry the reduction $C_{i,o}/\bar{C}_i$ is equal to that obtained in a standard cylinder after the same heating time t_h if the value R_s of the standard cylinder is taken so that the mean of the mass averaged temperature and the center temperature in that standard cylinder after heating time t_h is equal to the same mean in the container of different geometry and size after the same heating time. R_s is called the characteristic radius of a container.

Consequently the results for the standard cylinder are valid for any geometry if the characteristic radius R_s of the pack is used in Figures 1 and 2. The relationship between R_s and the pack dimensions for the main geometries have been calculated analytically. The results are given by Eq (19a), (19b), (20) and (21):

sphere with diameter d

$$\frac{1}{R_s^2} = \frac{6.16}{d^2} \quad \text{for Foh} = 0.4 \tag{19a}$$

$$\frac{1}{R_s^2} = \frac{5.84}{d^2} \quad \text{for Foh} = 0.5 \tag{19b}$$

cylinder with diameter d and length l

$$\frac{1}{R_s^2} = \frac{2.80}{d^2} + \frac{1.20}{l^2} \tag{20}$$

rectangular body of dimensions b, h and l

$$\frac{1}{R_s^2} = \frac{1.20}{b^2} + \frac{1.20}{h^2} + \frac{1.20}{l^2} \tag{21}$$

Effect of β

It is obvious that when $T_o > T_c$ and retort and cooling water temperatures are kept constant, the value of Foh has to be reduced with increasing T_o in order to keep the reduction $C_{i,o}/\bar{C}_i$ constant. It has been derived from the computer calculations that Figures 1 and 2 can also be used for values of $\beta > 0$. From Figures 1 and 2 the relationship between $C_{i,o}/\bar{C}_i$, $N_{i,1}$ and $N_{i,2}$ for a given value of Foh is first calculated for $\beta = 0$ ($T_o = T_c$). Subsequently the value of Foh is converted into a new value corresponding to the value for $\beta > 0$. A constant spore reduction can also be obtained by lowering the retort temperature at a constant Foh number. However, the HTST principle is taken better advantage of when a lower Foh is taken at constant retort temperature.

Table 1—Value of Foh at $\beta \geq 0$ and at Bi = ∞ required for constant values of $N_{i,1}$, $N_{i,2}$ and $C_{i,o}/\bar{C}_i$

β	Foh for $\beta \geq 0$
0	0.50
0.1	0.49 + 0.01 Fo*
0.2	0.47 + 0.02 Fo*
0.3	0.45 + 0.04 Fo*
0.4	0.43 + 0.06 Fo*
0.5	0.41 + 0.09 Fo*
0.6	0.38 + 0.12 Fo*
0.7	0.35 + 0.15 Fo*
0.8	0.29 + 0.20 Fo*
0.9	0.24 + 0.22 Fo*
1.0	Fo* − 0.06/$N_{i,2}$

The value of Foh corresponding to $\beta \geq 0$ at Bi = ∞ can be obtained from Table 1. In the Table Fo* is defined by

$$Fo^* = \frac{-\ln (\bar{C}_i/C_{i,o})}{N_{i,1} \exp N_{i,2}} \quad \text{at } \beta = 0, \text{ Foh} = 0.5$$

Fo* is the dimensionless heating time required to obtain the reduction $C_{i,o}/\bar{C}_i$ for $N_{i,1}$ at a constant temperature of the product. The value Foh = 0.5 is chosen because, as will be shown later, this results in maximum quality retention.

Effect of Biot

The Biot value expresses the ratio of the resistance for heat transfer of the food in the container and the total resistance for heat transfer of the heating phase outside the container (continuous phase) and the container wall.

$$Bi = \frac{\alpha R_s}{\lambda} \tag{22}$$

in which α is the heat transfer coefficient of the continuous phase and the container wall, λ is the effective thermal conductivity of the food in the container and R_s again the characteristic radius of the container.

It has been concluded from computer calculations that for values smaller than infinity but larger than 10 at constant values of T_o, T_h and β the logarithm of the reduction at Bi ≤ ∞ is a factor [(2/Bi) + 1] larger than the logarithm of the reduction corresponding with Bi = ∞ if the heating time corresponding to a Biot number is chosen so that for the different Bi numbers the mean of the mean product temperature and the center temperature remains constant. The mean of the center temperature and the mean product temperature is constant if at constant values of T_o, T_h and β Fourier satisfies the following condition:

$$Foh_{Bi \leq \infty} = \left(\frac{2}{Bi} + 1\right) Foh_{Bi = \infty} \tag{23}$$

Eq (23) can be analytically derived. Because the basis of the present calculation procedure is the reduction at $\beta = 0$ and Bi = ∞ the reduction corresponding to Bi = ∞ has to be calculated from the required reduction at the actual value of Bi according to Eq (24):

$$\ln (\bar{C}_i/C_{i,o})_{Bi = \infty} = \left(\frac{1}{\frac{2}{Bi} + 1}\right) \ln (\bar{C}_i/C_{i,o})_{Bi \leq \infty} \tag{24}$$

QUALITY OPTIMIZATION

WITH FIGURES 1 AND 2 and Eq (19a) through (21) the process conditions can be calculated that are required to obtain a desired reduction of the number of microorganisms for any combination of a_i and k_{i,T_c} (or corresponding z and D_{250} values). This can be done for any Foh value between 0.3 and

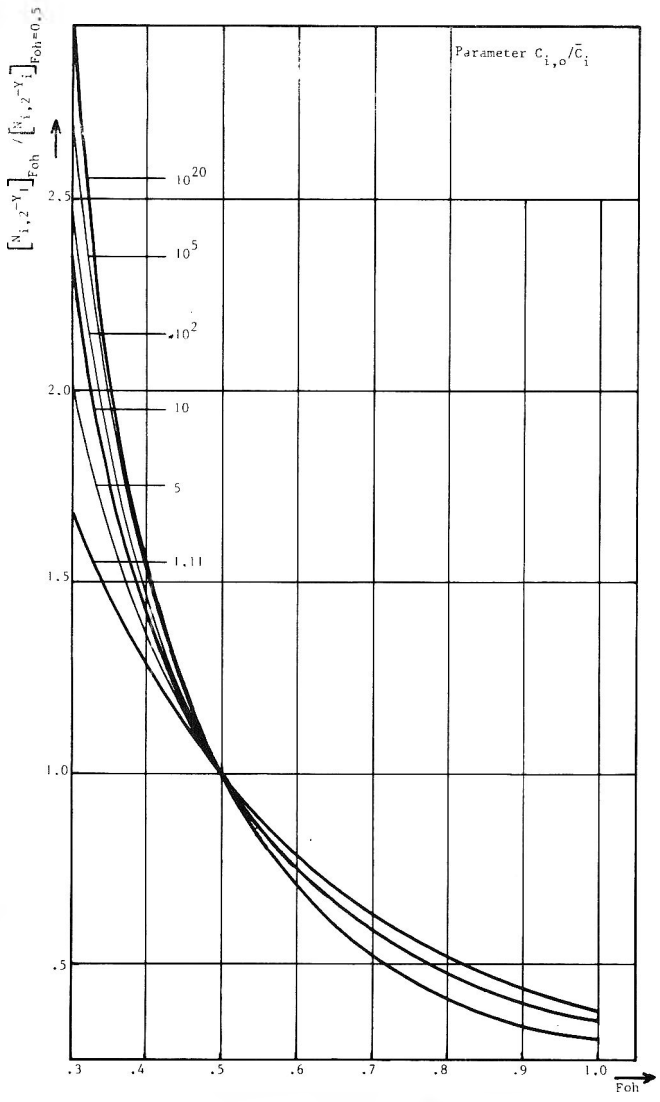


Fig. 2—Effect of Foh on the ratio of $(N_{i,2}-Y_i)$ at any Foh value between 0.3 and 1.0 and $(N_{i,2}-Y_i)$ at Foh = 0.5 for Bio = ∞ and β = 0.

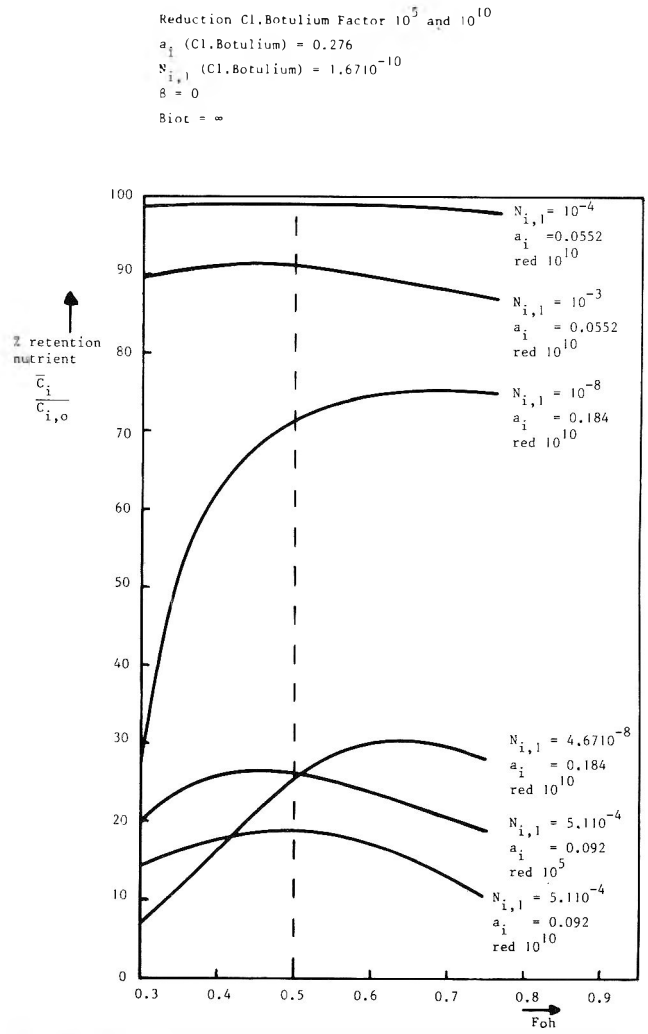


Fig. 3—Effect of Foh number on quality retention for various values of $N_{i,1}$ and a_i of the quality factor and a reduction of the spore concentration of C. botulinum of 10^5 and 10^{10}.

1.0. With the calculated process conditions the loss of nutrient or of any other quality factor can subsequently be calculated, provided that the a_i and k_{i,T_c} values of the nutrient are known. This has been done with Foh as variable for one N_1 and a_i value for the microorganisms (Cl. botulinum) and a range of $N_{i,1}$ and a_i, values for the nutrients. The calculated nutrient retentions (100 × $\bar{C}_i/C_{i,o}$) are presented in Figure 3 with the value of the reduction of the microorganisms and the $N_{i,1}$ and a_i values for the nutrients as parameters. The results are in accordance with the nutrient retentions calculated by Teixeira et al. (1969). The optimum in nutrient retention shifts to higher Foh values and consequently longer sterilization times at correspondingly lower retort temperatures with increasing values of the ratio a_i of the nutrient and a_i of the microorganism.

a_i (nutrient) / a_i (microorganism)	Foh (optimum)
0.20	0.425
0.33	0.450
0.67	0.625

An increase in the reduction of the number of microorganisms or an increase in $N_{i,1}$ and consequently of k_{i,T_c} of the nutrient only results in a decrease of the optimum retention but does not shift the place of the optimum with respect to Foh.

If for β = 0 and Bi = ∞ the Foh value should be fixed at 0.5, the deviation from the optimally possible retention is still very small. From Figure 3 it can be seen that at Foh = 0.5 the maximum deviation from the optimal nutrient (or more in general quality) retention is smaller than 5% absolute. Consequently at β = 0 and Bi = ∞, a heating time corresponding to Foh = 0.5 results in almost optimum quality retention over a wide range of a_i and thus also of E or z values of the heat labile components. This conclusion makes it possible to rapidly calculate the sterilization conditions that yield optimum quality retention at the required reduction of the concentration of microorganisms.

CALCULATION PROCEDURE

THE SUCCESSIVE STEPS will be given of the calculation of the process conditions that result in the required reduction of the number of microorganisms at minimum quality loss and of the calculation of the accompanying quality loss.

Calculation of optimum sterilization conditions

Step 1: Calculate both the value of k_{i,T_c} at cooling water

Table 2—Comparison of methods for predicting thiamine retention in 211 X 300 cans

Product	Temperature: Process time (min)	Initial (°C)	Retort (°C)	Cooling (°C)	z (°F)	$D_{250°F}$ min	Thiamine retention %: Ball & Olson[a]	Hayakawa[a]	Jen[b]	Teixeira[b]	Present short-cut	Analysis[a]
Carrot puree	60	28.89	121.11	11.66	46.7	160	53.9	59.0	–	–	63.5	66.8
Pea puree	60	13.06	121.11	12.22	46.7	160	55.2	59.5	61.4	61.0	64.6	65.6
Pork puree	40	20.55	126.67	11.39	55.3	140.4	49.2	52.2	–	–	57.3	53.0

[a] Results reported by Hayakawa (1969)
[b] Results reported by Y. Jen (1971)

temperature T_c and the value of a_i from the values z and D_{250} with Eq (4), (5) and (6).

Step 2: Calculate the characteristic radius R_s for the geometry of the pack and the pack dimensions with the aid of Eq (19b), (20) or (21).

Step 3: Calculate the value of $N_{i,1}$ for the microorganisms with definition (13a). The value of the thermal diffusion coefficient D′ can be calculated from the food composition or be determined experimentally.

Step 4: Calculate the Bi number from Eq (22). If Bi $<<\infty$ calculate the reduction of the number of microorganisms at Bi = ∞ from Eq (24).

Step 5: From Figure 1 read the corresponding value of $N_{i,2}$ for the calculated reduction of the number of microorganisms obtained from Step 4 at $\beta = 0$. From $N_{i,2}$ the value of $T_h - T_c$ and consequently the required temperature of the heating medium can be obtained.

Step 6: Calculate the value of β with Eq (17). If $\beta > 0$, calculate the Foh value corresponding to that value of β using Table 1.

Step 7: If Bi$<<\infty$ calculate the value of Foh corresponding to Bi $<<\infty$ from Foh obtained from step 6 with Eq (23).

Step 8: Calculate the heating time t_h between onset of heating and onset of cooling from the Foh obtained in Step 7 with Eq (12a). In these calculations it is assumed that at t = 0 the temperature of the heating medium is instantly raised to the desired retort temperature T_h. If it takes some time to attain the retort temperature, t_h should be calculated according to Stumbo (1973) as follows:

$$t_h = 0.4\, t_1 + t_2$$

in which t_1 is the time required to bring the heating medium to temperature T_h and t_2 is the time the retort remains at temperature T_h.

Calculation procedure for nutrient retention

Step 9: Calculate the values of a_i and k_{i,T_c} from the kinetic parameters such as the z and D value of the nutrient or any other quality factor.

Step 10: Calculate $N_{i,1}$ for the nutrient and calculate the value of $N_{i,2}$ for the nutrient from the value of $T_h - T_c$ obtained in Step 5. Calculate the reduction of nutrient with these values in first approximation by assuming that the nutrient reduction is equal to exp (Foh·$N_{i,1}$·exp $N_{i,2}$)$^-$ Subsequently this approximated value of the reduction is used to calculate the abscissa value in Figure 1. For that abscissa value the accurate value of the reduction is obtained from the corresponding ordinate value, again using the first approximation of the reduction as parameter.

The whole calculation requires about 5 min on a pocket calculator. For routine calculations the procedure can conveniently be programmed.

Table 3—Comparison of methods for calculating the reduction of microorganisms in standard cans

No.	z (°F)	$D_{250°F}$ (sec)	Reduction of microorganism, $C_{i,o}/\bar{C}_i$: Stumbo	Short-cut method
1	10	600	1×10^2	1×10^2
2	10	300	2×10^3	2×10^3
3	20	300	4×10^6	4×10^6
4	20	100	1×10^{17}	1×10^{17}

COMPARISON OF CALCULATED RESULTS OF THE SHORT-CUT METHOD WITH OTHER CALCULATION METHODS AND EXPERIMENTAL DATA

THE RESULTS of the short-cut method for nutrient retention are compared with the calculated results of other methods, including those of Ball and Olson (1957), Hayakawa (1969), Jen et al. (1971) and Teixeira et al. (1969) and also with experimental data. For the reduction of the number of microorganisms the method is compared with the calculation procedure of Stumbo (1973).

Nutrient retention

Some calculations were made to predict thiamine retention in 211 X 300 cans. For carrot puree and pea puree a D′-value of 0.001573 cm^2/sec (Jen et al., 1971) and for pork puree the D′-value of beef puree being 0.00243 cm^2/sec (Mulley et al., 1975) was used. Table 2 shows the calculated results of the present method and of the other authors and some experimentally obtained data. The short-cut method appears to be in good agreement with the experimental data.

Reduction of microorganisms

A comparison of the results of the Stumbo-method and the present method is made for the prediction of microorganism retention in standard cans. Table 3 shows the results for Foh = 0.5, initial temperature = 20°C, heating temperature = 121, 11°C, $\beta = 0$ and $\frac{R_s^2}{D'} = 2.10^4$ sec. It can be concluded that the present method leads to results which are identical to the results of other more time consuming methods.

EXPERIMENTAL DETERMINATION OF D′, a_i and k_{i,T_c}

IF THE THERMAL DIFFUSIVITY D′ of the product is not known or some convective heating is to be expected and/or D_{250} and z values (a_i and k_{i,T_c} values) of the microorganisms to be destroyed are not available, two simple laboratory experiments are sufficient to obtain these data. To this end a laboratory cylindrical standard can (height = diameter) with a thermocouple in the can center is used. The initial concentration of spores $C_{i,o}$ of the relevant microorganisms (e.g. C. botulinum) is measured in the food under consideration.

Alternatively the food is first sterilized and subsequently inoculated with a known concentration of spores. The can is heated (Bi = ∞) with steam of a constant relatively low temperature ($T_{h,1}$) say 95°C for a time corresponding to Foh = 0.5. This value is reached when:

$$\frac{T_{Center} - T_o}{T_{h,1} - T_o} = 0.967$$

T_{Center} = temperature of can center.

From the heating time t_h required to reach the value 0.967 prior to cooling and the characteristic radius R_s of the can the thermal diffusifity D' can be calculated.

After cooling the residual spore concentration is determined. The sterilization experiment is repeated at a heating temperature $T_{h,2}$, which is 5°C higher and for Foh = 0.5. Again the spore reduction is measured. Thus the spore reductions will be known for Foh = 0.5 at $T_{h,1}$ and $T_{h,2}$. From these values a_i is calculated in first approximation by assuming that

$$a_i = \frac{\ln \ln (\text{reduction})_{T_{h,2}} - \ln \ln (\text{reduction})_{T_{h,1}}}{T_{h,2} - T_{h,1}}$$

which equation becomes valid for large values of Foh. With this approximate value of a_i the approximate values of Y_i belonging to $T_{h,1}$ and $T_{h,2}$ are calculated by assuming that:

$$Y_{i,1} = a_i (T_{h,1} - T_c) \qquad \text{at } T_{h,1}$$

and

$$Y_{1,2} = a_i (T_{h,2} - T_c) \qquad \text{at } T_{h,2}$$

With these approximated values we obtain from Figure 1 the accurate value of

$(N_{i,2} - Y_i)$, corresponding to the reduction at $T_{h,1}$

and

$(N_{i,2} - Y_i)$, corresponding to the reduction at $T_{h,2}$

Thus we have obtained two equations from which we can calculate the two unknowns a_i and $N_{i,1}$. From $N_{i,1}$ the value of k_{i,T_c} is calculated.

The advantage of the procedure is that it provides the desired information about the values of D', a_i and k_{i,T_c} under sterilization conditions in the actual food. Because of the moderate temperature used, the spore reduction in the heated foods remains so small that the spore concentration still can be measured.

NOMENCLATURE

a_i	the dependence of k_i on the temperature	$°K^{-1}$
Bi	Biot-number	–
$C_{i,o}$	concentration at t = 0	kg^{-1} or –
$\bar{C}_{i,t}$	mass average concentration at t	kg^{-1} or –
$\bar{C}_{i,Fo}$	mass average concentration at Fo	kg^{-1} or –
D'	thermal diffusivity of the food in the pack	m^2/sec
D_i	decimal reduction time	sec
d	diameter	m
E_i	activation energy	kJ/kmol
Fo	Fourier number at t	–
Fo*	dimensionless heating time required to obtain the reduction $C_{i,o}/\bar{C}_{i,Fo}$ for $N_{i,1}$ at a constant temperature of the food	–
Foh	Fourier number at t_h	–
h	height	m
k_i	reaction rate coefficient	sec^{-1}
$k_{i,Tr}$	reaction rate coefficient at temperature T_r	sec^{-1}
l	length	m
$N_{i,1}$	dimensionless group	–
$N_{i,2}$	dimensionless group	–
R	gas-low constant	kJ/kmol °K
R_s	characteristic radius	m
t	time	sec
t_h	heating time	sec
T	temperature	°K
T_c	temperature of the cooling medium	°C
T_{center}	temperature in the center of the standard cylinder	°C
T_h	temperature of the heating medium	°C
T_o	initial temperature of the food	°C
T_r	reference temperature	°K
v	volume-unit	m^3
Y_i	dimensionless group	–
z_i	increase in temperature that is required to reduce the value of D_i by one order of magnitude	°F
α	heat transfer coefficient	kJ/m^2 °K sec
β	dimensionless initial temperature	–
λ	heat conduction coefficient	kJ/m °K sec
ϕ	dimensionless temperature	–

REFERENCES

Ball, C.O. and Olson, F.C.W., 1957. "Sterilization in Food Technology." McGraw-Hill Book Co., New York.

Carslaw, H.S. and Jaeger, J.C. 1959. "Conduction of Heat in Solids." Clarendon Press, Oxford.

Fennema, O.R. 1975. "Principles of Food Science. Part 2. Physical Principles of Food Preservation." Marcel Dekker Inc., New York and Basel.

Hayakawa, K. 1969. New parameters for calculation mass average sterilizing value to estimate nutrient retention in thermally conductive food. Can. Inst. Food Technol. J. 2: 165.

Jen, Y., Manson, J.E., Stumbo, C.R. and Zahradnik, J.W., 1971. A procedure for estimating sterilization of and quality factor degradation in thermally processed foods. J. Food Sci. 36: 692.

Luikov, A.V. 1968. "Analytical Heat Diffusion Theory." Academic Press, New York.

Mulley, E.A., Stumbo, C.R. and Hunting, W.M. 1975. Thiamine: A chemical index of the sterilization efficacy of thermal processing. J. Food Sci. 40: 993.

Stumbo, C.R. 1973. "Thermo Bacteriology in Food Processing," 2nd ed. Academic Press Inc., New York and London.

Teixeira, A.A., Dixon, J.R., Zahradnik, J.W. and Zinsmeister, G.E. 1969. Computer optimization of nutrient retention in thermal processing of conduction-heated foods. Food Technol. 23: 845.

Ms received 6/16/77; revised 10/15/77; accepted 10/20/77.

PRODUCTION OF AFLATOXINS DURING STORAGE OF GAMMA–IRRADIATED WHEAT

A. G. BEHERE, ARUN SHARMA, S.R. PADWALDESAI and G. B. NADKARNI

ABSTRACT

A correlation between relative humidity (RH) during storage and moisture content was obtained in wheat subjected to gamma irradiation at 20 krad. The samples were assessed for storage up to 6 months with and without artificial loading of grains with conidia of *Aspergillus flavus*. The mycotoxin production seemed to be determined by a critical level of moisture in the grain (13%) at RH over 80% at 28° ± 2°C. The total aflatoxin produced in the irradiated grains was observed to be lower than in the unirradiated controls. The amount of toxin contained in grains, artificially infected with *A. flavus* before or after irradiation, did not show appreciable differences. The results, while defining the storage conditions with reference to humidity, did not indicate any alterations in wheat relating to aflatoxin producing potential.

INTRODUCTION

THE LOSSES DURING STORAGE of food grains in tropical countries are essentially due to adverse climatic conditions of high humidity and temperature which accentuate the proliferation of spoilage microorganisms and insects (Rao et al., 1976). The ability of ionizing radiations to destroy these pests has been recognized in the context of extending the storage life of foods (Sreenivasan et al., 1972). Gamma-irradiation in the dose range 10–100 krad has been found to be effective in the disinfestation of grains (Cornwell and Bull, 1960; Rao et al., 1976). Low level pasteurizing doses are also known to prevent aflatoxin production in bread (Bullerman et al., 1973). Some reports on the other hand have suggested enhanced toxin formation by the fungi exposed to radiation (Applegate and Chipley, 1974). Though the extended storage has been the main objective of the radiation treatment, relatively little information is available as to whether the grains are rendered more susceptible to the attack of toxigenic fungi. Recently, the regrowth of fungi on irradiated wheat has been reported to be slower than on nonirradiated wheat and that toxin-producing fungi are more susceptible to irradiation (Ingram, 1975). It was also observed earlier in this laboratory that total microbial population showed a decline in wheat exposed to disinfestation doses of gamma-irradiation (Rao et al., 1976). The present studies were undertaken to ascertain the extent of aflatoxin production in gamma-irradiated and nonirradiated wheat stored at various relative humidity (RH) levels.

EXPERIMENTAL

Irradiation of wheat

Locally purchased wheat was divided into 5 lots of 6 kg each. One of them served as control and the other was irradiated with gamma rays at a dose of 20 krad which has been found to be effective for disinfestation of insect pests (Sreenivasan et al., 1972). Of the remaining three, one was kept as artificially infected control, the second was infected prior to irradiation and the third one was infected after irradiation.

Artificial inoculation

For the purpose of infection of wheat, conidia of *Aspergillus flavus* (ATCC 15517) were used. Conidia were harvested as described earlier (Padwal-Desai et al., 1976) and the conidial suspension containing 7.5 × 10^7 spores/kg were thoroughly mixed with wheat using a rotary mixer.

All authors are with the Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

0022-1147/78/0004-1102$02.25/0
© 1978 Institute of Food Technologists

Storage of wheat

Perforated polyethylene bags (300 gauge) containing 250g of wheat from the above lots, were stored in humidity controlled chambers (28 × 24.5 × 27.5 cm). The relative humidity levels at 20, 40, 60, 75, 90 and 100% were maintained by varying the proportions of glycerol and water as suggested by Osborne and Bacon (1958). For storage studies up to 6 months, 18 bags from each treatment were tied on the inner side of the lid of the chamber with the help of built-in hooks. Samples in triplicate from each bag were drawn at monthly intervals for analysis.

Moisture content in the grains

Percent moisture was determined by drying wheat samples in duplicate at 100°C until a constant weight was obtained (AOAC, 1960).

Estimation of aflatoxin

Wheat flour (10g) was washed twice with 50 ml of distilled hexane in a conical flask to remove fats and pigments. After discarding the hexane layer, samples were dried using nitrogen. Five ml of distilled water was added to each sample in order to make a thick slurry. The aflatoxins were extracted by two washes of 50 ml each of distilled chloroform. The chloroform extracts were pooled together and dried on a boiling water bath. The residue was then exactly made to 1 ml with chloroform, from which an appropriate quantity was spotted on TLC plates (0.5 mm thickness, Keiselgel G, E. Merck). TLC plates were given a "clean up" by developing the plates in pure diethyl ether prior to running the chloroform-acetone solvent (9:1) for chromatography of aflatoxins. The quantitative estimation of aflatoxins was carried out by comparison of fluorescence with appropriate standards (Jones, 1972).

RESULTS & DISCUSSION

THE EFFECT of relative humidity on the moisture content in the grain observed during storage up to 6 months is shown in Figure 1. An upward trend in the grain moisture content was observed only above 80% RH. A comparison of aflatoxin production in the uninfected control and irradiated wheat at 90% RH is presented in Figure 2, since this was observed to be optimal RH for maximal biogenesis of the toxin. It is striking that the irradiated wheat invariably showed lower aflatoxin levels than in the corresponding controls. It was observed earlier that even at the disinfestation dose there was reduction in the microbial count (Rao et al., 1976). The total toxin production showed reduction with concomitant decrease in the relative humidity. Experiments with grains, artificially infected before and after irradiation also revealed that there was little aflatoxin production at lower RH values despite long storage. On the other hand, storage up to 5–6 months at higher RH values alone caused significant aflatoxin production as shown in Figure 2. No appreciable differences were noticed in the levels of aflatoxin whether the conidia were added before or after irradiation. It is worth noting that at the end of 6 months storage at 90% RH the grains infected after irradiation seemed to yield lower amounts of toxin, as compared to infected control and grains loaded with spores prior to irradiation. The moisture content of 13–14% in the grains was a critical factor for promoting toxin production as shown in Figure 3. The extent of aflatoxin production did not vary significantly beyond this apparently critical moisture level. The initial levels showed higher values since the conidial suspension itself had some aflatoxin already present.

These experiments would therefore suggest that the disinfestation dose had little effect on the grains to make them more susceptible to attack by aflatoxin producing fungi. The correlation between RH of storage with the moisture content in the grain indicates that only these factors are critical for safe storage. It is apparent that below 13% moisture level in the grain, the fungus could not grow and hence biogenesis of aflatoxin was not detectable even in the deliberately infected

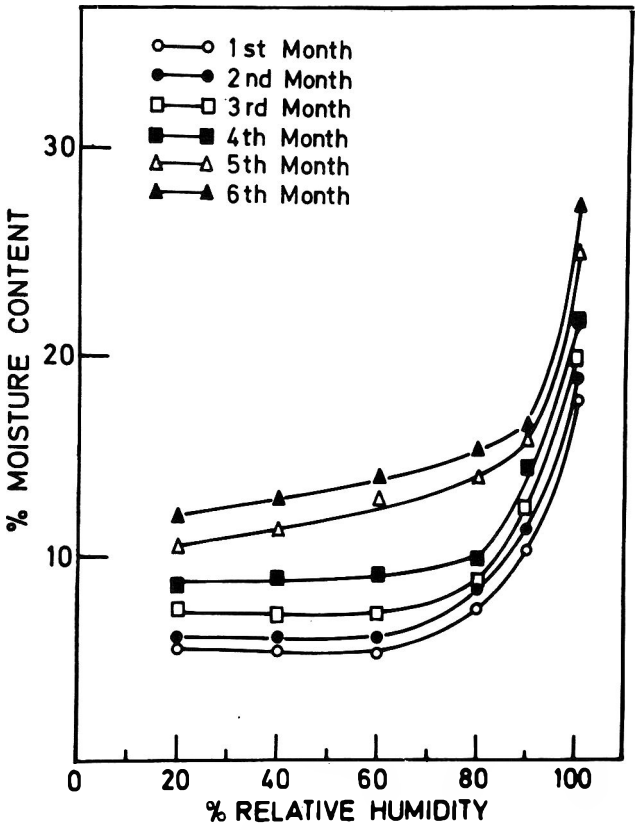


Fig. 1—Moisture content in wheat stored up to 6 months at various relative humidity (RH) levels. The experimental details are stated in the text.

wheat. These results are at variance with those reported by Priyadarshini and Tulpule (1976) who incubated spores with autoclaved wheat (irradiated at 75 krad) at 60% moisture level. These experiments may not therefore reflect the conditions normally used for storage of grains. The importance of humidity factors has also been emphasized by earlier authors (Boller and Schroeder, 1973; Mirocha and Christensen, 1976). The aflatoxin problem may not arise if irradiated grain is stored under sufficiently dry conditions (Ingram, 1975; WHO, 1977). The ability of the fungus to produce toxin has also been reported to be reduced after radiation exposures (Hartung et al., 1973). It has therefore been suggested that irradiation was an effective means for preservation of bread (Hartung et al., 1973; Bullerman et al., 1973).

Most of the earlier studies on toxigenic molds have provided useful information on radiation sensitivities of various strains, correlation of physiological state with radiation responses, relative inactivation of conidia or mycelia or the effects pertaining to virulence (Ingram, 1975). However, the doses employed were far beyond the range recommended for irradiation of wheat (WHO, 1977). The present attempt was essentially to simulate humidity conditions of storage and to evaluate the responses of irradiated wheat to fungal attack.

REFERENCES

AOAC. 1960. "Methods of Analysis," Horowitz, W. p. 283. Assoc. Official Anal. Chem., 9th ed. Ed. Washington, DC.

Applegate, K.L. and Chipley, J.R. 1974. Daily variations in the production of aflatoxins by Aspergillus flavus NRRL 3145 following exposure to Co^{60} irradiation. Appl. Bact. 37: 359.

Boller, R.A. and Schroeder, H.W. 1973. Influence of Aspergillus chivalieri on production of aflatoxin in rice by Aspergillus parasiticus. Phytopathology 63: 1507.

Bullerman, L.B., Bannhart, H.M., Hartung, T.E. 1973. Use of gamma-irradiation to prevent aflatoxin production in bread. J. Food Sci. 38: 1238.

Cornwell, P.B. and Bull, J.O. 1960. Insect control by gamma irradiation. An appraisal of the potentiation and problems involved. J. Sci. Fd. Agric. 11: 754.

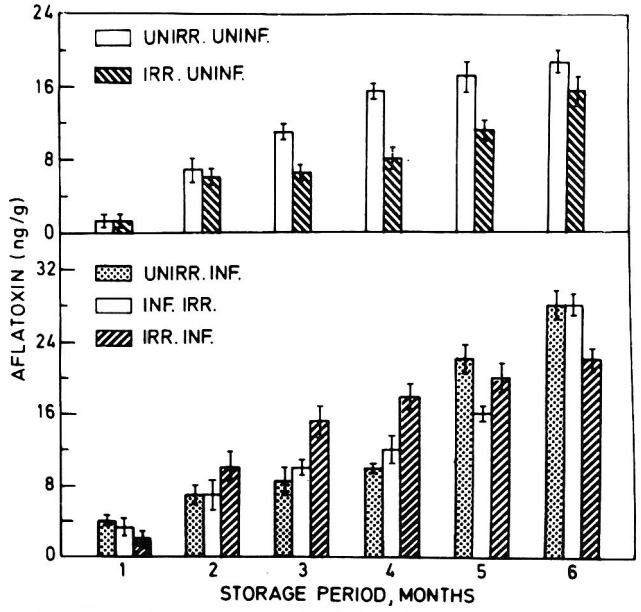


Fig. 2—Aflatoxin levels in control and gamma-irradiated (20 krad) wheat during storage at 90% RH, including deliberate infection by the spores of A. flavus. The sequence of treatments are indicated by abbreviations. Irr—irradiated, Unirr—unirradiated, Inf—artificially infected and Uninf—uninfected. The method of sampling has been stated in the text.

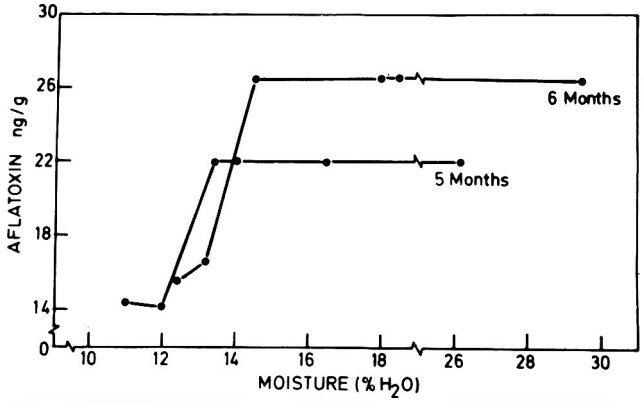


Fig. 3—Effect of grain moisture on biogensis of aflatoxin in wheat deliberately mixed with spores of A. flavus. The critical level of grain moisture above 13% could be observed only in wheat stored for 5 and 6 months at RH of 90% as shown.

Hartung, T.E., Bullerman, L.B., Arnold, R.G. and Heidel Baugh, N.D. 1973. Application of low dose irradiation to fresh bread system for space flight. J. Food Sci. 38: 129.

Ingram, M. 1975. Technical report series. International project in the field of food irradiation. IFIP R 33: 14.

Jones, B.D. 1972. Methods of aflatoxin analysis. Tropical Product Institute, London, Report No. G 70.

Mirocha, C.J. and Christensen, C.M. 1976. Mycotoxins and fungi that produce them. Proc. Amer. Phytol. Soc. 3: 118.

Osborne, T.S. and Bacon, J.A. 1958. Two improved and inexpensive systems for moisture stabilization in seed and other tissues. Plant Physiol. 36: 309.

Padwal-Desai, S.R., Ghanekar, A.S. and Sreenivasan, A. 1976. Studies on Aspergillus flavus. 1. Factors influencing radiation resistance of non-germinating conidia. Environment. Exptl. Bot. 16: 45.

Priyadarshini, E. and Tulpule, P.G. 1976. Aflatoxin production on irradiated foods. Fd. Cosmet. Toxicol. 13: 243.

Rao, V.S., Padwal-Desai, S.R., Bongirwar, D.R., Vakil, U.K. and Sreenivasan, A. 1976. Comparative storage studies on fumigated and irradiated wheat. Acta Alimentaria 5: 253.

Sreenivasan, A., Aiyar, A.S., Bongirwar, D.R., Kumta, U.S., Rahalkar, G.W., Sudersan, P., Thomas, P. and Vakil, U.K. 1972. Proceedings of the fourth conference on peaceful uses of atomic energy, Geneva, 12: 265.

WHO, 1977 Technical Report series No. 604. FAO/IAEA/WHO report of joint committee on "Wholesomeness of irradiated food".

Ms received 10/15/77; revised 1/21/78; accepted 1/30/78.

FEASIBILITY OF HOME FERMENTATION TO IMPROVE THE AMINO ACID BALANCE OF CORN MEAL

YUH-YUN DAVID WANG and M.L. FIELDS

ABSTRACT

The relative nutritiive value (RNV) of corn meal, as determined by *Tetrahymena pyiformis* W method, was improved by fermenting corn meal with *Saccharomyces cerevisiae* and *Candida tropicalis* ATCC 1369. Fermentation of corn meal with *Candida tropicalis* ATCC 1369 without prior enzyme hydrolysis of the starch with corn malt and adjusting the pH one time appeared the most feasible method. Evaluation of the nutritive value of corn malt indicated that it had the same RNV as casein.

INTRODUCTION

IN MANY PARTS of the world corn or maize constitutes a major part of the diet of the peoples. Corn is deficient in the amino acids lysine and tryptophan. Since yeasts are good sources of lysine and since they have been mixed with corn to improve the protein efficiency ratio (Bressani, 1968), an investigation into home fermentation of corn meal by yeast to improve the amino acid balance seemed desirable.

Home fermentations are not dependent upon industry. The raw materials and simple equipment are all that are needed to produce a product that looks like corn meal and that can be used in the native foods without major educational efforts to get the new food material acceptable. This research was conducted to determine the feasibility of home fermentation of corn meal by two yeasts to increase its amino acid balance.

MATERIALS & METHODS

Production of corn malt

The Ball jar method was used to germinate corn to produce corn malt. The method was essentially that of Chen (1962). The seeds were soaked in the jar. After washing the grain, a piece of cheesecloth was secured by a metal ring cap to the jar so that the cloth covered the mouth of the jar. The jar was placed in an inverted position so that the excess water drained from the jar. The jar was held at 30°C. The germinating grains were washed with tap water twice a day to provide moisture and to keep microbial growth on the seeds to a minimum. After 3–4 days of germination, the seeds were dried at 50°C under flowing air. The dried seeds were ground in a Thomas-Wiley mill with a 1 mm screen.

Slurry preparation and hydrolysis of starch with corn malt

To prepare a 10% corn meal slurry, 225g of whole corn meal (not germinated) was suspended in 2.25L of tap water. The slurry was boiled for 10 min to gelatinize the starch and to kill nonsporeforming microorganisms. After cooling, pH of the slurry was adjusted to 5.5 with reconstituted lemon juice.

Five grams of corn malt were mixed into the slurry. The mixture was incubated at 40°C for 30 min to hydrolyze the starch for the production of sugars.

Slurry inoculation and fermentation

Candida tropicalis ATCC 1369 was chosen since this yeast had been used in the production of food yeast. This species also is capable of utilizing maltose and soluble starch. *Saccharomyces cerevisiae* was also used because it is the common bread yeast and is readily available in most countries.

Before inoculation of the corn malt slurries, the pH of the slurries was adjusted to pH 3.5 with reconstituted lemon juice. The slurries were poured in about equal volumes into three aluminum baking pans. Two pans were each inoculated with 2 ml of a 24–36 hr malt extract broth culture of *C. tropicalis* or *S. cerevisiae*. One pan was not inoculated (control). After the inoculum and the slurries were mixed the pans were covered with a piece of cheese cloth and incubated at about 25°C for 3 days.

Slurry drying and regrinding

The fermented corn slurries were oven dried at 55–65°C under flowing air in the same pans used for fermentation. The fermented corn meal was weighed to obtain the dry weights. The dried material was removed from the pans and reground in a Wiley mill.

Analyses of fermented corn meal

The protein content of the fermented corn meal was determined by the AOAC (1975) micro-Kjeldal method. Crude protein content of the product was calculated by multiplying the nitrogen content by 6.25.

Microbiological analyses for lysine, tryptophan and methionine followed the method of Difco (1971). The sample preparation containing 100 mg N was predigested with 1 ml of 2% (w/v) papain solution for 3 hr at 56°C, as described by Ford (1964). These values are referred to as the available amino acids.

Total amino acids were determined by the method of Benson and Patterson (1971). These samples were hydrolyzed with 6N HCl for 22 hr at 110°C followed by automated cation exchange chromatography.

Relative nutritive value (RNV) of proteins was determined using *Tetrahymena pyriformis* W according to Stott and Smith (1963). RNV was calculated according to the equation of Helms and Rølle (1970).

Table 1—Percent nitrogen and crude protein of corn, corn malt, and fermented corn meal (3 days at room temperature)

Product	N%[a]	Crude protein %[a]
Corn meal	1.22	7.63
Corn malt	1.37	8.54
Fermented corn meal (Control, *S. cerevisiae*)	1.30	8.9[b]
Fermented corn meal (malt + *S. cerevisiae*)	2.63	16.4[c]
Fermented corn meal (Control, *C. tropicalis*)	1.34	8.36[d]
Fermented corn meal (malt + *C. tropicalis*)	2.31	14.5[e]

[a] N = 3.
[b] + 2.8% weight gain
[c] 52.5% weight loss
[d] 10% weight loss
[e] 14% weight loss

RESULTS & DISCUSSION

Analyses of the fermented meals

Chemical analyses on the % N and crude protein are given in Table 1. In the two treatments in which malt was used, the analyses show considerably higher crude protein content. However, these treatments showed considerable weight loss during the fermentation as contrasted to the controls. It would appear that the fermentation produced volatile products that were lost either during the fermentation or during the drying process.

Authors Wang and Fields are with the Dept. of Food Science & Nutrition, University of Missouri, Columbia, MO 65201

0022-1147/78/0004-1104$02.25/0
© 1978 Institute of Food Technologists

Table 2—Relative nutritive value (RNV) and lysine, methionine and tryptophan contents of corn meals

Product	Biologically available amino acids mg/gN			Total amino acids by chemical analysis mg/gN		
	Lysine[a]	Methionine[a]	Tryptophan[a]	Lysine[a]	Methionine[a]	RNV %[a]
Corn meal (Control)[b]	22.5	9.0	2.0	205.0	147.5	66.8
Corn malt	56.5	45.0	13.0	211.9	109.4	99.5
Control, *S. cerevisiae*[c]	e	e	e	223.1	138.1	74.1
Corn malt + *S. cerevisiae*[d]	e	e	2.0	216.8	121.9	87.9
Control, *C. tropicalis*[c]	e	e	e	216.2	119.4	82.6
Corn malt + *C. tropicalis*	e	e	2.0	229.4	125.6	87.9

[a] N = 2
[b] Not fermented
[c] No malt used
[d] Corn meal plus malt and fermented 3 days at room temperature
[e] Analysis not done

Data in Table 2 show that all fermentation treatments were higher in RNV than the whole corn meal control which had an RNV of 66.8 (RNV of casein, the reference protein in this method of analysis is 100%). When both species of yeasts were used without the corn malt, there was only a small increase in the % protein over the nonfermented control (Table 1). However, there was an improvement in the RNV of the proteins (66.8% for the control, 74.1% for *S. cerevisiae*, 82.6% for *C. tropicalis* ATCC 1369). With malt added to the slurry the % crude protein increased from 7.63 to 16.4% in the slurry fermented by *S. cerevisiae*. However only about one-half of the solids were recovered. The RNV of this product was 87.9%.

When malt was added to the slurry fermented with *C. tropicalis* ATCC 1369, the % crude protein increased from 7.63% (control) to 14.5%. Only 14% of the weight was lost during this fermentation. This smaller weight loss, as contrasted to the weight loss by fermentation with *S. cerevisiae*, is probably due to the fact that *S. cerevisiae* produced alcohol and carbon dioxide during its growth which was lost during the drying process. With the weight loss in mind and with the fact that the RNV of the *C. tropicalis* ATCC 1369 fermented slurry was 87.9%, *C. tropicalis* ATCC 1369 fermented slurry was 87.9%, *C. tropicalis* ATCC 1369 obviously would be the better yeast to use.

Corrobarative evidence (other than RNV) of the increased nutritive value was also obtained by assaying for lysine, tryptophan and methionine (Table 2). The microbiological analysis was obtained after enzyme hydrolysis, thus giving biologically available lysine, tryptophan and methionine. Data in Table 2 indicate that in the corn malt sample which has the highest RNV the lysine content increased from 22.5 mg/gN to 56.5 mg/gN; methionine increased from 9.0 mg/gN to 45 mg/gN and tryptophan from 2.0 mg/gN to 13.0 mg/gN.

The total lysine and methionine contents were measured and are given in Table 2. It is obvious that the total lysine and methionine contents were much higher than the biologically available lysine and methionine (by a factor of 9.1 × for lysine and 16.4 × for methionine). Although the increase was much less when the total lysine content was measured, there was an increase of about 11% when corn meal (control) is compared to the fermentation in which malt and *C. tropicalis* ATCC 1369 were used. All treatments were lower in methionine than the control corn meal. However, it is the biologically available amino acids and the amino acid balance that are important for animal growth.

Both yeasts used in this research are well known food yeasts. In other research conducted in our laboratory, it was shown that rats fed on a 1:1 protein mixture of *C. tropicalis* ATCC 1369 and corn grew well. The calculated rat PER of the protein mixture was 2.45, showing that there was no toxic material produced in the yeasts (Wang, 1977). Bressani (1968) showed that a mixture of *Candida utilis* (torula yeast) in a 1:1 protein distribution and corn resulted in PER values greater than 2.

In special toxicological studies also performed in our laboratory (Wang, 1977), it was shown that oil and water extracts of *C. tropicalis* ATCC 1369 had no ill effects when inoculated into fertile chicken embryos or when fed to rats or when rats were skin tested.

CONCLUSIONS

INCREASED NUTRITIVE VALUE of corn meal was achieved by fermentation with yeasts. *Candida tropicalis* ATCC 1369 appeared to be more efficient in increasing the RNV as compared to *S. cerevisiae*. When corn malt was used, the pH was adjusted twice (pH 5.5 and 3.5). However, an improvement in the RNV of 15.8% (82.6–66.8) was achieved by simply inoculating whole ground corn meal with *C. tropicalis* ATCC 1369. This was feasible with only one pH adjustment.

The experiments showed that another method to increase the RNV of corn was to germinate the corn. This process increased the available lysine, methionine and tryptophan contents of the corn. The corn meal can be used as a source to enrich corn meal products. With this procedure the RNV increased 32.7%. Additional research is needed to show application for the fermentation process under home conditions.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Agricultural Chemists, Washington, D.C.
Benson, J.V. Jr. and Patterson, J.A. 1971. Chromatographic advances in amino acids and peptide analysis using spherical resins and their applications in biochemistry and medicine. In "New Techniques in Amino Acid, Peptide, and Protein Analyses," Ed. Niederweiser, A. and Pataki, G., p. 1. Ann Arbor Sci. Pub., Ann Arbor, MI.
Bressani, R. 1968. The use of yeast in human foods. In "Single-cell Protein," Ed. Mateles, R.I. and Tannenbaum, S.R. The MIT Press, MIT, Cambridge, MA.
Chen, P.S. 1962. Soybeans for health, longevity and economy, p. 117. The Chemical Elements, South Lancaster, MA.
Difco. 1971. Technical information. Media for the microbiological assay of vitamins and amino acids, p. 59. Difco Laboratories Inc., Detroit, MI.
Ford, J.E. 1964. A microbiological method for assessing the nutritional value of protein. 3. Further studies on the measurement of available amino acids. Brit. J. Nutr. 18: 449.
Helms, P. and Rølle, G. 1970. Nutritive evaluation of protein quality with Tetrahymena pyriformis W. In "Evaluation of Novel Protein Products," Ed. Benden, A.Z.; Kehlberg, R; Lofquist, B. and Munck, L. Pergamon Press, N.Y.
Stott, J.A. and Smith, H. 1963. Microbiological evaluation of protein quality with Tetrahymena pyriformis W. 3. A simplified assay procedure. Brit. J. Nutr. 17: 227.
Wang, Y. 1977. Enrichment of ingredients for fabricated foods by fermentation and germination of corn and sorghum. Ph.D. thesis, University of Missouri, Columbia, MO.
Ms received 9/26/77; revised 12/9/77; accepted 12/14/77.

Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 7890, approved 6/17/77.

AVAILABLE LYSINE IN HEATED FORTIFIED RICE MEAL

TER-FUNG TSAO, ANTHONY L. FREY and JUDSON M. HARPER

ABSTRACT

The objective of the study was to evaluate the effect of thermal processing on available lysine retention in fortified rice meal. Ranges of temperature (115–185°C), moisture (15–20%) and lysine fortification level (2–4%) were used in the investigation. The kinetic constants of degradation were determined, postulating a first order model. Results indicate that available lysine degradation can be described by the monomolecular reaction and the effect of temperature on the reaction rate constant is described by the Arrhenius equation. Results also indicate that moisture level does not have significant effect on the rate constants in the degradation reaction.

INTRODUCTION

CEREALS are the main source of protein intake for two-thirds of the world population. As compared with the reference amino acid pattern, all cereal proteins are deficient in the essential amino acid lysine, which is required for growth. Therefore, lysine fortification of cereals could become an effective approach to improve the protein quality of cereals and help solve the protein malnutrition problem.

One of the practical problems in the nutrient fortification of cereals is thermal destruction of the added and original nutrients in processing (Aylward and Morton, 1970). This is a serious problem for lysine fortification, because lysine is considered to be nutritionally available only if its ϵ-amino group is free or unbound. Lysine is very susceptible to nutritive loss during heat treatment because of the well known Maillard, or browning, reaction involving its ϵ-amino groups (Hannan and Lea, 1952; Carpenter, 1973).

Eichner and Karel (1972) suggested that the browning rate decreased with increasing water content. However, work done by Thompson et al. (1976) indicates temperature and glucose content effects are greater and mask the effect of water content.

One potential way to solve the thermal destruction problem of available lysine is to use high-temperature, short-time (HTST) processes which will tend to maximize nutrient retention. Because the higher temperature effect on the inactivation of microorganisms usually increases more rapidly than its effect on the destruction of nutrients (Berk, 1970), the HTST system can be used to destroy microorganisms while nutrient composition is not altered to the same extent.

In order to use the HTST system effectively, it is desirable to understand the thermal effects on the kinetics of available lysine degradation.

Therefore, the objective of this study is to determine the kinetic model of the thermal destruction of available lysine in a system of rice meal. The effects of water content and level of fortified lysine on the lysine retention were also studied.

Author Harper is with the Dept. of Agricultural & Chemical Engineering, Colorado State University, Fort Collins, CO 80523. Author Tsao, formerly with Colorado State Univ., is now with Quaker Oats Co., Barrington, IL 60010. Author Frey, formerly with Colorado State Univ., is now with the College of Medicine, Univ. of Iowa, Iowa City, IA 52240.

0022-1147/78/0004-1106$02.25/0
© 1978 Institute of Food Technologists

THEORETICAL CONSIDERATION

Kinetics of available lysine degradation

In this study, the degradation reaction of available lysine was assumed to follow a monomolecular reaction, and the equation would be written as:

$$\text{Available lysine (AL)} \xrightarrow{k} \text{Degradation product} \qquad (1)$$

The rate expression is:

$$\frac{-d[AL]}{dt} = k[AL] \qquad (2)$$

If the initial concentration $[AL_o]$ is reduced to $[AL_t]$ after time t, then Eq (2) can be integrated and rearranged resulting in

$$k = \frac{-1}{t} \ln \frac{[AL_t]}{[AL_o]} = \frac{-1}{t} \ln (LR) \qquad (3)$$

where LR = available lysine retention, %.

A plot of the natural logarithm of percent available lysine retention at constant temperature against time would yield a straight line for a monomolecular reaction, with a slope of negative k, the rate constant at this reaction temperature.

Effect of temperature on reaction rate

Eq (3) would describe the degradation of available lysine as a function of time at constant temperature. The reaction rate is a function of temperature:

$$\ln k = \ln k_o - (E/RT) \qquad (4)$$

where: k = reaction rate constant; k_o = pre-exponential factor; R = gas constant, 11987 Kcal/°K · g mole; T = absolute temperature, °K; and E = activation energy, Kcal/g mole.

The constants k_o and E are independent of temperature, and they are determined by the properties of the reacting molecules. From Eq (4), a plot of logarithm of k versus 1/T should give a straight line and allow the estimation of E and k_o. The higher the value of E the greater the effect of increasing temperature on the reaction.

MATERIALS & METHODS

Available lysine analysis - Laboratory procedures

The available lysine analysis procedures were developed by Ousterhout and Wood (1970). Ten ml of 4% $NaHCO_3$ (pH 8.3) solution was added to 10 mg of sample in a 125 ml flask, followed by shaking in a 40°C incubator shaker for 10 min, then the addition of 10 ml of 1% trinitrobenzene sulfonic acid (TNBS) solution followed by shaking for an additional 2 hr at 40°C. Then 30 ml of concentrated HCl was added to the flask, and the flask was covered with a watch glass and autoclaved for 1 hr at 115°C. The autoclaved flask was then cooled to room temperature, diluted with 50 ml of water, and filtered.

A 10 ml aliquot of the filtrate was extracted twice with approximately 10 ml of diethyl ether, and then transferred to a stoppered test tube. Residual ether was eliminated from the aqueous phase by placing each tube in hot water for at least 5 min. The absorbance of the aqueous solution at a wavelength of 346 mμ was read using a Bausch and Lomb Spectronic-700 spectrophotometer, against a blank carried through the same procedure. However, concentrated HCl was added to the blank prior to the addition of the TNBS reagent as per suggested procedure (Ousterhout and Wood, 1970).

Table 1—Rate constant of available lysine degradation at given fortification level, moisture content and heating temperature

i	Fortification level % added L-lysine monohydrochloride	Moisture content (%)	Temp (°C)	Rate constant k_i, min^{-1}	Std error of k_i, min^{-1}
1	2	15	115	0.00404	0.00005
2	2	15	150	0.01590	0.00013
3	2	15	185	0.04816	0.00119
4	2	20	115	0.00410	0.00007
5	2	20	150	0.01615	0.00029
6	2	20	185	0.04882	0.00125
7	4	15	115	0.00405	0.00007
8	4	15	150	0.01621	0.00012
9	4	15	185	0.04751	0.00117
10	4	20	115	0.00411	0.00007
11	4	20	150	0.01591	0.00012
12	4	20	185	0.04888	0.00152

A standard curve was constructed from analyses of blanks to which various levels of L-lysine monohydrochloride had been added and then analyzed with the procedure as described. The resulting standard curve was linear over the range of 0–5% added L-lysine monohydrochloride. Estimation of the available lysine content of the unknown samples was accomplished by interpolation from the standard curve to within 0.1% of the lysine level in the sample.

Calculation of available lysine content

A standard curve was constructed from analyses of blanks to which various levels of L-lysine monohydrochloride had been added and then analyzed with the procedure described previously. Estimation of the available lysine content of the unknown samples was accomplished by interpolation from the standard curve.

In order to calculate the percent retention of available lysine, the moisture content of the samples was determined using a vacuum oven. Samples were dried at 70°C for 24 hr under 58.5–63.5 cm of mercury vacuum. The moisture content was calculated from the weight loss.

Sample preparation and experimental design

Using an Osterizer Dual Range Ten kitchen blender, rice meal No. 2 (Riviana Foods, Houston, TX) was blended with L-lysine monohydrochloride and water to arrive at the desired lysine fortification levels and moisture contents. These mixtures were heated at temperatures and conditions as described below:

Fortification levels–
2 and 4% added L-lysine monohydrochloride
Moisture content–
15 and 20%
Heating temperatures and heating times–
115°C – 30 min, 60 min, 120 min
150°C – 15 min, 30 min, 60 min
185°C – 7.5 min, 15 min, 30 min

It was necessary to use different heating times at the different temperatures to keep the lysine retention at reasonable levels. The experimental conditions selected were at temperatures and moistures which usually occur in extrusion processing of cereal grain. These retention times were substantially longer than those experienced in extruders. The necessity of the longer experimental retention times occurs because the influence of long times in the heat up of sample could be minimized in this fashion. Since models were used to correlate the data, the results can be extrapolated to the shorter retention times typical of extrusion processes.

For heating purposes, each sample was placed in a pipe nipple approximately 0.6 cm inside diameter and 7.5 cm long, then sealed with a pipe cap screwed on each end. At 115°C, the samples were heated in an autoclave. At 150°C and 180°C, an oil bath using diphenol, heated by an electrical heater, maintained the temperature. The heat transfer coefficient between the sample container and the heating medium was very high in both cases relative to the conductive heat transfer in the sample, so that changing the heating medium had very little effect. The small sample container reduced temperature variability within samples. After heating, the samples were cooled rapidly using an ice water bath. The available lysine contents of the heated and unheated samples were then analyzed by the method described previously. The percent retention of available lysine during heating at various temperatures for different lengths of time was calculated. These data were then analyzed using Eq (3) and (4) to determine the kinetic constants for lysine degradation.

The resulting full factorial experimental design was mixed level with two levels in lysine fortification and moisture content and three levels in heating temperature and heating time. The total number of experiments was therefore 2 × 2 × 3 × 3 or 36.

Table 2—t values for evaluating the effect of lysine fortification level on rate constant of degradation[a]

t	Value
1, 7	0.1224
2, 8	1.7635
3, 9	0.3892
4,10	0.1016
5,11	0.7546
6,12	0.0304
Degrees of freedom	10

[a] The superscript of t refers to the i in Table 1.

Table 3—Rate constant of available lysine degradation at given moisture content and heating temperature

Moisture content (%)	Temp (°C)	Rate constant, $k_{i,j}$, min^{-1}	Std error of $k_{i,j}$, min^{-1}
15	115	0.00404	0.00004
15	150	0.01606	0.00010
15	185	0.04783	0.00080
20	115	0.00410	0.00005
20	150	0.01603	0.00016
20	185	0.04885	0.00094

RESULTS & DISCUSSION

Kinetics of available lysine degradation

The percent lysine retention of duplicate rice samples showed a standard deviation of 1.16% retention. Each fortification level, temperature, and moisture content combination was considered as a separate data set. The rate constant, k, for each set was calculated by multiple regression using Eq (3). The calculated reaction rate constants are given in Table 1. Available lysine degradation rates increase with increasing temperature.

The effect of the lysine fortification level on the rate constant of available lysine degradation was statistically evaluated by a t test for the equality of two slopes. The calculated t values, as given in Table 2, support the hypothesis that the lysine fortification level has no significant effect on the rate constant of available lysine degradation. The values of t comparing k_i with k_j are all below 2.0 which is the 10% confidence level for finding a larger value of t. Since fortification level had no significant effect on the degradation rate constant, the data were combined and k determined as a function of temperature and moisture content alone using multiple linear regression with Eq (3), thus giving each condition quadruplicate replication (Table 3).

The effect of moisture content on the rate constant was also statistically examined by the t test for the equality of two slopes. The calculated t values, as tabulated in Table 4, indicate that the effect of moisture content on the rate constant of available lysine degradation was much less than the effect of temperature. Thus, the rate constant at each temperature was calculated combining all data at like temperatures and is summarized in Table 5. In this case, a further reduction in the standard error was obtained.

The activation energy of available lysine degradation was calculated from the reaction rate constants in Table 5 using

Table 4—t values for evaluating the effect of moisture content on rate constant of degradation[a]

t	Value
(1,7)(4,10)	0.9859
(2,8)(5,11)	0.1641
(3,9)(6,12)	0.8257
Degrees of freedom	22

[a] The superscript of t refers to i,j in Table 3.

Table 5—Rate constant of available lysine degradation at given heating temperature

Temp (°C)	Rate constant, k, min^{-1}	Std of error k, min^{-1}
115	0.00407	0.00003
150	0.01604	0.00009
185	0.04834	0.00061

the Arrhenius equation and multiple regression. The calculated values were:

Activation energy, E = 12.5 Kcal/mole
Pre-exponential factor, k_o = 745 hr^{-1}

The value of activation energy found would indicate that higher temperature processes would cause significant increases in lysine degradation. However, the value of k_o would indicate little lysine degradation would occur in a high-temperature, short-time process such as extrusion.

Using the regression values for k in Table 5, Figure 1 shows the degradation data for available lysine along with the estimated curve. The data points are the values of k from Table 5. This figure shows that the first order rate reaction model postulated for the degradation of available lysine fit the experimental data well. The Arrhenius plot in Figure 2 was derived from the values of E and k_o from the regression analysis.

CONCLUSIONS

1. The thermal loss of available lysine in a rice meal system can be described by the monomolecular reaction very well. Temperature has positive effect on reaction rate. The reaction rate equals 0.00407 min^{-1} at 115°C, 0.01604 min^{-1} at 150°C and 0.04834 min^{-1} at 185°C.

2. The Arrhenius equation suitably correlated the reaction rate constant with temperature. Activation energy was 12.5 Kcal/mole for lysine.

3. Moisture content and lysine fortification level do not have significant effect on the rate constants of available lysine degradation.

REFERENCES

Aylward, F. and Morton, I.D. 1970. Vitamin fortification of foods. In "Proceedings, SOS/70, Third International Congress, Food Science and Technology," p. 192.

Berk, Z. 1970. Processing and storage damage to nutritional value of foods. In "Proceedings, SOS/70, Third International Congress, Food Science and Technology," p. 189.

Carpenter, K.J. 1973. Damage to lysine in food processing — Its measurement and its significance. Nutr. Abs. & Review 43: 424.

Eichner, K. and Karel, M. 1972. The influence of water content and water activity on the sugar-amine browning reaction in model systems under various conditions. J. Agr. Food Chem. 20: 8.

Hannan, R.S. and Lea. C.H. 1952. Studies of the reaction between proteins and reducing sugars in the "dry" state. 6. The reactivity of the terminal amino groups of lysine in model systems. Biochem. Biophys. Acta 9: 293.

Ousterhout, L.E. and Wood, E.M. 1970. Available lysine in fish meals: Chemical (TNBS) method compared with a chick assay. Poul. Sci. 49: 1423.

Thompson, D.R., Wolf, J.C. and Reineccius, G.A. 1976. Lysine retention in food during extrusion-like processing. Transactions of the ASAE 19: 989.

Ms received 9/16/77; revised 12/24/77; accepted 12/30/77.

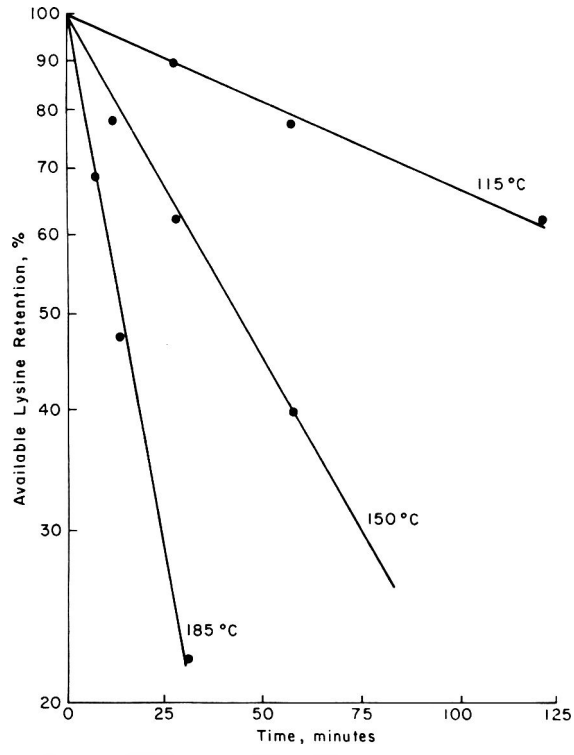


Fig. 1—Percent retention of available lysine in heated rice meal as a function of time and temperature. Data for varying fortification levels and moistures are combined.

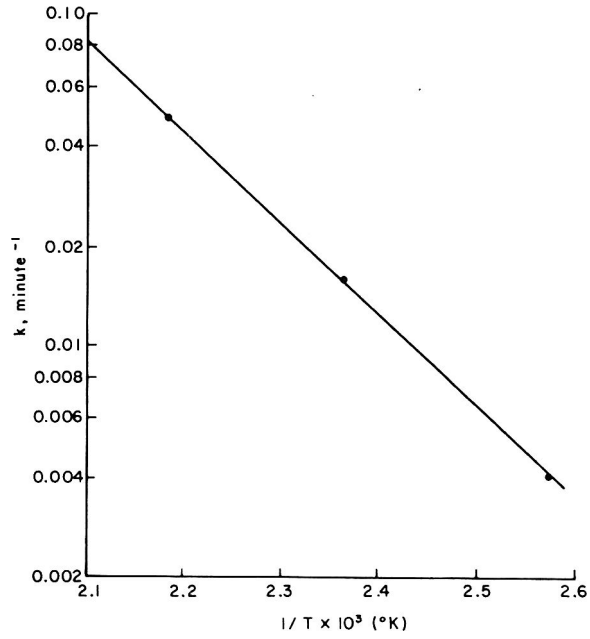


Fig. 2—Arrhenius plot of available lysine degradation.

FERMENTATION OF PEANUT MILK WITH *Lactobacillus bulgaricus* AND *L. acidophilus*

L. R. BEUCHAT and B. J. NAIL

ABSTRACT

Several extraction procedures were examined for their suitability to yield desirable peanut milks for fermentation by four strains of lactic acid bacteria. Studies revealed that a procedure in which peanuts were soaked in 1.0% sodium bicarbonate for 16–18 hr, drained, washed with tap water, ground, steeped for 4–5 hr in tap water, and filtered resulted in milk most desirable for fermentation. The addition of lactose (2%) to pasteurized peanut milk before fermenting with *Lactobacillus bulgaricus* NRRL B-1909 and *L. acidophilus* NRRL B-1910 for 3 days at 37°C resulted in a custard-like product having 0.38–0.53% titratable acidity at pH 4.76–4.43, respectively. Sensory panel evaluations of blended, fermented peanut milks containing added sucrose (2%) and fruit flavorings showed that the products were acceptable and competed favorably with flavored buttermilk. Fermented peanut milk substituted for buttermilk in a corn muffin recipe resulted in products with organoleptic characteristics not significantly ($P < 0.05$) different from those of the control. Further investigations are required to determine the optimum conditions for preparing fermented peanut milk products.

INTRODUCTION

THE SHORT SUPPLY of animal proteins in some parts of the world has created a need for developing processes by which plant proteins can be economically incorporated into diets. Research efforts in the United States have been directed mainly toward evaluating procedures for separating high-protein fractions from soybeans. Seeds of other plants have received less attention, not because they contain protein which may be functionally or nutritionally inferior to soybeans, but rather because of economics. However, this situation may change and it seems advisable at present to explore the development and utilization of a variety of foods derived from or containing a wide range of plant proteins.

Although soybean and peanut milks can be made more nutritious by supplementing with limiting amino acids, it is difficult to completely eliminate undesirable minor flavor components. This problem was reported to minimized in a soybean beverage by hydrolyzing soybean protein (Sugimoto et al., 1971). Mustakas (1974) heated a lipid-protein concentrate from soybeans at acid pH to inactivate lipoxygenase so that lipid oxidation would not occur and thereby develop off-flavors in beverages produced from it. Miltone, a toned peanut protein milk having a highly acceptable flavor was developed by the Central Technological Research Institute in Mysore, India. A review of oilseed and nut milk preparation and characteristics was presented by Swaminathan and Parpia (1967).

Fermentation of soybean and peanut milks has been investigated as an alternative for improving or masking undesirable ones. Mital et al. (1974) and Mital and Steinkraus (1976) stated that fermentation of soy milks with lactic acid bacteria resulted in a gelatinous curd with an acceptable flavor. A yogurt-like product was developed by fermenting soybean milk with species of *Lactobacillus* (Kanda et al., 1976; Wang et al., 1974). More recently Schmidt and Bates (1976) reported on the use of natural fruit flavorings to improve the acceptability of yogurt-like products prepared by lactic fermentation of soybean and peanut milks.

This report describes procedures for preparing peanut milk suitable for fermenting with lactobacilli. Fermented products were then evaluated for sensory characteristics to determine whether they could compete favorably with buttermilk and yogurt.

Authors Beuchat and Nail are with the Dept. of Food Science, University of Georgia Agricultural Experiment Station, Experiment, GA 30212.

0022-1147/78/0004-1109$02.25/0
© 1978 Institute of Food Technologists

EXPERIMENTAL

Preparation of peanut milk

Florunner cv. peanuts (*Arachis hypogaea* L.) served as the raw material from which peanut milk was prepared. Several extraction procedures were examined for their suitability to yield desirable milks for fermentation. Peanuts (1 kg, skins attached) were submerged in 2L of tap water containing 0.2, 0.6, and 1.0% sodium bicarbonate or citric acid and soaked for 16–18 hr at room temperature (22°C). A control consisted of peanuts soaked in water containing no added chemicals. Peanut mixtures were then either brought to boiling or allowed to remain at room temperature. Following soaking or immediately after heating (for some tests), fluid was drained and peanuts were washed with tap water and ground using an Oster Kitchen Center (Oster Corp., Milwaukee, WI). The ground peanuts were combined with 5L of tap water and steeped at room temperature for 4–5 hr before filtering through eight layers of cheesecloth. The filtrate is referred to as peanut milk throughout this report.

Cultures and media

Two strains of *Lactobacillus bulgaricus* (NRRL B-548 and NRRL B-1909) and two strains of *L. acidophilus* (NRRL B-1910 and NRRL B-2092) were kindly supplied by Dr. L.K. Nakamura, Northern Regional Research Center, SEA/USDA, Peoria, IL. Lyophilized cultures were activated in a deep liver medium (DLM) described by Haynes et al. (1955). The medium formula consisted of 100 ml beef liver extract, 5.0g yeast extract, 10.0g Tryptone, 2.0g K_2HPO_4, and 5.0g glucose in 900 ml distilled water, pH 7.4. Several small pieces of dried liver were added to each tube of medium (10 ml) before sterilizing. Stock cultures were maintained in a mixture of DLM and peanut milk (1:1, vol/vol) at 37°C, transfers being made every 5–7 days.

Inocula for fermented peanut milk studies consisted of 1-day-old cultures grown in DLM-peanut milk medium. Care was taken not to include liver particles as part of the inoculum.

Enumeration of lactic acid bacteria in fermented peanut milk was made by plating diluted aliquots on lactic agar (Elliker et al., 1956). Counts were made after incubation at 37°C for 2–3 days.

Procedure for fermentation

Peanut milk (100 ml) was heated for 10 min at 121°C, cooled at 37°C, and inoculated (1%) with lactic acid bacteria cultures. Strains of *L. bulgaricus* and *L. acidophilus* were studied separately and in combination. The effects of supplementing peanut milk with 2% (wt/vol) glucose, sucrose, lactose, and gelatin and with up to 4% cheese whey solids (acid) (CWS) on fermentation and end-product characteristics were also examined. Sugars (filter-sterilized) and gelatin (heat-sterilized) were added to autoclaved peanut milk, and CWS was dispersed in the milk before heating.

Fermentation was carried out at 37°C and products were analyzed after various periods of incubation up to 4 days.

Chemical analyses

The protein content (Kjeldahl N × 5.47) and titratable acidity (calculated as lactic acid) of peanut milk were determined using methods of the AOAC (1975). The procedure for quantitating lipids was outlined

in an earlier report (Beuchat and Worthington, 1974); pH values were monitored during the course of fermentation.

Evaluation of fermented milks

The fermented, coagulated peanut milk initially containing 2% lactose was blended sufficiently to yield a homogenous, pourable product. Various amounts of coconut, lemon, banana and vanilla extracts (McCormick & Co., Inc., Baltimore, MD), orange extract and pineapple flavor (Kroger Co., Cincinnati, OH) diluted in ethanol were added to the blended ferment. The buttermilk-like beverage was chilled to 4°C and evaluated for organoleptic properties before and after the addition of 2% (wt/vol) sucrose and commercial flavoring agents.

Peanut milk to which lactose (2%) had been added before fermentation was substituted for commercial buttermilk in two bakery products. A corn muffin recipe consisted of 1 cup (236.6 cm^3) corn meal, 1 tsp (14.8 cm^3) baking powder, 1 tsp salt, 0.25 tsp (3.7 cm^3) baking soda, 1 egg, 1 cup buttermilk and 1 tbp (44.4 ml) peanut oil. Dry ingredients were sifted together and combined with blended wet ingredients. Batter was poured into muffin tins and baked for 15 min at 232°C (450°F). A second recipe for a layer cake consisted of 2.5 cups (591.5 cm^3) plain wheat flour (pre-sifted), 1.5 cups (354.9 cm^3) sugar, 1.5 tsp (22.2 cm^3) baking soda, 0.75 tsp (11.1 cm^3) salt, 1.5 cups buttermilk, 0.75 cup (177.4 cm^3) shortening, 3 eggs (blended) and 1.5 tsp vanilla extract. Dry ingredients were sifted with pre-sifted flour, combined with remaining ingredients, mixed thoroughly, and poured into two 8-in. (20.3-cm) diameter by 1.5-in. (3.8-cm) deep pans. Cakes were baked at 177°C (350°F) for 30 min, removed from the oven, and cooled on racks. Both bakery products were stored at room temperature for 20–22 hr before subjecting to organoleptic evaluation.

A 10-member sensory evaluation panel was instructed to assign scores of 1–9 (1 = extremely poor; 5 = borderline; 9 = excellent) for appearance, color, aroma, texture, and flavor of fermented peanut milk and of products containing fermented peanut milk. Buttermilk obtained from a local grocer and nonfermented peanut milk were used as controls for sensory panel evaluations.

Significance of mean differences in values was determined by Duncan's multiple range test and reported at $P \leq 0.05$.

RESULTS & DISCUSSION

THE EFFECTS of soaking and boiling peanuts in $NaHCO_3$ and citric acid on protein content, pH, and color of resultant peanut milk are summarized in Table 1. The addition of $NaHCO_3$ to soak water resulted, in the absence of heat, in substantially higher amounts of protein in peanut milk and the addition of citric acid reduced the amount of protein extracted. As expected, the pH of milks moved upward in samples containing $NaHCO_3$ and downward in those containing citric acid. Since the isoelectric points of the main peanut proteins, arachin and conarachin, are in the pH 4–5 range, it is not surprising that protein solubility was highest in $NaHCO_3$ samples. Upon heating, proteins were denatured and solubility was drastically reduced in all samples. Color of resultant peanut milks was also adversely affected.

Table 1—Effect of soaking and boiling peanuts in $NaHCO_3$ and citric acid on protein content, pH and color of resultant peanut milk

	No heat			Heated		
Soak treatment	Protein (%)	pH	Color	Protein (%)	pH	Color
Control	1.73	6.70	White	0.85	6.86	Eggshell white
$NaHCO_3$						
0.2%	1.79	6.98	White	0.90	7.24	Eggshell white
0.6%	1.95	7.07	Eggshell white	0.90	7.57	Cream
1.0%	2.21	7.18	Eggshell white	0.88	7.90	Brownish
Citric acid						
0.2%	1.33	6.52	White	0.28	6.55	Cream
0.6%	0.77	6.01	Cream	0.22	5.96	Cream
1.0%	0.61	5.73	Cream	0.22	5.44	Cream

The addition of $NaHCO_3$ to soybeans during soaking in the preparation of Oriental fermented foods is believed to reduce undesirable beany or grassy flavor characteristics. Heating of legume seeds is also a recognized method for altering flavor. In the present study, treatment of peanuts with 1% $NaHCO_3$ but without heat prior to extraction of protein was judged as the most desirable procedure. Higher levels of $NaHCO_3$ in soak water may have resulted in higher protein concentrations in peanut milk; however, these milks would have required pH adjustment to an initial value more suitable for lactobacilli. Samples treated with 1% $NaHCO_3$ exhibited less beany and green flavor characteristics than did control or citric acid treated samples.

Incorporation of $NaHCO_3$ in soybean soak water was also reported to improve the flavor of extracted soybean milk (Kanda et al., 1976). These authors showed that ultrasonic treatment of soaked soybeans increased the amount of protein in resultant soybean milk by more than two-fold. Optimum sterilization or pasteurization parameters were 20 min at 100°C. Although relationships between texture and pasteurization conditions for peanut milk were not examined, various time/temperature experiments revealed that best overall color and flavor properties were achieved when milk was heated at 121°C for 10 min. This treatment resulted in slightly cream-gray (intense eggshell white) peanut milk without the harsh, beany, grassy, flavor characteristic of the raw milk. The product used as a substrate for fermentation contained 2.11% protein, 4.8% oil and 0.04% titratable acidity.

Of the four lactic acid bacterial strains tested, *L. bulgaricus* B-1909 and *L. acidophilus* B-1910 grew most rapidly, producing the greatest amounts of acid in peanut milk. Maximum acid production (0.38%) occurred between 48 and 72 hr of fermentation in lactose-supplemented (2%) milk cultured with *L. bulgaricus* B-1909. Milk fermented with *L. acidophilus* B-1910 contained 0.53% titratable acidity after this fermentation period. Respective pH values for these cultures at 72 hr dropped to 4.76 and 4.43. Viable cell counts were highest after 17 hr of fermentation, reaching maximum levels of 7.3×10^8 per ml for *L. bulgaricus* B-1909 and 9.9×10^7 per ml for *L. acidophilus* B-1910. After 72 hr, counts decreased to 1.5×10^6 and 9.3×10^4 per ml, respectively. It was not possible to achieve higher acidity by inoculating with various combinations of two strains (1:1, vol/vol) selected from the four lactobacilli.

Other researchers have reported variations in the ability of lactic acid bacteria to ferment soybean milk (Angeles and Marth, 1971; Wang et al., 1974). Mital et al. (1974) examined growth and acid-producing characteristics of *Streptococcus thermophilus* and several species of *Lactobacillus* in cows' milk and in soybean milk prepared by two techniques. Lactobacilli, with the exception of *L. bulgaricus*, showed greater acid production in soy milks than in cows' milk. In general, acid production was related to ability of strains to utilize sucrose, the major fermentable sugar. In a later study, Mital and Steinkraus (1976) reported that fermented soy milks with a yogurt-like gelatinous curd could be produced from soy milk using *S. thermophilus*, *L. acidophilus*, or *L. plantarum*. Kothari (1975) showed that supplementation of soy milk with cows' or buffaloes' skim milk or lactic acid whey stimulated acid production by streptococci and *L. bulgaricus*.

All test strains grew in peanut milk not fortified with carbohydrates. *L. bulgaricus* B-548 and *L. acidophilus* B-2092 were not significanlty affected by the addition of 2% glucose, sucrose, or lactose; however, strains B-1909 and B-1910 were stimulated by glucose and lactose. Kanda et al. (1976) reported that growth of *L. acidophilus* B-1910 and B-2092 in soybean milk was not significantly affected by added sucrose.

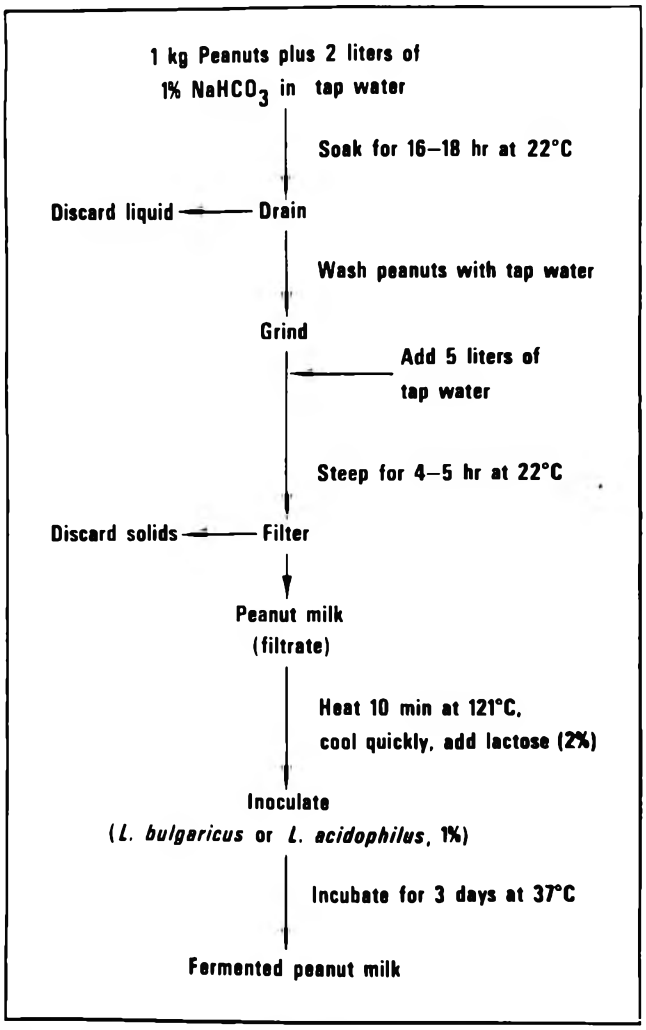


Fig. 1—Flow diagram describing the preparation of fermented peanut milk.

Table 2—Sensory evaluation scores for fermented milks[a]

Product	Flavoring added	Sensory characteristic: Appearance	Aroma	Texture	Flavor
Buttermilk	Coconut	8.0a	7.0a	7.4a	6.1abc
	Lemon	8.0a	7.1a	7.5a	6.6a
	Crange	6.1ab	5.9a	6.2a	6.3ab
	Banana	6.9ab	5.6a	6.5a	5.0abcde
	Pineapple	6.3ab	6.0a	6.0a	6.0abc
	Vanilla	6.1ab	6.3a	6.0a	5.6abcde
Peanut milk					
L. bulgaricus	Coconut	6.0ab	7.0a	4.9a	4.7abcde
	Lemon	6.1ab	6.4a	5.1a	4.4bcde
	Orange	5.9ab	6.1a	5.3a	4.0de
	Banana	5.0ab	5.4a	4.9a	4.5bcde
	Pineapple	4.9b	5.3a	4.9a	4.3cde
	Vanilla	4.8b	5.3a	4.6a	4.0de
L. acidophilus	Coconut	6.3ab	6.6a	5.8a	5.2abcde
	Lemon	6.4ab	6.4a	5.9a	5.5abcde
	Orange	6.4ab	5.5a	6.2a	5.7abcd
	Banana	6.4ab	5.5a	5.7a	3.7e
	Pineapple	6.4ab	6.2a	6.5a	4.9abcde
	Vanilla	6.2ab	5.9a	6.1a	4.3cde

[a] Values in the same column not followed by the same letter are significantly different ($P \leq 0.05$).

Table 3—Sensory evaluation scores for corn muffins[a]

Liquid ingredient	Sensory characteristic: Appearance	Color	Aroma	Texture	Flavor
Buttermilk	7.6a	7.5a	7.1a	7.4a	7.2a
Peanut milk					
Unfermented	6.4b	5.3b	6.9a	6.8a	6.4b
L. bulgaricus	7.1ab	7.1a	6.7a	7.1a	6.9ab
L. acidophilus	7.1ab	7.7a	7.3a	7.3a	7.3a

[a] Values in same column not followed by the same letter are significantly different ($P \leq 0.05$).

Since *L. bulgaricus* does not readily metabolize sucrose, its lack of response to this sugar was expected. The ability of *L. acidophilus* to readily utilize raffinose and stachyose (Stern et al., 1977) may enhance the digestibility of peanut milk. It should be noted that after several transfers in the DLM-peanut milk maintenance medium all test strains appeared to have a shorter lag time once inoculated into peanut milk substrate. This adaptation period may also be required by other lactics and should be considered by researchers exploring the area of legume seed milk fermentation.

The addition of CWS at concentrations of 2 and 4% to peanut milk before pasteurizing caused some separation of solids. Fermented milks containing CWS tended to develop defective curds with excessive acidity and low pH. For example, *L. bulgaricus* B-1909 produced 0.60% titratable acidity (pH 4.01) in peanut milk supplemented with 2% CWS. The latter observation is in general agreement with that reported by Kanda et al. (1976). The addition of up to 4% CWS to soybean milk fermented with *L. acidophilus* increased the titratable acidity from 0.40 to 0.74%. They selected a level of 2% CWS for producing a soybean yogurt-like product.

Peanut milk containing gelatin (2%) enhanced curd formation; however, texture was undesirably firm. Even at 1%, although the amount of free whey in ferments was reduced, gelatin caused the product to develop an undesirable texture.

As a result of the above studies on various extraction, pasteurization, supplementation, and fermentation procedures, a final scheme was developed. A flow diagram summarizing the process is shown in Figure 1.

Although an acceptable custard-like texture developed in peanut milk fermented with *L. bulgaricus*-1909 and *L. acidophilus* B-1910, excessive whey on the surface of cultures made the products appear somewhat undesirable as yogurt simulates. The ferments were therefore blended to form beverages having a consistency similar to that of buttermilk. Sucrose (2%) and flavoring agents were added to enhance the flavor of the ferments and the resultant chilled products were evaluated for organoleptic characteristics. Sensory scores are shown in Table 2. No significant differences ($P < 0.05$) were noted for appearance, aroma and texture between fermented peanut milk and buttermilk when comparing specific flavors. However, appearance scores for peanut milk fermented with *L. bulgaricus* B-1909 and flavored with pineapple or vanilla were significantly lower than those assigned to buttermilk flavored with coconut or lemon. Unflavored fermented peanut milks exhibited a slight beany aftertaste and were considered inferior to buttermilk. Upon addition of flavorings, fermented peanut milks competed favorably with flavored buttermilk. Overall, coconut and lemon flavors were considered to be most complementary to fermented peanut milk. Sugimoto et al. (1971) reported on the acceptability of a lemon-flavored beverage containing hydrolyzed soybean protein and Kanda et al. (1976) reported that lemon was extremely complementary to soybean yogurt.

Unfermented and fermented peanut milks were substituted for buttermilk in a corn muffin recipe. Sensory evaluation data (Table 3) show that fermentation significantly improved the color of muffins containing peanut milk. Scores for appear-

ance were increased in muffins containing fermented peanut milks to levels which were not significantly different from those recorded for buttermilk. The flavor of muffins containing fermented peanut milk was not significantly different from that of the buttermilk recipe, and muffins containing unfermented peanut milk were scored significantly lower for flavor. Muffins containing fermented peanut milk had a desirable golden brown color as compared to a slightly grayish coloration noted for muffins containing unfermented milk. Improved flavor of muffins containing fermented peanut milk was attributed to the loss of beany, green characteristics associated with raw peanut milk.

Substitution of peanut milks for buttermilk in a layer cake recipe caused the product to have an undesirably darkened color. However, scores for appearance, aroma, and texture were not significantly different among various test recipes. Flavor of cake containing peanut milk fermented with *L. acidophilus* was significantly better than that of cake containing unfermented milk. Because of the white or cream color associated with the cake recipe, the use of peanut milk as a buttermilk substitute appears undesirable.

In summary, we have demonstrated that a water extract from peanuts fermented with *L. bulgaricus* or *L. acidophilus* will produce an acceptable product which, when blended, flavored, and consumed as a beverage, will compete favorably with buttermilk. Fermented peanut milk can be substituted for buttermilk in a corn muffin recipe without significant sacrifice of sensory characteristics. Additional research to examine procedures for more efficient extraction of protein from peanuts which would result in milk more suitable for fermentation should be conducted. Also, optimum conditions of inoculum size, and incubation time and temperature for a larger number of strains of lactic acid bacteria should be ascertained. The use of stabilizers to preserve the custard-like texture and to control whey accumulation in peanut milk ferments should be explored. Finally, storage stability of ferments needs to be determined. It appears likely that improvements can be made in procedures for preparing fermented peanut milks and that products having various textural and flavor characteristics can be achieved.

REFERENCES

Angeles, A.G. and Marth, E.H. 1971. Growth and activity of lactic acid bacteria in soymilk. 1. Growth and acid production. J. Milk Food Technol. 34: 30.

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.

Beuchat, L.R. and Worthington, R.E. 1974. Changes in the lipid content of fermented peanuts. J. Agric. Food Chem. 22: 509.

Elliker, P.R., Anderson, A.W. and Hannesson, G. 1956. An agar culture medium for lactic streptococci and lactobacilli, J. Dairy Sci. 39: 1611.

Haynes, W.C., Wickerham, L.J. and Hesseltine, C.W. 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. 3: 361.

Kanda, H., Wang, H.L., Hesseltine, C.W. and Warner, K. 1976. Yoghurt production by Lactobacillus fermentation of soybean milk. Process Biochem. 11(4): 23.

Kothari, S.L. 1975. Studies on fermented soymilk products. 3. Activity of cheese and yogurt cultures in soymilk and fortified soymilk. Indian J. Microbiol. 15: 18.

Mital, B.K. and Steinkraus, K.H. 1976. Flavor acceptability of unfermented and lactic-fermented soy milks. J. Milk Food Technol. 39: 342.

Mital, B.K., Steinkraus, K.H. and Naylor, H.B. 1974. Growth of lactic acid bacteria in soy milks. J. Food Sci. 39: 1018.

Mustakas, G.C. 1974. A new soy lipid-protein concentrate for beverages. Cereal Sci. Today 19(2): 62.

Schmidt, R.H. and Bates, R.P. 1976. Sensory acceptability of fruit flavored oilseed milk formulations. Proc. Fla. State Hortic. Soc. 89: 217.

Stern, N.J., Hesseltine, C.W., Wang, H.L. and Konishi, F. 1977. Lactobacillus acidophilus utilization of sugars and production of a fermented soybean product. Can. Inst. Food Sci. Technol. J. 10: 197.

Sugimoto, H., Van Buren, J.P. and Robinson, W.B. 1971. An enzymatic process for a protein-containing beverage based on soybean protein and lemon juice. J. Food Sci. 36: 729.

Swaminathan, M. and Parpia, H.A.B. 1967. Milk substitutes based on oilseeds and nuts. World Rev. Nutr. Diet. 8: 184.

Wang, H.L., Kraidej, L. and Hesseltine, C.W. 1974. Lactic acid fermentation of soybean milk. J. Milk Food Technol. 37: 71.

Ms received 11/1/77; revised 1/12/78; accepted 1/18/78.

This investigation was supported in part by the Georgia Agricultural Commodity Commission for Peanuts. We are grateful for its financial assistance.

GERMINATION OF CORN AND SORGHUM IN THE HOME TO IMPROVE NUTRITIVE VALUE

YUH-YUN DAVID WANG and M. L. FIELDS

ABSTRACT

Corn and sorghum seeds were germinated at 25°, 30° and 35°C. Germinated seeds of both cereals had increased relative nutritive values (RNV) and increased levels of lysine, methionine, and tryptophan when compared to nongerminated seeds. The RNV's of the nongerminated corn and sorghum meals were 66.8 and 55.5% respectively while the RNV's of the germinated corn and sorghum were 99.5 and 62.9% respectively. The highest RNV for corn occurred after 4 days at 25°C, 2 days at 30°C and 3 days at 35°C. For sorghum, highest RNV was attained when the seeds were germinated for 5 days at 25°C, 6 days at 30°C and 3 days at 35°C. The highest RNV value achieved by germinating corn was 99.5%, whereas the highest for sorghum was 78.3%.

INTRODUCTION

IN MANY developing countries, corn and sorghum are the main staple of the diet of low income families. The protein quality of these two cereals is low because the protein of corn lacks adequate quantities of lysine and tryptophan while sorghum lacks lysine and total sulfur containing amino acids (FAO, 1970). Economical ways to correct these deficiencies are highly desirable.

One of the feasible methods is germinating the seeds. Recently researchers have studied the influence of germination upon the nutritive value of seeds. Whyte (1973) pointed out that during the germination process, stored materials are converted into more usable forms for both the plant and man. Chen et al. (1975) reported that the first stage in the germination of seeds involves the breakdown of seed reserves and their utilization by the growing root and shoot.

During the germination process, some constituents increase whereas others decrease. Fordham et al. (1975) found that ascorbic acid was higher in germinated beans and peas than in the nongerminated dry seeds. The amounts of niacin, pyridoxine, pantothenic acid, inositol, biotin, tocopherol and vitamin K increased in some germinated legumes (Banerjee et al., 1955). The contents of ascorbic acid, riboflavin, niacin and retinol increased in germinated soybeans (Chen, 1970).

Changes in the free amino acid content of germinated seeds have also been observed. Tsai et al. (1975) found that during the germination of corn, lysine and tryptophan increased, whereas the protein zein decreased. The amino acid composition of three varieties of sorghum was similar in total quantity but there was a considerable difference in the free and combined states (Horn and Schwartz, 1961). Glutamic acid and proline decreased during germination of barley while others, such as aspartic acid, alanine, glycine, lysine and arginine increased (Folkes and Yemm, 1958).

Srinivas et al. (1975) and Evancho et al. (1977) found that there was a good relation between the protein quality when measured by *Tetrahymena pyriformis* W method and the protein efficiency ratio (PER) which is determined by rat studies. Evancho et al. (1977) found that the relative nutritive value (RNV) and PER were related as follows: PER = 0.286 + 0.022 RNV. These values were correlated ($r = 0.90$) which was significant ($P < 0.01$).

In this paper, the conditions of time and temperature necessary to produce the highest RNV are presented. Since lysine, tryptophan and sulfur amino acids are limiting in corn and sorghum, the levels of these amino acids were determined as available amino acids (mg/gN).

Authors Wang and Fields are with the Dept. of Food Science & Nutrition, University of Missouri, Columbia, MO 65201.

0022-1147/78/0004-1113$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Corn and sorghum samples

Dried field corn (*Zea mays*) and milo sorghum (*Sorghum bicolor*) were obtained from a local grain dealer in Columbia, MO. The samples were cleaned by passing the grain over screens to remove broken grains and debris. Samples were stored under refrigeration (2.2°C) during the research. Commercially available high lysine corn was also obtained locally.

Soaking of seeds

Prior to germinating the seeds (either corn or sorghum), the cleaned corn and sorghum seeds were soaked in 4–5 volumes of water at room temperature for 16–18 hr. Soaking prior to incubating the seeds proved to give more uniform germination.

Washing the seeds

After soaking the seeds, they were washed, and re-washed 2 to 3 times a day thereafter until the last day of the germination period. Washing prevented the build-up of mold on the grains during germination.

Experiments with germinated seeds

The seeds were germinated by the jar method of Chen (1962). After soaked seeds were washed, they were placed in a quart glass jar. A piece of cheese cloth was used to cover the mouth of the jar and was secured by the metal ring cap of the jar. The cheesecloth allowed oxygen to enter the jar for the germinating seeds.

There were two experiments. There was one sample for each germination time and temperature for each experiment ($N = 1$). In the first experiment, the seeds were held at 30°C in an incubator for 3–4 days. In this experiment, the germinated seeds were separated from the nongerminated kernels and only the germinated kernels were used for the analyses. The nongerminated soaked seeds were discarded.

In the second experiment, the seeds were germinated by the jar method at 25°, 30° and 35°C in incubators for 2–6 days depending upon the temperature used. In this experiment, the germinated and the nongerminated seeds were not separated as in the first experiment but were dried and analyzed together. The percent germination and coleoptile length were determined as a measure of the germination process.

In both experiments, the seeds were dried overnight at 50°C in flowing air in a Freas model 835 dryer. Nonsoaked and nongerminated seeds served as the control for the two experiments.

Grinding of seeds and storage of samples

The dried seeds were ground in a Thomas-Wiley Laboratory Mill (model 4) using a 1 mm screen. The dried, ground corn and sorghum meals were placed in jars and stored at 2.2°C in glass jars until analyzed.

Nitrogen determination and relative nutritive value

The micro-Kjeldahl method of the AOAC (1975) was used to determine the nitrogen content of the ground seeds. The relative nutritive value (RNV) of the meals was determined using *Tetrahymena pyriformis* W. (ATCC 10542) according to Stott and Smith (1963).

Certified casein (Fisher Scientific Co., Fairlawn, NJ) was used as a reference protein. A test sample containing 12 mg N was weighed into a 100 ml beaker. The sample was extracted three times with diethyl ether at room temperature and once with 95% ethanol. The sample was finally washed with ether and air-dried at room temperature. A suspension of the washed sample in distilled water was made to give a N content of 3 mg/4 ml, and the pH was adjusted to 8.2.

Four milliliters of the sample suspensions were introduced into each of 3, screw-capped culture tubes (20 × 150 mm). Working solutions of minerals, nucleic acids, glucose and vitamins were then added in the sequence described by Stott and Smith (1963). *Tetrahymena* medium, prepared following the formula of ATCC, was used for maintaining the culture and for preparation of the *inoculum*. Each culture tube, containing a suspension of autoclaved test material and supplemented

Table 1—Influence of germination on relative nutritive value (RNV), calculated Tetrahymena PER, lysine, methionine and tryptophan contents of corn and sorghum

Treatment	% RNV	Calculated Tetrahymena PER	Available amino acid mg/gN Lysine	Methionine	Tryptophan
Corn meal[a] (Control)	66.8	1.8	22.5	9.0	2.0
Corn meal[b] (from germinated corn)	99.5	2.5	56.5	45.0	13.0
Sorghum meal[a] (Control)	55.3	1.5	13.5	8.5	3.4
Sorghum meal[b] (from germinated sorghum)	62.9	1.7	30.0	15.0	9.5

[a] Meal prepared from whole seed not germinated N = 1
[b] Seeds germinated at 30°C for 3–4 days

nutrients, was inoculated with 3 drops of a 3-day broth culture of the organism. After inoculation, the tubes were incubated in inclined position (about 15°) at room temperature (25°C) for 4 days.

After incubation, the culture tube was shaken on a Vortex Genie vibrator (Scientific Industries, Springfield, MA) for a few seconds, and 1 ml of the culture was then transferred to a 15 × 150 mm screw-capped culture tube containing 1 ml of 36% formaldehyde preserving fluid. *Tetrahymena* cells were counted in a double-cell Lumicyte hemacytometer (Propper Manufacture Co., Inc., Long Island City, NY). Cells in eight alternate mm^2 squares were counted, and the mean number per mm^2 was used to calculate the population of the test culture in units of 10^4 organisms/ml. The RNV in percentage was calculated according to the formula derived by Helms and Røllе (1970):

$$RNV = \frac{\log (\text{count for test protein}) - \log (\text{count for inoculum})}{\log (\text{count for casein}) - \log (\text{count for inoculum})} \times 100$$

The equation of Evancho et al. (1977) was used to estimate the PER value.

Determination of available lysine, methionine and tryptophan

The microbiological assay method of Difco (1977) was employed for the determination of available lysine, methionine, and tryptophan. *Pediococcus cerevisiae* ATCC 8042 was the test organism for the lysine and the methionine assays. *Lactobacillus plantarum* ATCC 8014 was used for the tryptophan assay. Reagent-grade L (+)-lysine monohydrochloride, DL-tryptophan (both of Eastman Kodak Co.) and DL-methionine (Fisher Scientific Co.) were used in preparing standards.

Samples of germinated and nongerminated cereals containing 100 mg N were predigested with 1 ml of 2% (w/v) papain solution for 3 hr at 56°C, as described by Ford (1964). After enzymic digestion, each preparation was adjusted to pH 7.2 and diluted with distilled water to 100 ml. They were then shaken vigorously and 20 ml portions were diluted to 100 ml with distilled water. This dilution was added to tubes in amounts of 1, 2, 3, 4 and 5 ml. Five milliliters of the amino acid assay medium (Difco Lab., Detroit, MI) were then added to each assay tube and all the tubes were autoclaved for 10 min at 121°C. After cooling, each tube was inoculated with one drop of diluted suspension of test culture and the tubes were incubated at 35–37°C for 72 hr. The acidity of each culture was titrated with 0.1N NaOH to pH 6.8, the original pH of the media, at the end of the incubation period. The standard curves for lysine, methionine and tryptophan were constructed following the Difco Technical Information (1977). The amino acid content of each sample was calculated based on the respective standard amino acid curve.

RESULTS & DISCUSSION

THE RESULTS of the first experiment are given in Table 1. Corn meal (control) made from whole kernel, field corn had an RNV of 66.8% or a calculated *Tetrahymena* PER of 1.8. Available lysine, methionine and tryptophan values were 22.5, 9.0 and 2.0 mg/gN, respectively.

In this experiment, only the germinated corn and sorghum seeds were used for analysis. The % RNV increased from 66.8 to 99.5 (100 is the value for casein). Lysine increased 2.5 times in corn meal made from germinated seeds as compared to the control, and the tryptophan increased 6.5 times. The methionine content also increased 5 times over the control (Table 1).

The increase in RNV was not as great with sorghum as with corn after germination. The RNV increased from 55.3% to 62.9% or from a *Tetrahymena* PER of 1.5 to 1.7. Lysine increased 2.2 times, methionine 1.8 times and tryptophan 5.7 times over the same amino acid values of the control samples.

The RNV is an indication of the amino acid balance. *T. pyriformis* W measures amino acids that are required by humans and rats (Røllе, 1975; Kidder and Dewey, 1961). Since RNV is correlated well with rat feeding studies (PER), the increases found in RNV values indicate that the amino acid balance had been improved by germination.

Since this research was being related to developing countries, as well as in the United States, in which temperature might vary from 25°C to 35°C, germination was measured at 25°, 30° and 35°C. In the second experiment data in Table 2 indicate that RNV values for the germinated corn were higher than control corn at each temperature. The amino acid values were also greater in the germinated samples, although the values differ depending on the temperature of germination. Calculated *Tetrahymena* PER values had an increase from 1.7 to a maximum of 2.3 at each temperature.

The trends of increased RNV and increased available lysine, tryptophan and methionine were the same for corn in both experiments (Table 1 and 2). These data are similar to Tsai et al. (1975) in that increased lysine and tryptophan occurred.

FAO (1970) reported rat PER values for maize as follows: 1.05, 1.13, 1.18 and 1.41. The calculated *Tetrahymena* PER in this research was higher than those reported by the FAO. The calculated *Tetrahymena* PER values for high lysine corn were less than the two values (2.31 and 2.57) reported by FAO but the germinated value (2.3) was in agreement.

The data for sorghum in the second experiment are given in Table 3. The percent germination was much lower than that of the corn. The increase in RNV was from 55.3 to 62.9% but these seeds were incubated at 30°C for 3–4 days (Table 1), whereas data in Table 3 show that after 6 days a maximum RNV of 78.3 (calculated *Tetrahymena* PER of 2.0) was obtained (Table 3). Even in only 30% of the seeds germinated at 30°C for 6 days, the RNV was greater (78.3%) than the RNV was greater (78.3%) than the RNV of the seeds incubated at 30°C for 3–4 days with all germinated seeds being used. This indicates that more biochemical changes occurred in the 6-day germinated seeds to bring about the observed RNV.

Again, there was a trend for an increase in the available lysine, tryptophan and methionine contents in the germinated sorghum seeds (Table 3) as in experiment 1.

FAO (1970) listed rat PER values of 1.61, 1.78 and 1.95 for sorghum. The calculated *Tetrahymena* PER for the control was 1.5 while the highest calculated PER after germination was 2.0.

From the data in Table 2, a germination period of 4 days at 25°C, 2 days at 30°C and 3 days at 35°C increased the nutritive value of corn in the shortest period of time. For sorghum the times and temperatures of germination which produced the highest RNV values were: 5 days at 25°C, 6 days at 30°C and 3 days at 35°C (Table 3).

The data in this study suggest that germination of corn and sorghum is a procedure that can be used at home to produce a more nutritious ingredient from which to produce foods in developing countries or in the United States. Further research on germination should be carried out in a home environment to establish its practical value.

Table 2—Effect of time and temperature on relative nutritive value (RNV), available lysine, tryptophan and methionine content of germinated and nongerminated corn seeds[a]

Treatment	Temp °C	Time days	% Germination	Coleoptile length cm	RNV %	Calculated Tetrahymena PER[b]	Available amino acid[c] (mg/gN) Lysine	Tryptophan	Methionine
Control nongerminated	–	0	–	–	62.2	1.7	22.5	4.0	9.0
Germinated	25	2	20–30	0.2–1.0	70.8	1.8	36.0	6.4	10.8
		3	30–40	1.0–1.5	76.7	2.0	45.0	12.4	20.4
		4	70–80	1.5–4.0	89.4	2.3	80.0	38.0	59.0
		5	70–80	4.0–10.5	92.6	2.3	52.5	19.0	42.0
	30	2	40–50	0.1–1.0	89.7	2.3	26.3	12.0	24.6
		3	50–60	1.5–2.5	89.4	2.3	48.8	16.0	30.0
		4	60–70	3.5–6.0	88.8	2.2	33.8	22.0	46.4
		5	60–70	3.5–6.3	91.0	2.3	33.8	23.0	34.8
		6	60–70	–	84.9	2.2	38.8	18.0	35.4
	35	2	40–50	0.5–1.5	79.6	2.0	36.0	9.6	17.4
		3	50–60	2.0–3.0	89.7	2.3	37.5	13.0	24.0
		4	50–60	2.5–4.5	88.4	2.2	45.0	23.0	22.2
High lysine, nongerminated		0	–		71.8	1.9	24.0	5.6	6.0
High lysine, nongerminated	30	4	90	3.0–6.5	90.7	2.3	97.5	38.0	46.4

[a] N = 1. Seeds were germinated in quart glass jars (a mixture of germinated and nongerminated), dried at 50°C and ground in a Wiley mill.
[b] PER = 0.286 + 0.022 (RNV). Values rounded to nearest one-tenth of PER.
[c] Expressed as mg of L-Lysine, DL-tryptophan and DL-methionine.

Table 3—Effect of time and temperature on relative nutritive value (RNV), available lysine, tryptophan, and methionine content of germinated and nongerminated sorghum seeds[a]

Treatment	Temp °C	Time days	% Germination	Coleoptile length cm	RNV %	Calculated Tetrahymena PER[b]	Available amino acid[c] (mg/gN) Lysine	Tryptophan	Methionine
Control, nongerminated	–	0	–		54.6	1.5	13.5	6.8	8.5
Germinated	25	2	10–15	0.2–0.4	48.6	1.4	24.0	4.8	8.4
		3	15–20	0.5–1.0	54.0	1.5	33.0	7.6	11.5
		4	25–35	2.5–5.5	67.8	1.8	45.0	15.2	18.6
		5	25	2.5–8.5	68.9	1.8	28.0	15.0	15.3
	30	2	10	0.0–1.0	52.4	1.4	15.0	7.2	7.2
		3	10–15	2.5–4.5	62.1	1.7	21.0	8.8	7.5
		4	20–30	2.5–7.0	58.0	1.6	33.0	12.0	13.8
		5	30	3.5–7.5	62.4	1.7	33.0	15.2	14.3
		6	30	5.0–10.0	78.3	2.0	69.0	18.6	19.5
	35	2	15–20	2.0–3.0	54.7	1.5	30.0	9.4	14.0
		3	10	3.5–5.5	62.4	1.7	26.3	8.0	10.2
		4	10	4.0–7.0	63.0	1.7	24.0	12.0	10.0

[a] N = 1. Seeds were germinated in quart glass jars, dried at 50°C and ground in a Wiley mill.
[b] PER = 0.286 + 0.022 (RNV). Values rounded to nearest one-tenth PER.
[c] Expressed as mg of L-lysine, DL-tryptophan and DL-methionine.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Agricultural Chemists, Washington, DC.

Banerjee, S., Rohatgi, K., Banerjee, M. and Chattopadhyay, H. 1955. Pyridoxine, inositol and vitamin K contents of germinated pulses. Food Res. 20: 545.

Chen, P.S. 1962. "Soybeans for Health, Longevity and Economy," p. 117. The Chemical Elements, South Lancaster, MA.

Chen, P.S. 1970. "Soybeans for Health, Longevity and Economy," 3rd ed., p. 117. Proocker Press, St. Catherine, Ontario, Canada.

Chen, L.H., Wells, C.E. and Fordham, J.R. 1975. Germinated seeds for human consumption. J. Food Sci. 40: 1290.

Difco. 1977. "Difco Technical Information. Media for the Microbiological Assay of Vitamins and Amino Acids," p. 59; p. 67. Difco Laboratories Inc., Detroit, MI.

Evancho, G.M., Hurt, H.D., Devlin, P.A., Landers, R.E. and Ashton, D.H. 1977. Comparison of Tetrahymena pyriformis W and rat bioassays for the determination of protein quality. J. Food Sci. 42: 444.

FAO. 1970. "Amino Acid Content of Foods and Biological Data on Proteins." Food and Agriculture Organization of the United Nations, Rome, Italy.

Folkes, B.F. and Yemm, S.W. 1958. The respiration of barley plants. 10. Respiration and the metabolism of amino acids and protein in germinated grain. New Phytologists 57: 106.

Ford, J.E. 1964. A microbiological method for assessing the nutritional value of protein. 3. Further studies on the measurement of available amino acids. Brit. J. Nutr. 18: 449.

Fordham, J.R., Wells, C.E. and Chen, L.H. 1975. Sprouting of seeds and nutrient composition of seeds and sprouts. J. Food Sci. 40: 552.

Helms, P. and Rølle, G. 1970. Nutritive evaluation of protein quality with Tetrahymena pyriformis W. In "Evaluation of Novel Protein Products," Ed. Benden, Z.A., Kehlberg, R., Lofquist, B. and Munck, L. Pergamon Press, New York.

Horn, P.J. and Schwartz, H.M. 1961. Kaffir corn malting and brewing studies. 9. Amino acid composition of kaffir corn grain and malt. J. Sci. Food Agri. 12: 457.

Kidder, G.W. and Dewey, V.C. 1961. Cited in "Biochemistry and Physiology of Protozoa," Vol. 1, p. 323, Ed. Lwoff, A. Academic Press, Inc., New York.

Rølle, G. 1975. Research on the quantitative requirements of essential amino acids by Tetrahymena pyriformis W. Acta. Agric. Scan. 25: 17.

Srinivas, H., Vakil, U.K. and Sreenivasan, A. 1975. Evaluation of protein quality of irradiated foods using Tetrahymena pyriformis W and rat assay. J. Food Sci. 40: 65.

Stott, J.A. and Smith, H. 1963. Microbiological evaluation of protein quality with Tetrahymena pyriformis W. 3. A simplified procedure. Brit. J. Nutr. 17: 227.

Tsai, C.Y., Dalby, A. and Jones, R.A. 1975. Lysine and tryptophan increased during germination of maize seed. Cereal Chem. 52: 356.

Whyte, K.C. 1973. "Complete Sprouting Cookbook," p. 14. Troubador Press, San Francisco, CA.

Ms received 9/26/77; revised 1/14/78; accepted 1/18/78.

Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 7900.

CORNMEAL/PEANUT FLOUR BLENDS AND THEIR CHARACTERISTICS

G. N. BOOKWALTER, K. WARNER, R. A. ANDERSON and E. B. BAGLEY

ABSTRACT

The effect of fortifying degermed cornmeal with defatted peanut flour (DPF) was evaluated by studying the nutritional quality, color properties, flavor, storage stability and product applications. Protein efficiency ratio (PER) increased from 0.3–1.4 with 15% DPF. A PER of 2.0 resulted with the addition of 0.18% *L*-lysine HCl to the blend. Color changes are minimized by levels of 15% or less DPF. Taste panel results indicated no significant change in preference, acceptability and similarity to cornmeal when as much as 20% peanut flour was contained in the blend. Storage stability was satisfactory as indicated by changes in flavor, peroxides, free fatty acids and available and added lysine after storage for 2, 6 and 12 months at 49, 37 and 25°C, respectively. Leavened and unleavened breads were acceptable when prepared with blends containing up to 20% DPF.

INTRODUCTION

DEGERMED, YELLOW CORNMEAL has been provided to malnourished people by the United States for more than a generation through various foreign donation programs. While cornmeal is very palatable when used in a variety of food applications, consideration was given to improving its food value by protein supplementation. It was recognized that corn and soybean proteins complement each other (Bressani and Elias, 1966; Dimler, 1967). This prompted the development of a soy-fortified cornmeal (SFCM) by Bookwalter et al. (1971a) which was included in the Food for Peace Program (USDA, 1975a). This blend is composed of degermed cornmeal and 15% defatted toasted soy flour plus added calcium, iron, thiamine, riboflavin, niacin and vitamin A. SFCM was more nutritious than degermed cornmeal, although both were interchangeable in common and traditional food preparations.

In 1976, 36 million pounds of SFCM, valued at $3.3 million was donated to recipient countries under the "International Development and Food Assistance Act of 1975." All current shipments of cornmeal are soy-fortified as a result of the removal of nonfortified cornmeal from the commodity availability list, effective for fiscal year 1977. This action will result in substantially increased shipments of SFCM. According to data from the Agency for International Development, U.S. Department of State, SFCM is currently utilized in overseas maternal child feeding (23%), school feeding (23%), food for work projects (48%) and disaster relief (6%).

In addition to soy, peanuts are also recognized as a valuable source of vegetable protein (Harris et al., 1972). Peanuts are grown primarily for human food in the United States. The major portion of the U.S. crop is used in the manufacture of peanut butter or is consumed directly (USDA, 1976). Although variable, about one-fifth of the crop is crushed, resulting in an edible oil and a meal residue which is generally used for animal feed. Work by Ayres et al. (1974) has shown that the meal residue can be further processed under sanitary conditions to achieve edible peanut flour and grits products.

Specific objectives of the experiments reported here were to evaluate an edible peanut flour as a protein source in SFCM-type formulations. Side-by-side comparisons were made with soy flour in order to evaluate the nutritional characteristics, color, flavor, storage stability and performance in food applications.

All authors are with the USDA Northern Regional Research Center, ARS, Peoria, IL 61604.

Table 1—Analyses[a] of corn, peanut and soy products and their formulations[b]

Base materials and combinations	Moisture (%)	Protein[c] (%)	Fat (%)	Ash (%)	Crude fiber (%)
Base materials:					
Cornmeal, degermed (C)	7.9	8.3	1.4	0.5	0.5
Peanut flour, defatted (P)	8.6	68.6	0.6	5.3	3.8
Soy flour, defatted, toasted (S)	7.2	54.8	1.4	7.0	3.3
5% P-95% C	7.7	11.7	1.2	0.7	0.6
15% P-85% C[d]	7.9	17.0	1.2	1.3	1.0
25% P-75% C	7.0	22.7	1.1	1.7	1.1
35% P-65% C	7.0	29.7	1.0	2.2	1.4
50% P-50% C	8.2	38.4	1.0	2.9	2.2
5% S-95% C	7.8	10.6	1.4	0.8	0.6
15% S-85% C[d]	7.9	15.4	1.4	1.5	0.9
25% S-75% C	6.9	20.3	1.2	2.1	1.1
35% S-65% C	7.0	25.0	1.3	2.7	1.5
50% S-50% C	7.5	31.5	1.4	3.7	1.9
7.5% P-7.5% S-85% C	7.9	16.1	1.5	1.4	1.1
12.5% P-2.5% S-85% C	7.9	16.4	1.4	1.3	1.1

[a] Dry matter basis
[b] Enrichment per pound added: 500 mg calcium, 2 mg thiamine, 1.2 mg riboflavin, 16 mg niacin, 13 mg iron and 4000 IU vitamin A.
[c] Nitrogen X 6.25.
[d] *L*-lysine HCl (0.18–0.71%) added to some formulas.

MATERIALS & METHODS

Ingredients and formulations

Commercial and laboratory-prepared ingredients were utilized in the preparation of the experimental blends. They are listed with their analyses in Table 1. The degermed yellow cornmeal was dry-milled to the specifications shown in Announcement CM-7 (USDA, 1975b). The defatted peanut flour was prepared from selected peanuts by cleaning, dehulling, blanching (skin removal), cracking, tempering, cooking, prepressing, flaking, defatting with hexane, desolventizing and grinding according to Ayres et al. (1974). The toasted defatted soy flour was prepared to meet specifications of Announcement SFCM-2 (USDA, 1975a). Other ingredients used in this study complied with commercial standards of identity.

Blends of degermed cornmeal containing 5–50% of defatted peanut flour along with paired samples containing defatted toasted soy flour were prepared. Table 1 shows the analytical profiles of the various combinations.

Analytical tests

Peroxide values, free fatty acids of the pentane-hexane extracted fat and other fat assays were determined by standard procedures (AOCS, 1970). Available lysine was measured by a method based on reaction with dinitrofluorobenzene (Rao et al., 1963). Added lysine was measured according to the method of Finley et al. (1972). Moisture analyses were made on a Brabender moisture tester (120°C, 60 min). Other chemical analyses were determined by standard procedures (AACC, 1962). Protein efficiency ratio (PER) was determined by the method outlined in AOAC (1975), with the diets standardized to 10% protein except for the cornmeal which was tested "as is" and compared to an Animal Nutrition Research Council casein reference standard adjusted

to 2.5 PER. Digestibility data was obtained from the 7th through 14th day of the PER tests. Color comparisons were made by visual observation and color measurements with a Hunter Model D-25 color difference meter.

Flavor evaluation

Tests were conducted to determine the amount of defatted peanut flour which can be added to degermed cornmeal without changing the organoleptic characteristics. Paired, randomized, coded samples, one containing all cornmeal and the other containing a blend of up to 50% peanut flour, were presented as cooked gruels containing 10% solids, to each of 14 taste panelists for organoleptic evaluation. Each pair of samples was rated for preference, acceptability and identification of the all-cornmeal control. This procedure was followed for each of the blends of degermed cornmeal containing from 5 to 50% defatted peanut flour.

Stoarge stability

Stability tests were made on blends of degermed cornmeal with 15% peanut or soy flours, with and without the inclusion of 0.18% *L*-lysine-HCl. The mixtures were packaged in glass containers with foil-lined screw closures. Such containers maintained moisture at constant levels in samples stored at 37 and 49°C and at approximately 25°C room temperature. Controls were held at −18°C. Withdrawals from storage were made at 49°C after 0, 1 and 2 months; at 37°C after 3 and 6 months; and at 25°C after 6 and 12 months. The samples were evaluated for changes in peroxide values, free fatty acids, available lysine, added *L*-lysine HCl and flavor.

The samples were made into cooked gruels (10% solids) by stirring into boiling water and cooking for 2 min. Flavor changes were evaluated by a trained, experienced 14-member taste panel during the 12 months of storage. The product combinations, stored at −18°C and at an elevated temperature, were randomized and compared for flavor at each time interval. Samples were rated for flavor according to the 10-point scoresheet described by Bookwalter et al. (1971b). Scores of 6 and above were considered acceptable. Samples with objectionable flavors were assigned scores of 5 and below. Statistical evaluations were made on the experimental data after completion of each taste panel series. Analysis of variance, regression analysis (Snedecor and Cochran, 1968) and Duncan's multiple range test (Duncan, 1955) were used to assess results.

Table 2—PER values and digestibility of various combinations of degermed cornmeal, defatted peanut flour and defatted, toasted soy flour, with or without L-lysine HCl

Blends of cornmeal with peanut and/or soy flour (%)	Added *L*-lysine-HCl (% of blend)	PER[a]	Digestibility (%)[b] Diet	Nitrogen
Peanut, 15	–	1.42 D	94	86
Soy, 15	–	2.09 A	93	84
Peanut, 12.5; soy, 2.5	–	1.62 CD	93	84
Peanut, 7.5; soy, 7.5	–	1.79 BC	94	85
Peanut, 15	0.18	2.02 A	94	86
Peanut, 15	0.36	1.95 AB	94	88

[a] PER (protein efficiency ratio) = gram weight gain per gram protein intake. Animal Nutrition Research Council casein = 2.50. Two PER values with no common letter, within A-D, differ significantly (5% level).

[b] Digestibility: Diet = (feed intake − fecal weight)/feed intake X 100. Nitrogen = (N intake − fecal N)/N intake X 100.

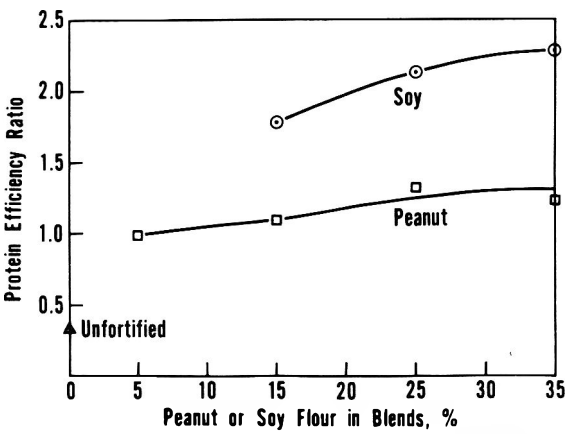


Fig. 1—Protein efficiency ratios in various blends of degermed cornmeal with defatted peanut and defatted, toasted soy flour. ANRC casein = 2.50. All samples were tested in the same feeding study except the unfortified degermed cornmeal.

Food applications

Leavened and unleavened breads constitute major food uses for SFCM. The experimental cornmeal blends with 0–50% peanut flour were compared side by side with the same levels of soy flour in unleavened and leavened breads. Chapattis were chosen as an example of an unleavened bread while northern corn bread was prepared to exemplify a leavened bread. The products were prepared according to Bookwalter et al. (1971a).

RESULTS & DISCUSSION

Nutritional aspects

The quantity of protein in the blends increased with increasing amounts of either peanut or soy flour and decreasing levels of cornmeal (Table 1). Higher protein contents were achieved with peanut than with soy flour at the same levels. The protein quality of the unfortified cornmeal was improved (Fig. 1) by the inclusion of either peanut or soy flour. However, PER values of blends containing peanut were significantly lower than those containing soy at the same addition levels. The nutritive quality of peanut protein is limited primarily by its content of lysine and methionine (Milner, 1962). Soy protein is also deficient in methionine but is high in lysine, the first limiting amino acid in degermed cornmeal (Dimler, 1967; Kato and Muramatsu, 1971). We found the sulfur amino acid levels to be similar in both peanut and soy proteins. Available lysine values for the corn, peanut and soy products were 2.3, 3.2 and 6.1% of protein, respectively. Available lysine of cornmeal containing 15% of peanut and soy flours was approximately 2.3 and 3.2% of protein, respectively. The available lysine differences could account for the PER differences shown in Figure 1. We found threonine levels of 2.5, 4.2 and 3.1% for peanut, soy and corn proteins, respectively, which could also contribute to differences in PER values. Total sulfur amino acid levels of peanut and soy proteins were approximately 2.0% and those of corn protein were about 3.4%.

Blends of cornmeal and 15% peanut flour were tested for PER with and without the inclusion of 0.18% *L*-lysine HCl (Table 2). There were no significant differences in PER values of a 15% peanut-fortified cornmeal with added 0.18% *L*-lysine HCl and a 15% soy-fortified cornmeal. There was no further improvement when the level of added *L*-lysine HCl was increased to 0.36% or as much as 0.71%. When soy was substituted for one-half of the peanut flour, a significantly improved PER value was obtained (Table 2).

Color properties

By visual observation, the yellow color of degermed yellow cornmeal changes to a light yellowish buff color with the addition of increased levels of either peanut or soy flour. Moreover, the product texture changes from a granular to floury appearance. At peanut or soy flour levels of 15% or less, color and texture differences are minimized. However, at levels of 20–50% the products are characterized by decreasing yellow color and a more floury texture, noticeably different from the all-yellow cornmeal sample. Blends of peanut or soy flour with cornmeal at the same addition level had a similar appearance. Hunter color measurements were determined for blends of cornmeal containing 0–50% peanut or soy flours as well as for all peanut and soy flour. Figure 2 shows plots of L (lightness), *a* (+red, −green) and *b* (+yellow, −blue) values. L-values increased to about the same extent with increased levels of either peanut or soy flour. The L-values were practically identical for the same levels of peanut and soy flour blended with

Table 3—Organoleptic comparisons of degermed cornmeal with blends containing defatted peanut flour

Peanut flour in blend (%)	No. of panelists	Acceptability		Preference			Identificatior of all cornmeal sample	
		All cornmeal	Cornmeal-peanut blend	All cornmeal	Cornmeal-peanut blend	No preference	All cornmeal	Cornmeal peanut blend
5	13	13[a]	13[a]	3	7	3	9	4
10	13	13[a]	13[a]	4	7	2	9	4
15	12	12[a]	12[a]	8	4	–	8	4
20	13	13[a]	13[a]	8	4	1	9	4
35	14	14[a]	14[a]	8	5	1	14[a]	0
50	13	13[a]	13[a]	12[b]	1	–	13[a]	0

[a] Significant (1% level)
[b] Significant (5% level)

cornmeal. The *a*-values of the blends were also very similar at the same level of fortification and decreased to about the same extent to near zero with additions of the same amount of peanut or soy flour. Changes which were most apparent by visual observation were associated with the Hunter +*b*-values, the indicator of yellowness. The +*b*-values were changed to the greatest extent by increased amounts of either peanut or soy flour in the blends. The *b*-values of the blends were very similar at the same fortification level and decreased to about the same extent with additions of the same amount of peanut or soy flour. The L, *a*, *b* values for all peanut or soy flour are very similar as shown in Figure 2.

Flavor evaluation

Flavor comparisons of degermed cornmeal with blends containing defatted peanut flour are summarized in Table 3. All coded, randomized combinations, including the all cornmeal sample and blends with up to 50% peanut flour, were found acceptable at the 1% significance level. There was no significant preference between all cornmeal and blends with up to 35% peanut flour. There was a significant preference for the all cornmeal sample when compared to the blend containing 50% peanut flour. When panelists were told to identify one of the two coded, randomized samples as being most like a control sample of cornmeal, significantly correct identifications were not made when the amount of peanut flour was at the 20% level or lower. Significantly correct identification of the all cornmeal was made when compared with the blends containing 35 or 50% peanut flour. Changes in texture as well as in flavor of these two blends probably aided the panel members in identifying the all-cornmeal sample. Blending as much as 20% defatted peanut flour with degermed cornmeal resulted in no important change in organoleptic properties. This is in close agreement with similar tests with blends of degermed cornmeal and defatted, toasted soy flour (Bookwalter et al., 1971a).

Storage stability

Mean flavor scores of stored blends of degermed cornmeal containing 15% of either defatted peanut or defatted, toasted soy flour, with and without 0.18% *L*-lysine HCl are shown in Table 4. Mean flavor scores are shown for each time-temperature condition which were compared with a control sample stored at −18°C. All flavor scores were satisfactory (6.6 or above) during storage at these conditions. The overall standard deviation was 0.157, and the least significant difference for comparing any two mean flavor scores was approximately 0.5. A statistical analysis of the pooled data indicated no significant differences attributable to flour type or added *L*-lysine HCl. There were significant flavor changes associated with storage. However, satisfactory flavor stability is indicated for blends of degermed cornmeal fortified with peanut or soy flour, with or without added *L*-lysine HCl. These results are similar to previous work with SFCM (Bookwalter et al., 1971a).

Analyses of the various stored blend combinations are shown in Table 5. Initially, peroxide values and free fatty acids of the extracted fat were slightly higher for blends with peanut flour, but this trend did not continue after storage at elevated temperatures. At the various storage conditions, peroxide values were sufficiently low to indicate adequate stability. Free fatty acids were slightly lower for blends containing peanut flour, although all are similar to previous results with SFCM (Bookwalter et al., 1971a). The effect of free fatty acids is minimized because the fat content is low (Table 1). The presence of *L*-lysine HCl had no effect on stability as indicated by peroxide values and free fatty acids. Available lysine values for blends containing peanut flour were approximately 1% lower than those containing soy flour (Table 5). Similar lysine values were achieved by adding 0.18% *L*-lysine HCl to the blends containing 15% peanut flour. The blends with 15% soy flour were also given the same treatment to gain further information on stability. All lysine values after storage at these conditions are similar to their respective controls held at −18°C. There

Table 4—Mean flavor scores[a] of stored degermed cornmeal containing 15% defatted peanut or defatted, toasted soy flour, with and without 0.18% L-lysine HCl

Fortifying components	Temperature (months)						
	−18°C	49°C		37°C		25°C	
	0	1	2	3	6	6	12
Peanut flour (PF)	8.0	7.5	7.1	7.9	7.3	7.6	7.7
Soy flour (SF)	8.0	7.5	6.6	7.8	7.3	7.6	7.8
PF + *L*-lysine HCl	8.0	7.4	7.4	7.4	7.3	7.5	7.3
SF + *L*-lysine HCl	8.0	7.7	7.1	7.8	7.4	7.8	7.4

[a] Each mean score based on scores from 14 panelists. The least significant difference (5% level) for comparing any two values is approximately 0.5.

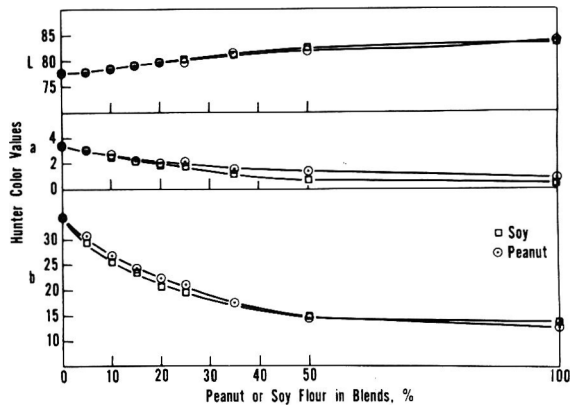


Fig. 2—Hunter color values (L = lightness; a = +red, −green; b = +yellow, −blue in various blends of degermed cornmeal with defatted peanut and defatted, toasted soy flour.

Table 5—Analyses of stored degermed cornmeal containing 15% defatted peanut or defatted, toasted soy flour, with and without 0.18% L-lysine HCl

Fortifying components	Storage		Peroxide value (meq/kg fat)	Free fatty acids (% oleic)	Available lysine (% of protein)	Added L-lysine HCl (% of blend)
	Months	Temp (° C)				
Peanut flour (PF)		−18	15	6.5	2.3	–
Soy flour (SF)		−18	4	6.0	3.2	–
PF + L-lysine HCl (L)		−18	12	7.0	3.3[a]	0.18
SF + L-lysine HCl (L)		−18	3	6.1	4.2[a]	0.17
PF	2	49	5	32	2.3	–
SF	2	49	3	34	3.5	–
PF + L	2	49	4	32	3.2[a]	0.17
SF + L	2	49	9	34	4.5[a]	0.16
PF	6	37	4	37	2.0	–
SF	6	37	1	41	3.2	–
PF + L	6	37	2	37	3.1[a]	0.20
SF + L	6	37	2	39	4.2[a]	0.18
PF	12	25	3	33	2.2	–
SF	12	25	3	35	3.3	–
PF + L	12	25	4	35	3.2[a]	0.18
SF + L	12	25	2	36	4.3[a]	0.19

[a] Total available lysine (available plus added L-lysine HCl)

were no differences in stability as indicated by changes in either available lysine values or added L-lysine HCl. These data indicate the practicality of adding L-lysine HCl to blends with peanut flour in order to achieve improved nutritional characteristics.

Food applications

Test results showing the characteristics of unleavened bread made with cornmeal blended with up to 50% peanut or soy flours are shown in Table 6. Water absorption increased with additional amounts of peanut or soy flour in the blends. Therefore, it was necessary to add extra water to maintain workable doughs. More water correction was necessary with blends containing 35% or more soy flour than with comparable levels of peanut flour. Improvements in dough cohesion and pliability were noted with increased levels of peanut or soy flour in the blends. Both the dough color and final cooked crumb color became increasingly darker with increased amounts of peanut or soy in the blends. Color changes were less with cornmeal-peanut than with comparable levels of cornmeal – soy mixtures. The external appearance of cooked unleavened bread was similar at each level of peanut or soy flour. Increased levels of both types of blends resulted in increased gas retention as evidenced by internal areas, or bubbles, of steam. The surfaces of the bubbles became darker during cooking which gave a blistered appearance of a typical Indian chapatti. Product texture was similar for each level of peanut or soy flour. Texture became less grainy and smoother and the final cooked products became more tender with increased amounts of peanut or soy flours. Acceptable unleavened bread was made with blends containing as much as 20% defatted peanut flour.

Baking results for chemically leavened corn bread are contained in Table 7. Batter viscosity increased with increasing levels of either peanut or soy flour in the formulations. Viscosity was changed to a lesser extent by peanut than by soy flour. Loaf volume, as indicated by center height, increased slightly by the inclusion of either type of flour. Crumb color became progressively darker with both flour types; however, this changed to a lesser extent in corn bread made with the cornmeal-peanut flour blends. Grain and texture were slightly more open and slightly coarser with increased levels of both flour types. Acceptable corn bread was made with blends containing as much as 20% peanut flour.

CONCLUSIONS

FOODS with improved nutritional value, satisfactory quality

—Text continued on page 1120

Table 6—Characteristics of blends of degermed cornmeal fortified with up to 50% of defatted peanut or defatted, toasted soy flour in unleavened bread

Fortifying components (%)	Increased[a] dough water (g)	Dough properties	Dough color[b]	Cooked external appearance	Crumb color	Texture
0 – (All-degermed cornmeal)	None	Short-grainy	100	Smooth	100	Grainy
5 – Peanut	4	Short-grainy	100	Trace blistered	100	Grainy
10 – Peanut	8	Sl. short-grainy	99.5	Sl. blistered	100	Grainy
15 – Peanut	12	Pliable-smooth	98.5	Trace blistered	99.5	Sl. grainy
20 – Peanut	16	Pliable-smooth	98	Sl. blistered	98.5	Sl. grainy
35 – Peanut	22	V. pliable-v. smooth	97.5	Blistered	97	Sl. smooth
50 – Peanut	34	Soft-v. smooth	96	Blistered	96	Smooth
5 – Soy	4	Short-grainy	100	Trace blistered	100	Grainy
10 – Soy	8	Sl. short-grainy	99	Trace blistered	99	Grainy
15 – Soy	12	Sl. pliable-sl. smooth	98	Trace blistered	98.5	Sl. grainy
20 – Soy	16	Pliable-smooth	97	Sl. blistered	97	Sl. grainy
35 – Soy	28	Pliable-smooth	94	Blistered	95	Sl. smooth
50 – Soy	40	Sl. soft-v. smooth	93	Blistered	93	Smooth

[a] Increased water to maintain similar handling properties
[b] 100 = light yellow; 93 = tan.

and storage stability can be prepared with blends which utilize degermed cornmeal and defatted peanut flour.

REFERENCES

AACC. 1962. "Cereal Laboratory Methods." 7th ed. American Association of Cereal Chemists, St. Paul, MN.

AOAC. 1975. "Official Methods of Analysis." Ed. Horwitz, W., 12th ed. Association of Official Agricultural Chemists, Washington, DC.

AOCS. 1970. "Official and Tentative Methods," Ed. Mehlenbacher, V.C., Hopper, T.H. and Sollee, E.M., 3rd ed. American Oil Chemists' Society, Chicago, IL.

Ayres, J.L., Branscomb, L.L. and Rogers, G.M. 1974. Processing of edible peanut flour and grits. J. Am. Oil Chem. Soc. 51: 133.

Bookwalter, G.N., Kwolek, W.F., Black, L.T. and Griffin, E.L. 1971a. Cornmeal/soy flour blends: Characteristics and food applications. J. Food Sci. 36: 1026.

Bookwalter, G.N., Moser, H.A., Kwolek, W.F., Pfeifer, V.F. and Griffin, E.L. 1971b. Storage stability of CSM: Alternate formulations for corn-soy-milk. J. Food Sci. 36: 732.

Bressani, R. and Elias, L.G. 1966. All-vegetable protein mixtures for human feeding. The development of INCAP vegetable mixture 14 based on soybean flour. J. Food Sci. 31: 626.

Dimler, R.J. 1967. Soybeans and corn join forces in food! Soybean Dig. 27: 50.

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

Finley, J.W., Ferrel, R.E. and Fellers, D.A. 1972. Determination of added lysine in fortified wheat and bulgar. Cereal Chem. 49: 514.

Harris, H., Davis, E.Y., Van de Mark, M.S., Rymal, K.S. and Spadro, J.J. 1972. Development and use of defatted peanut flours, meals and grits. Auburn University, Agricultural Experiment Station, Bulletin 431, Auburn, AL.

Kato, J. and Muramatsu, N. 1971. Amino acid supplementation of grain. J. Am. Oil Chem. Soc. 48: 415.

Milner, M. 1962. Peanuts as a protein resource in international feeding programs. Food Technol. 16(7): 46.

Rao, S.R., Carter, F.L. and Frampton, B.L. 1963. Determination of available lysine in oilseed meal proteins. Anal. Chem. 35: 1927.

Snedecor, G.W. and Cochran, W.G. 1968. "Statistical Methods," 6th ed., Iowa State College Press, Ames, IA.

USDA. 1975a. Purchase of soy-fortified corn meal for use in export programs. Announcement SFCM-2. Agricultural Stabilization and Conservation Service, ASCS Commodity Office, P.O. Box 8377, Shawnee Mission, KS 66208.

USDA. 1975b. Purchase of corn meal for use in export programs. Announcement CM-7. Agricultural Stabilization and Conservation Service, ASCS Commodity Office, P.O. Box 8377, Shawnee Mission, KS 66208.

USDA. 1976. Agricultural Statistics, U.S. Government Printing Office, Washington, DC 20402.

Ms received 9/12/77; revised 12/13/77; accepted 12/20/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

Table 7—Characteristics of blends of degermed cornmeal fortified with up to 50% of defatted peanut or defatted, toasted soy flour in corn bread

Fortifying components (%)	Batter viscosity	Volume (center ht) (in.)	Crumb color[a]	Grain and texture
0 — (all degermed cornmeal)	Normal	2.9	100	Med. cells Sl. coarse
5 — Peanut	Normal	3.1	100	Med. cells Sl. coarse
10 — Peanut	Normal	3.1	99.5	Med. cells Sl. coarse
15 — Peanut	Normal	3.1	99	Med. cells Sl. coarse
20 — Peanut	Normal	3.1	98.5	Med. cells Sl. coarse
35 — Peanut	Sl. thick	3.1	97.5	Sl. open cells Med. coarse
50 — Peanut	Thick	3.1	96	Med. open cells Coarse
5 — Soy	Normal	3.0	99.5	Med. cells Sl. coarse
10 — Soy	Normal	3.1	98.5	Med. cells Sl. coarse
15 — Soy	Normal	3.1	98.0	Med. cells Sl. coarse
20 — Soy	Sl. thick	3.1	97.5	Med. cells Sl. coarse
35 — Soy	Thick	3.2	95	Sl. open cells Med. coarse
50 — Soy	V. thick	3.1	92	Open cells Coarse

[a] 100 = yellow; 92 = tan.

Dr. W.F. Kwolek, Biometrician, USDA, North Central Region, ARS, stationed at the Northern Regional Research Center, provided statistical evaluation, and Dr. W.R. Gumbmann, Research Leader, Pharmacology Unit, Western Regional Research Center, Berkeley, CA, provided protein efficiency ratios.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

TENSILE STRENGTH OF PROCESSED MEATS DETERMINED BY AN OBJECTIVE INSTRON TECHNIQUE

T. A. GILLETT, C. L. BROWN, R. L. LEUTZINGER, R. D. CASSIDY and S. SIMON

ABSTRACT

Union Carbide has designed an accessory for securing meat slices while tensile strength determinations are being made using an Instron Universal Testing Machine. When compared to pneumatic jaws, the slice-holding accessory (SHA) gave smaller standard deviations, handled larger sample sizes, weakened the sample less and could be used to evaluate a wider range of products. The SHA was successfully used to demonstrate the positive effects of salt, phosphate, and dry skim milk on the binding characteristics of processed meats. In addition, it illustrated the effect of sample storage temperature and slice thickness on tensile strength (g/cm^2) of selected meat items. Recommendations are discussed for determining, reporting, and comparing tensile strength values on processed meats.

INTRODUCTION

THE TERM "BINDING" has different meanings in the meat processing field. It could refer to water-holding capacity (Hamm, 1960), emulsifying capacity (Saffle, 1968), or the "knitting together of chunks of meat to produce a unit system" (Vadehra and Baker, 1970). Reference to "binding" in this paper will refer to the latter meaning, where adhesion of pieces of meat to each other is initiated by the mechanical formation of a protein exudate, followed by protein coagulation during thermal processing.

Hansen et al. (1966) patented the principle of binding meat chunks utilizing salt soluble proteins. Froning (1965) demonstrated superior binding in turkey rolls containing food grade phosphates. Mass (1963) patented procedures for mechanically mixing pieces of meat to promote binding. Renewed interest in measuring meat binding occurred with the advent of mechanical massaging and tumbling techniques in the U.S. (Anonymous, 1977). A sensitive, objective, measure of bind would help investigators evaluate those parameters that are important in assessing such characteristics as sliceability and consistency of meat products.

Texture of meat has been evaluated employing shear, elasticity, compression and tensile strength values (Warner, 1928; Hashimoto et al., 1959; Kulwich et al., 1963; Pool, 1967; Schnell et al., 1970; Bouton and Harris, 1972; Stanley et al., 1971, 1972; Huang et al., 1977). Tensile strength per se is a direct measure of binding strength in a chunked and formed product and, therefore, will be the method of choice for this study. Two instrument systems lend themselves to automated measure of tensile strength. They are: (1) Ottawa Texture Measuring System devised by Voisey (1971) and, (2) The Universal Testing Machine (Hindmann and Burr, 1949; Bourne et al., 1966).

Pool (1967) was the first to use the Instron tensile tester for measuring tensile strength of meat. He measured connective tissue tenacity by cementing both ends of meat plugs to circular plates with methyl-2-cyano acrylate, then measuring the force required to separate the plates or connective tissue. Cementing was tedious and time consuming and has not been widely used. Maesso et al. (1970), used the Instron on ¼ inch slices of processed poultry meat for evaluating bind but did not describe how the slice was secured. Stanley et al. (1971) were able to reduce muscle slippage from the jaws of an Instron by wrapping the ends of the samples with moistened strips of fabric. They, however, were forced to reject data where breakage occurred at the jaws, presumably because of jaw compressior. weakening. Bouton and Harris (1972) reported reduced muscle breakage at the jaws when they employed pneumatically operated jaws where the air pressure could be adjusted so that a minimal force was used to clamp the muscle in place and still prevent slippage. Suter et al. (1976) described an apparatus for making tension tests on cooked ground meat patties employing plasma protein concentrates which they correlated with sensory panel results. From our experience with processed meats of soft texture (ham, liver sausage, etc.) jaw compression results in frequent breakage at the jaws. It is the purpose of this paper to describe an accessory to the Instron which allowed rapid determination of tensile strength for measuring bind in slices of processed meat which eliminated breakage due to jaw compression.

Authors Gillett, Leutzinger, Cassidy and Simon are with the Food Science Institute, Films-Packaging Division, Union Carbide Corp., 6733 West 65th St., Chicago, IL 60638. Author Brown, formerly with Union Carbide, is now affiliated with Land O-Frost, 621 131st Place, Hammond, IN 46327.

0022-1147/78/0004-1121$02.25/0
© 1978 Institute of Food Technologists

Table 1—Ham pickle formulation (15% pump)

Ingredient		Percent of pickle	Percent of finished product
Added water		76.0–81.05[a]	10.3–11.1
Salt		14.7	2.0
Sucrose		3.7	0.5
Sodium erythorbate		0.4	0.055
Sodium nitrite		0.15	0.02
Phosphate	Level 1	–	–
	Level 2	1.8	0.25
	Level 3	2.6	0.35
	Level 4	3.7	0.50
	Level 5	5.5	0.75

[a] Water content varied inversely with phosphate level.

EXPERIMENTAL

Ham preparation

Seventy-five kg of fresh boneless, trimmed whole hams were ground through a 2.54 × 3.18 cm kidney plate. Hams were then preblended 5 min at 7 ± 2°C in a Model RSV-10-K4402 Rietz double ribbon blender (Robert Reiser Co., Inc.; Boston, MA) before subdividing into five 15-kg batches. Each batch received 15% added curing pickle where salt, sucrose, erythorbate, and nitrite were held constant. Pickle phosphate level ranged from 0–5.5% and water was adjusted to balance other ingredients (see Table 1).

Each 15-kg batch was mixed 8 min in a 30 kg custom made paddle mixer, then stuffed via a model 1000S Vemag (Robert Reiser Co., Inc.; Boston, MA) into size 8 fibrous prestuck casing (Union Carbide) to recommended diameter (122 mm). Product was hung vertically and thermal processed. During the first 5 min of processing, smoke was generated from kiln dried maple sawdust (Frantz Co., Inc.; Milwaukee,

WI) with a Kartridg Pak smoke generator (Davenport, IA) set at 398°C with a sawdust feed setting of 7. The dry bulb temperature was programmed to raise from 60°C to 82°C until an internal temperature of 68°C was reached. The wet bulb temperature was programmed to maintain 30% relative humidity throughout the cycle. The product was then showered for 30 min in tap water and held overnight at 4°C.

Turkey roll preparation

One hundred fifty kg of frozen, boneless, skinless turkey breast meat was tempered and ground through a 2.54 × 3.18 cm kidney plate followed by 5 min of mixing in the Rietz double ribbon blender without vacuum (7 ± 2°C). Fifteen kg aliquots were then transferred to the 30 kg paddle mixer where individual treatments were prepared. The first five treatments contained 2% salt, while phosphate (Kena 28 or Brifisol D512) level was varied to produce products containing 0, 0.25, 0.35, 0.50 and 0.75% phosphate. The remaining five treatments contained 0.35% phosphate (Brifisol D512), while salt level was varied to obtain batches with 0, 0.5, 1, 2 and 3% salt. Salt was added dry while phosphate was dispersed in added water, which comprised 5% of each treatment. All ingredients were mixed for 7 min then stuffed to recommended diameter via a Model S1000 Vemag (Robert Reiser Co., Inc.; Boston, MA) into a size 8 opaque MP casing (Union Carbide). Three rolls of each treatment were processed in 82°C water to an internal temperature of 71°C then chilled for 36–48 hr in a 4°C cooler.

Liver sausage preparation

Pork livers, spices and cure were chopped at high speed for 2 min in a Model K9374359 Stuttgart Seydelmann (Robert Reiser Co., Inc.; Boston, MA) chopper. Bacon ends were then added to the mixture and chopped for an additional minute. Various levels of dry skim milk (1, 2, 3½%) were next added to three of the four 25 kg sausage batters and each was chopped to 10°C (see Table 2). Individual batches were emulsified in a Mincemaster equipped with a 1.7 mm plate to a temperature of 16–17°C. The product was hand stuffed in size 4 fibrous MP casing (Union Carbide) to a recommended 83 mm diameter via a Model S1000 Vemag. The product was water cooked (74°C) in a Jourdan cooker to 68°C internal temperature, showered in tap water, and held overnight at 4°C.

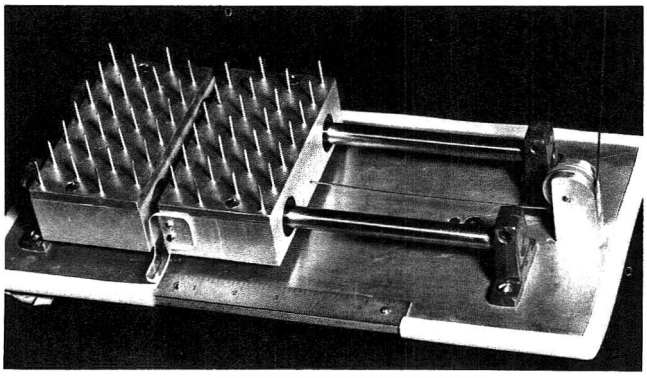


Fig. 1—Instron accessory for holding meat slices (SHA).

Table 2—Liver sausage formulation

Ingredient		Weight (kg)
Pork liver		13.25
Bacon ends		10.85
Salt		0.60
Braunschweiger seasoning		0.25
Prague powder (cure)		0.06
Dry skim milk	0%	–
	1%	0.25
	2%	0.50
	3.5%	0.875

Table 3—Mean proximate composition of products[a,b]

Product	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Turkey roll	73.2 ± 1.1	22.4 ± 1.0	1.35 ± 0.6	2.82 ± 0.2
Ham	66.4 ± 0.8	19.8 ± 0.4	9.48 ± 0.4	3.79 ± 0.2
Liver sausage	48.1 ± 1.0	14.0 ± 0.7	33.90 ± 1.6	3.96 ± 0.2

[a] All means are average of 16 or more samples.
[b] AOAC (1975)

Tensile strength or bind measurements using a slice-holding accessory (SHA)

Bind measurements were made on a Model TM Instron Universal Testing Machine with Union Carbide's newly designed slice-holding accessory (Fig. 1) for measuring the tensile strength of individual slices. The accessory was firmly attached to the moving cross head of the Instron by means of a ring and set screw. One end of a pulley cable was attached to a stationary 1 lb (454g) load cell by manually set jaws, while the other end passed around the pulley and was fastened by set screw to the moving sample holder. As the cross head of the Instron was activated (lowered) the cable tightened causing the moving slice holder (right) to travel along the parallel rods assisted by Thomson linear bearings. Since half the slice was impaled on the pins of the stationary holder and half on the moving holder a stress was placed upon the meat slice between the two slice holders as they were pulled apart into open position. The tensile strength of the meat slice was transmitted via the cable to the strain gauge of the tension load cell and its output was fed to a strip-chart recorder. The slice-holding pins were intentionally offset in rows of four to reduce sample stress along the pin lines of the front rows.

Inherent in the apparatus were frictional forces which had to be subtracted from each measurement. This deduction was equal to 60g.

Sample slice preparation and use

Triplicate slices of ham and turkey roll 2, 4, 6 and 8 mm thick were prepared from each of 3 rolls using a manually fed electric U.S. Berkley slicer (U.S. Slicing Mach. Co., LaPorte, IN), then sized to width (7.62 cm) with a knife and Plexiglas template. All slices were placed in plastic bags and equilibrated to −1°C prior to tensile strength measurement.

On liver sausage thicker slices (6, 8, 10 mm) were prepared which were not sized with a template and knife due to the characteristic soft texture of the product. Case or surface hardening did not occur with liver sausage in the MP casing therefore sizing was unnecessary and seemed to deform and weaken the product. Five whole slices (83 mm diam) of each thickness were used in the evaluation of liver sausage following equilibration in plastic bags at −1°C.

Comparison of pneumatic jaws and slice-holding accessory (SHA)

Six slices of 2, 4 and 6 mm were taken from each of 3 turkey rolls. Tensile strength determinations were made on adjacent slices comparing the pneumatic jaws and the SHA. The slices pulled using the SHA were prepared as described in the previous section, while the slices evaluated on the pneumatic jaws were cut to fit the jaw width (2.54 cm) and the pressure was set to secure the samples and minimize product deformation or compression weakening.

Effect of temperature on measurement of tensile strength in liver sausage

Temperature appeared to have a noticeable effect on tensile strength of liver sausage. In order to determine the optimum temperature for bind evaluation, ten replicate slices of liver sausage were held overnight at each of three temperatures: −1°C, 4°C and 27°C. Temperatures below −1°C were avoided since ice crystal formation could alter the tensile strength values.

Proximate composition

Fat, protein, moisture, and ash were determined by AOAC methods on samples of the final products. Means and ranges are shown in Table 3.

Statistical design and analysis

A 3 × 3 × 4 × 5 nested factorial design was utilized to evaluate the tensile strength of turkey rolls and ham where there were 3 rolls, 3 slices, 4 slice thicknesses and 5 levels of salt or phosphate. A 5 × 3 × 4 nested factorial design was utilized to evaluate liver sausage tensile strength where there were 5 slices, 3 slice thicknesses and 4 levels of dry skim milk. The effect of temperature upon the determination of tensile strength was evaluated using a 2 × 5 × 3 nested factorial design with 2 rolls, 5 slices and 3 temperatures. Comparisons between pneumatic jaws and the SHA for measuring tensile strength of turkey roll slices were

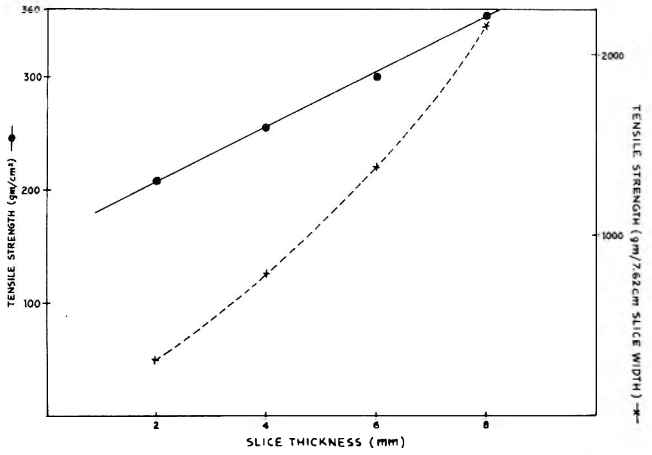


Fig. 2—Mean tensile strength vs slice thickness on phosphate treated hams. (Curve represents average tensile strength values for hams containing 0, 0.25, 0.35, 0.5 and 0.75% phosphate and 2% added NaCl.)

made using a standard two way analysis of variance. Multiple mean comparisons on appropriate data were made by the method of Duncan (1955) following a significant F ratio with a standard analysis of variance.

RESULTS & DISCUSSION

Pneumatic jaws vs slice-holding acessory (SHA)

A comparison was made on turkey rolls between the SHA and the standard pneumatic jaws furnished by Instron Corp. The overall mean for the pneumatic jaw was 289.7 g/cm^2 with a standard deviation of 106.1. The SHA had an overall mean of 497.41 g/cm^2 which was considerably higher; yet the standard deviation was only 89.7. The SHA gave more sensitive readings with smaller deviations. The differences were highly significant ($P \leq 0.01$).

The advantages of the SHA included a larger sample width (7.62 cm vs 2.54 cm), less sample destruction due to compression weakening, and simpler methodology. The compression weakening resulting from the pressure created by closing the jaws was evident in the sporadic breaking. Often breaks occurred at or in close proximity to the clamps while the tearing on the SHA was generally near the center of the meat slice. It was not possible to grip slices of liver sausage with the pneumatic jaws due to the softness of the product. Sample placement was simple with the SHA; since slices could be laid on the pins and pressed into position without deforming the slice.

Tensile strength measurements vs slice thickness

There appears to be some variability in the manner in which tensile strength is expressed. Break force has been reported for materials based on width, diameter and cross-sectional area (Barnes, 1968). Since width and diameter encompass only one dimension, cross-sectional area intuitively seems to be the best way to express tensile strength. Accordingly, tensile strength values for phosphate-treated hams were plotted versus slice thickness (Fig. 2) using two methods of expression: width, and cross-sectional area. The relationship between tensile strength and slice thickness was curvilinear when expressed on the basis of slice width (g/7.62 cm) and linear when expressed on the basis of cross-sectional area (g/cm^2). However, in the latter case the slope was not zero as would be expected if tensile strength was independent of slice thickness. The fact that it was not independent of slice thickness implies that direct comparison of data from different slice thicknesses can be misleading.

Table 4 illustrates that slice thickness does have a highly

Table 4—Effect of slice thickness on mean tensile strength (g/cm^2) of selected sliced meat items[a]

Item	Turkey rolls[b]	Ham[b]	Liver sausage[c]
2 mm	310d	208d	–
4 mm	351e	265e	–
6 mm	468f	300f	30d
8 mm	490g	354g	53e
10 mm	–	–	74f
Correlation	0.97	0.99+	0.96
Regression equations[d]	y = 32.6x + 240.5	y = 24.1x + 159	y = 11x – 35.67
Total observations	180	180	60

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.01$).
[b] Mean tensile strength for slices containing 0, 0.25, 0.35, 0.5 and 0.75% PO_4 (Kena 28).
[c] Mean tensile strength for slices containing 0, 1, 2 and 3.5% dry skim milk.
[d] Where: y = tensile strength; x = slice thickness.

Table 5—Effect of phosphate level on mean tensile strength (g/cm^2) of ham and turkey rolls[a]

Added phosphate (Kena 28) (%)	Tensile strength[b]	
	Turkey roll	Ham[c]
0.00	287c	122c
0.25	369d	342e
0.35	416e	322de
0.50	466f	295d
0.75	481f	315de
Total observations	180	180

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.01$).
[b] Mean tensile strengths include samples of 2, 4, 6 and 8 mm thick slices.
[c] Chunked and formed

significant effect on tensile strength (g/cm^2). This relationship was observed in turkey rolls, hams and liver sausage. Mean tensile strength values on the 6 mm and 8 mm slices can be used for bind comparison between products since these widths are common to all products tested. Turkey roll had considerably more bind than ham while the liver sausage bind was clearly inferior to both although processing conditions were different on each product.

Regression equations expressing the relationship between slice thickness and mean tensile strength (g/cm^2) are also shown in Table 4. These equations vary considerably between products and therefore any tensile strength predictions would be product specific. Consequently, there would be little value in comparing tensile strengths of unlike products where different slice thicknesses were used. Although we have chosen to report tensile strength on a cross-sectional basis, future reports should: (1) specify the slice thickness used and, (2) not assume any relationship in tensile strength values between different products when slice thickness varies. Nevertheless, the extremely high correlations (0.96–0.99) obtained between slice thickness and tensile strengths for any given product suggests that the SHA method accurately measures tensile strength.

Effect of added phosphate and sodium chloride on tensile strength

Table 5 demonstrates the effect of phosphate (Kena 28) on bind or tensile strength of turkey roll and chunked and formed ham containing 2% NaCl. As expected, the SHA values for

tensile strength indicated a dramatic increase in bind as phosphate (0.25%) was added to the formulation. Tensile strength of the turkey rolls increased as the phosphate level increased from 0.25 to 0.35% but began to plateau with additional phosphate. The results indicated that there was no significant advantage of adding over 0.35% phosphate (Brifisol D512) to hams, or more than 0.50% to turkey rolls as far as tensile strength was concerned. These trends were in general agreement with earlier studies on phosphate and water-holding capacity (Shults et al., 1972; Cohen et al., 1977).

Figure 3 illustrates the bind effect of various levels of phosphate (Brifisol D512) at a constant NaCl concentration (2%) and also the effect of various levels of added NaCl at a constant phosphate level (0.35%). The upper curve shows a sharp rise in tensile strength associated with the addition of 0.25% phosphate, a nonsignificant increase at 0.35%, a nonsignificant decline at 0.5%, and a significant increase in tensile strength with 0.75% added phosphate. The lower curve shows a significant increase in tensile strength with each additional increment of salt. Both curves illustrate expected responses and demonstrate how treatment effects can be monitored by use of the SHA where tensile strength of bind is the selected parameter.

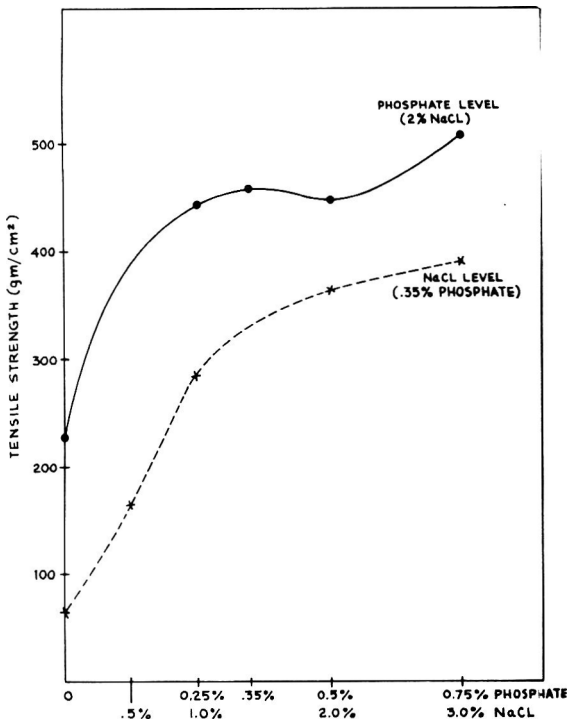


Fig. 3—Effect of phosphate (Brifisol D512) and sodium chloride concentration on mean tensile strength of turkey rolls where 2, 4, 6 and 8 mm slices were averaged (−1°C).

Table 6—Effect of holding temperature[a] and dry skim milk (DSM) level[b] on meat tensile strength of liver sausage[c]

Hold temp	Tensile strength (g/cm²)	DSM (%)	Tensile strength (g/cm²)
−1°C	42d	0	34d
4°C	34e	1	35d
27°C	20f	2	57e
		3.5	72f
Correlation	−0.98	Correlation	0.96
Total obs	30	Total obs	60

[a] All liver sausage samples for temperature evaluation contain 3.5% DSM.

[b] Mean tensile strengths include samples of 6, 8 and 10 mm.

[c] Means in the same column not followed by the same letter are significantly different ($P < 0.01$).

Effect of holding temperature on liver sausage

Storage temperature for sliced meat samples can play an important role in tensile strength determination. Frozen samples were nearly impossible to compress or pull apart. Conversely, samples held at elevated temperatures (>10°C) underwent rapid bacterial degradation, and in the case of soft textured products like liver sausage, samples became too mushy for evaluation. Table 6 shows that over the range −1 to 27°C, increases in temperature caused a significant decrease ($P < 0.01$) in tensile strength of liver sausage containing 3.5% dry skim milk. The relationship between temperature and tensile strength was highly correlated ($r = 0.98$).

The conclusion was drawn that sample storage temperature should be held constant if tensile strength data are to be compared for any meat product. Ideal storage temperature should be between −1° and 2°C to avoid bacterial degradation or freezing.

Effect of dry skim milk (DSM) in liver sausage

Binders such as DSM are added to sausage for the purpose of water binding and fat emulsification (Kramlich, 1971). These factors should have an observable effect on the tensile strength of a sliced sausage product. Table 6 shows that DSM level significantly ($P < 0.01$) effected tensile strength of liver sausage. As DSM level increased from 0 to 3.5% (USDA legal limit) the tensile strength of the slices increased. However, it should be pointed out that more than 1% DSM was needed to demonstrate a positive effect upon bind. Once again it was possible to objectively monitor the effect of treatment (DSM) on tensile strength in the liver sausage with the SHA.

CONCLUSION

THE SHA PROVED to be superior to standard pneumatic jaws for objectively measuring tensile strength (g/cm²) of processed meats. Using this laboratory accessory, we concluded that sample storage temperature, slice thickness, level of salt, phosphate, and dry skim milk each had a significant ($P < 0.01$) effect upon tensile strength of processed meat.

It appears that tensile strength determinations were not independent of slice thickness even when expressed on a cross-sectional area basis. Regression equations showing this relationship were highly correlated, but product specific.

Finally, the SHA should be a valuable tool for measuring tensile strength in processed meats over a wide range of products, processing conditions, formulations, and treatments.

REFERENCES

Anonymous. 1977. The massaging-tumbling revolution: an update on equipment and systems plus recommendations for ham curing. Meat Ind. Apr:36 (review of paper presented by Terry Lack at 57th Annual Meeting of the Meat Packers Council of Canada in Toronto, Feb. 1977).

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.

Barnes, C.R. 1968. The ABCs of package testing. In "Modern Packaging Encyclopedia," Vol. 41, No. 7A, p. 50. McGraw-Hill Inc. New York.

Bourne, M.C., Moyer, J.C. and Hand, D.B. 1966. Measurement of food texture by a universal testing machine. Food Technol. 20: 522.

Bouton, P.E. and Harris, P.V. 1972. The effects of cooking temperature and time on some mechanical properties of meat. J. Food Sci. 37: 140.

Cohen, J.S., Shults, G.W., Mason, V.C. and Wierbicki, E. 1977. Variables affecting the acceptability of radappertized ground beef products. J. Food Sci. 42: 338.

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

Froning, G.W. 1965. Effect of polyphosphates on binding properties of chicken meat. Poultry Sci. 44: 1104.

Hamm, R. 1960 Biochemistry of meat hydration. Adv. in Food Res. 10: 356.

Hansen, L.J., Hills, C. and Schwall, D.V. 1966. Method of preparing a poultry product. U.S. Patent 3,285,752.

—Continued on page 1129

FACTORS ASSOCIATED WITH MICROBIAL GROWTH IN GROUND BEEF EXTENDED WITH VARYING LEVELS OF TEXTURED SOY PROTEIN

J. T. KEETON and C. C. MELTON

ABSTRACT

Three separate batches of ground beef containing 25% fat were extended to 0, 10, 20 and 30% by weight with rehydrated defatted textured soy protein (TSP). Five samples of each soy level were wrapped in a polyethylene oxygen-permeable film and stored at 5°C for 4 days. Samples were analyzed daily for changes in bacterial numbers (total plate counts and coliforms), pH, titratable acidity, proximate analysis, ninhydrin reactive material, carbohydrate content, and gas liquid chromatographic (GLC) analysis of individual amino acids to assess spoilage in TSP extended ground beef. Total bacterial numbers increased significantly as days of storage progressed and in proportion to added TSP. Coliforms increased during storage, but not proportional to increased levels of soy protein. Spoilage in soy extended samples, as determined by off-odors and bacterial numbers, was evident by day 4 as total bacterial counts approached 10^8 bacteria/g. The differences of pH were significant among soy levels, but did not change appreciably during storage. Titratable acidities were not significantly changed with increases in soy protein. As soy level increased, percentages of moisture and ash increased, but percent fat decreased. No differences were noted for percent protein. Total carbohydrate as determined by proximate analysis increased with TSP levels. Concentration values for carbohydrates were proportionately larger with added amounts of TSP, but varied little during 4 days of storage. Ninhydrin reactive material differed significantly among samples, but not proportionate to added TSP, and decreased in the 30% soy level between days 3 and 4. Decreases in individual amino acids could not be shown from the qualitative results obtained by GLC analysis. Further investigation is suggested using more definitive measuring techniques to determine the factor(s) causing accelerated spoilage in soy extended ground beef.

INTRODUCTION

TEXTURED SOY PROTEINS (TSP) used in conjunction with meat products have been and continue to be an increasingly important adjunct food source because of their economic advantage and nutritive value (Hegarty and Ahn, 1976; Kotula et al., 1976; Smith et al., 1976; Twigg et al., 1977). Consumer acceptance or rejection of these soy extended fresh meat products can be directly attributable to the quality characteristics exhibited by these foods.

Ground beef provides an excellent environment for microbial growth and becomes contaminated as a result of grinding and mixing during the fabrication process (Emswiler et al., 1976). The overall bacteriological quality of ground beef depends upon the bacterial population of beef trimmings, sanitation during processing, type of packaging, storage time, and storage temperature. Judge et al. (1974) observed significant increases, after 7 days storage at 4°C, in total bacterial numbers of soy extended and all-beef patties when compared to day 0. However, comparisons among control and soy patties on day 7 resulted in no significant increases in bacterial numbers. Stansbury (1975) noted highly significant increases in aerobic and psychrophilic-mesophilic logarithmic counts in soy extended ground beef with increasing levels of textured vegetable protein (TSP) during storage at 0° and 6°C. The total effect was a reduction in shelf life from 7 to 4 days at refrigerator temperatures (3–6°C).

The current study was conducted to examine various levels of soy extended ground beef and to identify the factor(s) responsible for accelerated microbial growth at refrigerator temperature (5°C).

Author Melton is with the Dept. of Food Technology and Science, The University of Tennessee, Knoxville, TN 37901. Author Keeton, formerly with The University of Tennessee is now with the Dept. of Food Science, North Carolina State University, Raleigh, NC 27607.

0022-1147/78/0004-1125$02.25/0
© 1978 Institute of Food Technologists

EXPERIMENTAL

Sample preparation

Three separate 11.36 kg lots of flaked frozen beef containing 25% fat were extended 0 (control), 10, 20 and 30% by weight with a rehydrated defatted textured soy protein (TSP) product. The TSP, containing approximately 50% protein on a dry weight basis, was rehydrated at room temperature for 15 min with a 2:1 ratio of cold tap water to TSP, prior to combining with flaked beef. Proportionate amounts of beef and TSP were mixed individually for each percentage level, following accepted sanitary practices, and blended further by grinding through a 0.64 cm plate on a laboratory size Hobart grinder. Five 500g samples per soy level were packaged in 0.45 kg size plastic foam meat trays and wrapped with a polyethylene oxygen-permeable film. Each lot, representing one replication, was stored 4 days in a laboratory refrigerator at 5°C. Eight 50g samples from each level were taken daily (the first using sterile technique), placed in "Whirl-Pac" bags, and stored at −18°C until analyzed. The sample taken by sterile technique was plated immediately after sampling for bacterial enumeration.

Microbiological assays

Eleven grams of aseptically sampled meat from each soy level were weighed into a sterile blender jar, 99 ml of phosphate-buffered sterile, distilled water added, and the mixture blended for 45 sec. Serial dilutions from 1:10 to 1:1,000,000 were prepared for total plate counts and coliform counts as suggested by Ockerman (1972) and Sharf (1966). Plates were poured with Standard Methods and Violet Red Bile agar (VRBA) and incubated at 21°C for 3 days and 35°C for 18–24 hr, respectively. Total bacterial numbers and coliforms were determined using a Quebec dark field colony counter according to the guidelines of the American Public Health Association (APHA, 1972), and reported as logarithmic number of bacteria per gram of sample.

pH and titratable acidity

A 10g meat sample from each soy level (0, 10, 20, 30%) and each day of storage (0, 1, 2, 3, 4 days) was blended with 100 ml of deionized distilled water for 2 min. Duplicate pH values were read to the nearest 0.01 pH unit using a model 801 Orion digital pH meter fitted with a standard glass electrode.

Blended samples from the pH measurement were diluted to 250 ml volume with distilled water, mixed by inversion, and filtered through cheese cloth. Fifty ml of distilled water were added to 50 ml of dilute meat slurry and titrated with .1N NaOH to a phenolphthalein end-point. The red-pink phenolphthalein end-point was readily determined on dilute meat slurries of sample duplicates.

Proximate analysis

Percent protein, fat, moisture, and ash were determined on duplicate samples analyzed by AOAC (1965, 1970) methods. Carbohydrate material was determined by difference after totaling the proximate analysis.

Table 1—Analysis of variance for microbial and chemical determinations

		Mean squares									
	DF	TPC	Coliforms	pH	Titratable acidity	Ninhydrin material	Carbo-hydrate	% Protein	% Moisture	% Fat[d]	% Ash
Soy level[a]	3	0.5454*	0.5498*	0.7573***	0.0041	0.0023***	0.4427***	0 3640	11.7743**	124.9306***	0.4540***
Replication (Error A)	2	5.0794***	0.6498*	0.0815***	0.9504***	0.0028***	0.0317**	2 8562*	13.2946**	6.3573*	0.0109
Replication X soy level	6	0.1198	0.0916	0.0022	0.C114	0.0001	0.0032	0 2627	0.6625	1.0523	0.0074
Days storage[b]	4	11.7998***	2.7762***	0.0260	0.C529**	0.0006*	0.0023	0 3007	1.1463	0.7261	0.0136*
Days storage X soy level	12	0.0809	0.1481	0.0026	0.C066	0.0002	0.0017	0 2645	1.0694	0.8196	0.0031
Error B[c]	32	0.0476	0.2065	0.0126	0.C111	0.0002	0.0016	0 1778	1.2122	1.1970	0.0041

[a] 0, 10, 20 and 30% of added TSP
[b] 0, 1, 2, 3 and 4 days of storage
[c] Represents the replication X days storage and replication X days storage X soy level[a] interations
[d] Ether extract
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Ninhydrin reactive material and carbohydrate determinations

Soy extended meat extracts were prepared by blending 5g samples with 100 ml of cold 80% EtOH for 5 min. Extracts were centrifuged 15 min at 10,000 × G, then the supernatant was filtered through glass wool into "Whirl-Pac" bags and stored at 0°C until analyzed.

A relative measure of the total free amino acids was obtained by analyzing EtOH extracts for ninhydrin reactive material, α-amino groups (Mickelson et al., 1967). Triplicate samples were diluted to fall within a 0.005–0.025 mg/ml range of a glycine standard, then combined with ninhydrin reagents, and absorbance measurements determined on a Perkin-Elmer 124 double beam spectrophotometer at 570 nm.

Total carbohydrates were determined on EtOH extracts by the phenolsulfuric acid method of Dubois et al. (1956). Triplicate samples were diluted to fall within a 10–80 ppm range of glucose standard, mixed with phenol and concentrated H_2SO_4, and absorbance measurements determined on a Perkin-Elmer 124 double beam spectrophotometer at 485 nm.

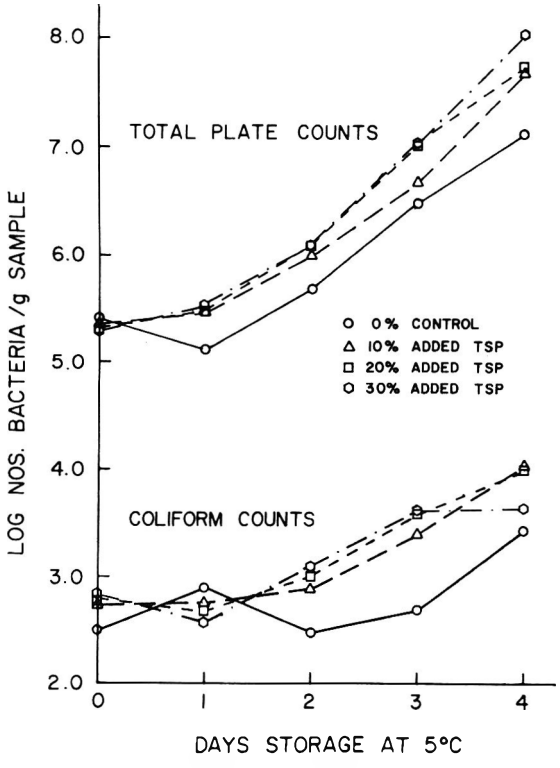


Fig. 1—Logarithmic numbers of bacteria in various levels of textured soy protein (TSP) extended ground beef.

Free amino acid analysis by gas liquid chromatography (GLC)

Free amino acids were extracted from thawed meat samples, using 1% picric acid, by the method of Field and Chang (1969) and compared with 80% EtOH extracts of the ninhydrin reactive material analysis. Preliminary chromatograms revealed no differences in extraction efficiencies, but the EtOH extraction method was chosen because fewer rinses were required to regenerate ion-exchange columns in the cleanup step.

Extract cleanup procedures using Dowex-X12 cation and Dowex 2-X8 anion exchange resins were adapted from Gehrke (1972) and n-butyl N-trifluoroacetyl esters of amino acids for gas liquid chromatography were prepared according to Lamkin and Gehrke (1965). Four to six μl of esterfied sample were chromatographed immediately after preparation, using a Bendix model 2500 GLC equipped with flame ionization detector and integrating recorder. Amino acid esters were separated on glass columns 182.88 cm × 4 mm i.d. containing 0.65% w/w ethylene glycol adipate (EGA) on AW chromosorb W and OV-17/OV-210, 2%/1% w/w siloxane on Supelcoport. Chromatographic conditions were as follows:

	0.65% EGA	2%/1% OV-17/OV-210
Initial temperature	100°C	110°C
Final temperature	210°C	235°C
Program rate	4°C/min	8°C/min
Attenuator	500-2K	500-2K
Carrier gas, N_2	50 ml/min	50 ml/min
H_2 flow rate	20 ml/min	50 ml/min
Air flow rate	75 ml/min	75 ml/min

Color and odor changes

Daily olfactive evaluations, using five descriptors ranging from no off-odor to very distinct spoilage odor, were made by the author immediately after opening individual samples. Detection of a pungent ammonia-like odor was recorded as an indication of spoilage, and later compared with bacterial numbers. Combined odor evaluations and numbers of bacteria were the determinants of spoilage.

Color appraisals were made at the time of odor evaluation and compared to the ground beef control. An arbitrary five point color scale ranged from no discoloration to very dark discoloration.

Statistical analysis

The main effects of time, soy level, and replication were arranged in a split-plot design and samples analyzed at random. Increased precision for subunit comparisons among soy levels and days of storage was attained by use of the split-plot design, but at the expense of whole unit comparisons among replications (Steele and Torrie, 1960). Significant attributes of the main effects were differentiated by analysis of variance.

RESULTS & DISCUSSION

Microbial assays

Mean values of logarithmic bacterial numbers/g in relation to soy levels and days of storage at 5°C are presented in Figure 1 and represent three combined replications. In Table 1, sig-

nificant differences were shown among respective soy levels ($P < 0.05$), replications ($P < 0.001$), and days of storage ($P < 0.001$) for total plate count values. Total plate counts increased with corresponding amounts of added TSP and length of the storage period. Stansbury (1975) also observed significant increases in log aerobic counts/g and psychrophilic-mesophilic counts/g as soy levels increased in fresh ground beef extended with TSP and stored at 6°C. Replication differences would be expected since separate lots of ground beef were used, each differing in the initial amount of bacteria present. Bacterial counts at day 0 ranged from 5.31–5.40 log bacteria/g. After 1 day of storage at 5°C, proliferation of bacteria was greatest in the soy protein samples and the same trend continued throughout storage. Log bacterial numbers at day 4 were proportional to the amount of added TSP and ranged from 7.70 (10% level) to 8.05 (30% level) in the soy extended samples, as compared to 7.13 in the control. Ayres (1960) demonstrated that "off-odors" are first noticed when the number of bacteria/cm^2 reach 10^7 (log 7.00) and that slime formation and spoilage occur at bacterial counts just under 10^8 bacteria/cm^2. Judge et al. (1974) reported increased bacterial counts in soy extended beef patties to be the result of variation in raw materials. However, Stansbury (1975) attributed increases in total aerobic counts and psychrophilic-mesophilic counts to the TSP extender. He also reported the dry TSP extender to contain almost no bacteria when examined for bacterial contamination prior to rehydration. It appears from observations made in this study that some TSP component or components is/are responsible for accelerated spoilage of fresh soy extended ground beef.

Significant differences were observed among soy levels, replications, and days of storage for log numbers of coliform bacteria/g (Table 1). Coliform counts for the TSP samples were greater than the control after storage day 1, but did not increase proportionately with increased soy protein. Initial log coliform counts were 2.50–2.83, but ultimately reached 3.70–4.05 for the TSP samples and 3.44 for the control at day 4. Emswiler et al. (1976) have shown coliforms to decrease in ground beef during storage at -1.7 ± 0.6°C and Stansbury (1975) reported no significant variation in coliform counts in TSP extended ground beef at 6°C. Additional mixing of the soy extended material was required during formulation prior to grinding and, as a result, bacterial contamination could have been greater.

Subjective evaluations were made daily by visual appraisal and odor determination of each sample to detect objectionable color and odor changes. TSP samples were initially darker than the control, having a discoloration similar to metmyoglobin. Soy extended samples became progressively darker with each day of storage and with increasing amounts of added soy protein. By day 3, sour-like odors were first noted in some extended samples, which corresponds to bacterial numbers reported to produce off-odors (Ayres, 1960). Definite spoilage odors were present on day 4 in all TSP samples.

pH and titratable acidity

Analysis of variance for pH in Table 1 shows differences among soy levels ($P < 0.001$) and replications ($P < 0.001$). Mean pH values plotted in Figure 2 indicate little change in pH within each respective soy level over the 4 day storage period. Shelef and Jay (1970) reported the difference between freshness and incipient spoilage usually does not exceed 0.30–0.50 of a pH unit during the first 4 days of spoilage. These researchers have also concluded that increased pH values after 4 days are partially the result of bacterial metabolic by-products, such as amino sugars. The increase in pH with increasing soy levels could partially explain the increase in bacterial numbers since growth of spoilage bacteria would tend to favor a neutral pH. Availability of proteins, amino acids, and carbohydrates might also be considered as potential growth stimulators since pH changes would cause different degrees of availability of these respective components.

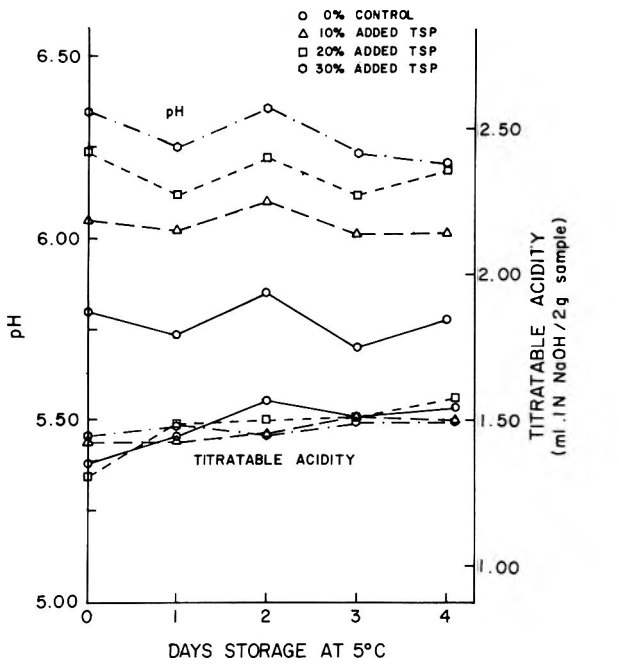


Fig. 2—pH and titratable acidity changes of various soy levels in relation to days of storage.

Table 2—Means of proximate analysis by soy levels and by days storage

Days storage	Soy level (%)	% Protein	% Moisture	% Fat[a]	% Ash	% Total	% Carbohydrate by difference[b]
	0	16.18	59.70	24.27	0.55	100.70	–
	10	16.39	60.30	22.15	0.69	99.53	0.47
	20	16.44	60.48	19.59	0.83	97.34	2.66
	30	16.56	61.80	17.68	0.95	96.99	3.01
0		16.17	60.72	21.10	0.79	98.78	1.22
1		16.56	60.89	21.03	0.78	99.26	0.74
2		16.35	60.69	20.84	0.76	98.64	1.36
3		16.35	60.45	20.53	0.73	98.06	1.94
4		16.54	60.10	21.12	0.71	98.47	1.53
Overall means		16.39	60.57	20.92	0.75	98.63	1.37

[a] Ether extract
[b] The remaining material unaccounted for after computing totals of the proximate analysis.

Titratable acidity increased ($P < 0.01$) with storage time (Fig. 2). Significant differences were observed among replications, but not among the various soy levels. Shelef and Jay (1970) have shown titratable acidity to be a relatively accurate method of determining spoilage in ground beef, but use of the method of Ockerman (1972) was not adequate in attributing spoilage to the various soy levels.

Proximate analysis

Analysis of variance and mean proximate analysis values are summarized in Tables 1 and 2, respectively. Percent protein was different ($P < 0.05$) among replications, but not among soy levels or days storage. Little variation in protein content was expected since rehydrated TSP is approximately 19% protein as compared to ground beef (16%).

Percent moisture (Tables 1 and 2) increased ($P < 0.01$) as the soy level increased, but little variation in mositure levels was noted for combined sample means over the storage period. Percent moisture was also significantly different among repli-

cations. Increased moisture availability as soy protein increases does not seem to be a factor contributing to accelerated spoilage since the water activity of fresh beef is approximately 0.99. The corresponding significant increase in moisture and decrease in fat with added TSP does indicate a dilution of ground beef fat tissue with hydrated soy protein.

Significant differences among replications were noted for percent fat.

Percent ash increased ($P < 0.001$) with increasing amounts of TSP and decreased ($P < 0.05$) as storage progressed. Stansbury (1975) reported increasing amounts of ash in soy extended ground beef corresponding to added amounts of soy protein. Residual material, designated as carbohydrate by difference, could explain in part the notable increases in bacterial numbers as the soy protein increased. Davidson et al. (1973) and Jay (1972) have shown meat spoilage bacteria to utilize carbohydrates and sugar derivatives from C_2 to C_{24} with preference to the short chain, readily available substrates. Lechowich (1971) stated that aerobic spoilage bacteria such as pseudomonads generally oxidize sugars completely to CO_2 and water, providing energy for large numbers of cells and essential slime formation. However, carbohydrate oxidation products are not responsible for the putrifactive off-odors of spoiled ground beef.

Ninhydrin reactive material and carbohydrates

Jay and Kontou (1967) reported decreases in quantity and types of amino acids and decreases in nucleotides as bacterial numbers increased beyond log 7.8 in ground beef held for 9 days at 7°C. Ninhydrin reactive material in this study was determined as a relative measure of free α-amino groups metabolized by bacteria during the storage period. Significant differences (Table 1) were noted among soy levels, replications, and days of storage. As shown in Figure 3, ninhydrin reactive material increased for all samples during storage except the 30% level between days 3 and 4, but not proportionate to the amount of added TSP. Jay and Kontou (1967) also observed an increase in ninhydrin reactive material which parallel the data of the 30% soy level. They reported a continual decrease in ninhydrin reactive material after day 3 until day 7, at which point no further decreases were noted. Accelerated spoilage in TSP extended ground beef cannot be directly attributed to bacterial utilization of free amino acids, based on these data. However, the decrease in ninhydrin reactive material for the 30% level does imply that this material may be involved in development of putrefactive off-odors.

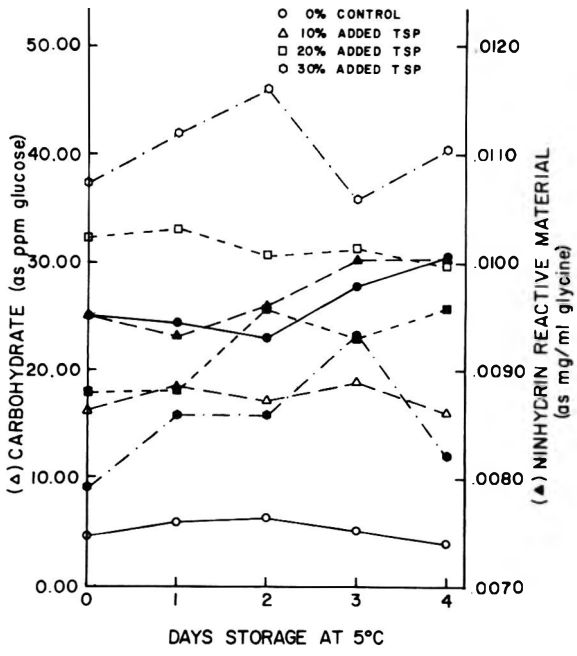


Fig. 3—Concentration values of carbohydrates and ninhydrin reactive material as related to days of storage (open symbols represent carbohydrate concentrations; closed symbols represent ninhydrin reactive material concentrations).

As shown in Table 1 and in Figure 3, total carbohydrates increased ($P < 0.001$) in conjunction with soy protein levels and differences ($P < 0.001$) were noted among replications. Carbohydrates within each soy level remained relatively constant during the storage period except for the 30% group. The relative amounts of carbohydrate utilized up to day 4 may represent only a small portion of the total carbohydrate available for use by meat spoilage bacteria.

GLC analysis of free amino acids

Meat spoilage bacteria apparently do not attack complex beef proteins until readily available nutrients have been depleted (Jay and Kontou, 1967), which appears to be consistant with the known facts concerning bacterial degradation of proteins. These bacteria secrete extracellular hydrolytic enzymes to produce mercaptans, amines, fatty acids, ammonia, hydrogen sulfide, indole and other foul odor compounds associated with putrefaction (Lechowich, 1971). If free amino acids were more abundant in the TSP material, then those samples containing large amounts of soy protein should show signs of spoilage quicker and have a corresponding decrease in free amino acids. This rationale was used when considering GLC analysis of free amino acids in attributing spoilage to these nitrogenous compounds. In addition, Satterlee and Lillard (1967) described GLC analysis of n-butyl N-trifluoroacetyl esters of free amino acids in meats and meat products to be a reliable method of measuring the degree of proteolysis.

Numerous chromatograms were run to quantitatively measure changes in individual free amino acids of the various soy levels for each day of storage. However, artifacts from interfering substances in the soy-extended samples prevented quantitative determinations. Qualitative separation of 17 free amino acids was accomplished, but no interpretation of the GLC data could be made to associate microbial spoilage with increased bacterial numbers and a corresponding decrease in individual amino acids. For this reason, no GLC data are presented.

Stansbury (1975) enumerated the types of bacteria present in soy extended ground beef and postulated that spoilage might be attributed to nutrients available in the TSP. From this study, results are inconclusive in attributing accelerated spoilage to specific component(s) of the soy protein. With refinement of the GLC technique or alternate analytical methods, a more precise evaluation could be made regarding the factor(s) causing accelerated microbial spoilage.

CONCLUSIONS

MEAT SPOILAGE BACTERIA were shown to increase in numbers more rapidly in TSP extended (up to 30%) ground beef than on ground beef containing 25% fat. Coliforms appear to grow better on soy extended beef samples, but extrinsic contamination should be considered also. Nonproportionate increases in coliform numbers were observed with added soy protein. Spoilage in the soy extended samples was evident by day 4 as total bacterial numbers approached 10^8 bacteria/g. With increasing amounts of soy protein, pH values were higher (6.5) providing more favorable growth conditions. Carbohydrate by difference from the proximate analysis indicates that additional nutrient sources are available to meat spoilage bacteria from the added TSP. However, carbohydrate as determined spectrophotometrically did not decrease significantly with increased bacterial numbers. Significant variations

were noted in the number of free α-amino groups among the different soy levels. Ninhydrin reactive material determinations of the 30% level parallel the observations of Jay and Kontou (1967) and suggest partial utilization of this material by spoilage bacteria.

Qualitative GLC determinations of individual amino acids did not provide adequate information to attribute microbial spoilage to changes in these nitrogenous compounds. However, use of analytical techniques with increased measurement sensitivity to determine relative changes in individual amino acids and sugars during longer storage periods should resolve whether these components are the primary contributing factor(s) to spoilage in TSP extended ground beef.

REFERENCES

AOAC. 1965. "Official Methods of Analysis," 10th ed. Association of Official Analytical Chemists, Washington, DC.

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

APHA. 1972. "Standard Methods for the Examination of Dairy Products," 13th ed. American Public Health Association, Washington, DC.

Ayres, J.C. 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res. 25: 1.

Davidson, C.M., Dowdell, M.J. and Board, R.G. 1973. Properties of gram negative aerobes isolated from meats. J. Food Sci. 38: 303.

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.

Emswiler, B.S., Pierson, C.J. and Kotula, A.W. 1976. Bacteriological quality and shelf life of ground beef. Applied Environ. Microbiol. 31(6): 826.

Field, R.A. and Chang, Y. 1969. Free amino acids in bovine muscles and their relationship to tenderness. J. Food Sci. 34: 329.

Gehrke, C.W. 1972. "Regis Amino Acid Analysis Operational Manual." Regis Chemical Co., Morton Grove, IL.

Hegarty, P.V.J. and Ahn, P.C. 1976. Nutritional comparison between a soy-based meat analog and ground beef in the unheated and heated states. J. Food Sci. 41: 1133.

Jay, J.M. 1972. Mechanism and detection of microbial spoilage in meats at low temperatures: A status report. J. Milk Food Technol. 35: 467.

Jay, J.M. and Kontou, K.S. 1967. Fate of free amino acids and nucleotides in spoiling beef. Appl. Microbiol. 15: 759.

Judge, M.D., Haugh, C.G., Zachariah, G.L., Parmlee, C.E. and Pyle, R.L. 1974. Soy additives in beef patties. J. Food Sci. 39: 137.

Kotula, A.W., Twigg, G.G. and Young, W.P. 1976. Evaluation of beef patties containing soy protein, during 12-month frozen storage. J. Food Sci. 41: 1142.

Lamkin, W.M. and Gehrke, C.W. 1965. Quantitative gas chromatography of amino acids. Anal. Chem. 37: 383.

Lechowich, R.V. 1971. Microbiology of meat. In "The Science of Meat and Meat Products," 2nd ed. W.H. Freeman and Co., San Francisco, CA.

Mickelsen, R., Fish, N.L. and Claydon, T.J. 1967. Some chemical and flavor characteristics of a milk proteolysate of Pseudomonas fluorescens. J. Dairy Sci. 50: 172.

Ockerman, H.W. 1972. "Quality Control of Postmortem Muscle Tissue," 7th ed. The Ohio State University.

Satterlee, L.D. and Lillard, D.A. 1967. A procedure for gas chromatographic analysis of free amino acids in meats. J. Food Sci. 32: 682.

Sharf, M.M. 1966. "Recommended Methods for the Microbiological Examination of Foods," 2nd ed. The American Public Health Association, Inc., Washington, DC.

Shelef, L.A. and Jay, J.M. 1970. Use of titrimetric method to assess the bacterial spoilage of fresh beef. Appl. Microbiol. 19: 902.

Smith, G.C., Marshall, W.H., Carpenter, Z.L., Branson, R.E. and Meinke, W.W. 1976. Textured soy proteins for use in blended ground beef patties. J. Food Sci. 41: 1148.

Stansbury, J.B. 1975. Quantitative determinations of selected bacteria in ground beef extended with varying levels of textured vegetable protein. Ph.D. dissertation, The University of Tennessee, Knoxville.

Steele, R.G.D. and Torrie, H.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Company Inc., New York.

Twigg, G.G., Kotula, A.W. and Young, E.P. 1977. Consumer acceptance of beef patties containing soy protein. J. Ani. Sci. 44: 218

Ms received 8/19/77; revised 11/10/77; accepted 11/17/77.

The authors express their appreciation to Mr. Joe Lonas of Travisco Meat and Seafood Co. for supplying ground beef used in this study; to Dr. Sharon L. Melton for recommendations concerning the chemical analyses; and to Dr. W.L. Sanders for assistance with the experimental design.

This paper represents a summary of part of the research done by the senior author to fulfill the requirements for the Ph.D. degree.

Hashimoto, Y., Fukazawa, T., Niki, R. and Yasui, T. 1959. Effect of storage conditions on some of the biochemical properties of meat and on the physical properties of an experimental sausage. Food Res. 24: 185.

Hindman, H. and Burr, G.S. 1949. The Instron tensile tester. Trans. Am. Soc. Mech. Engrs. 71: 789.

Huang, F., Dethmers, A.E. and Robertson, J.W. 1977. A rheological method for evaluating the textural qualities of cooked mutton. J. Food Sci. 42: 721.

Kramlich, W.E. 1971. Sausage products. In "The Science of Meat and Meat Products," Ed. Price, J.F. and Sweigert, B.S. W.H. Freeman and Co., San Francisco, CA.

Kulwich, R., Decker, R.W. and Alsmeyer, R.H. 1963. Use of a slice-tenderness evaluation device with pork. Food Technol. 17: 201.

Maesso, E.R., Baker, R.C. and Vadehra, D.V. 1970. The effect of vacuum, pressure, pH and different meat types on the binding ability of poultry meat. Poultry Sci. 49: 697.

Mass, R.H. 1963. Processing meat. U.S. Patent 3,076,713.

Pool, M.F. 1967. Objective measurement of connective tissue tenacity of poultry meat. J. Food Sci. 32: 550.

Saffle, R.L. 1968. Meat emulsions: Adv. in Food Res. 16: 105.

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

Shults, G.W., Russel, D.R. and Wierbicki, E. 1972. Effect of condensed phosphates on pH, swelling and waterholding capacity of beef. J. Food Sci. 37: 860.

Stanley, D.W., Pearson, G.P. and Coxworth, V.E. 1971. Evaluation of certain physical properties of meat using a universal testing machine. J. Food Sci. 36: 256.

Stanley, D.W., McKnight, L.M., Hindes, W.G.S., Usborne, W.R. and DeMan, J.M. 1972. Predicting meat tenderness from muscle tensile properties. J. Texture Studies 3: 51.

Suter, D.A., Sustek, E., Dill, C.M., 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.

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

Voisey, P.W. 1971. The Ottawa texture measuring system. Can. Inst. Food Technol. J. 3: 44.

Warner, K.F. 1928. Progress report of the mechanical test for tenderness of meat. Ann. Proc. Am. Soc. Animal Prod. 21: 114.

Ms received 10/20/77; revised 1/12/78; accepted 1/18/78.

The authors express their appreciation to Mr. Cliff Faust and his Engineering staff for assistance in the design and construction of the slice-holding accessory.

AN ASSESSMENT OF THE MEAT CHARACTERISTICS OF PRE-RIGOR LEG-TWISTED MUTTON CARCASSES

ADEMOLA OKUBANJO

ABSTRACT

Six good grade ewes were dressed conventionally with the hind hoofs retained on a randomly selected side of each carcass. Leg-twisting was effected by inserting one hoof into a slit made anterior to the flank muscle. Both sides were suspended by the achilles tendon. Generally, muscles evaluated post-rigor from the leg-twisted sides had lower shear force values, longer sarcomeres, thicker fibers and lower fragmentation values than corresponding muscles from the control sides except the semitendinosus and psoas major. The orientation angle of the femur on the pelvic axis was reduced by 50% bringing the conformation of the carcass close to that of the live animal in the resting prostrate form.

INTRODUCTION

VARIOUS STUDIES have been conducted on the physical and mechanical techniques for preventing postmortem shortening in most muscles on the intact carcass, particularly the commercially important muscles of the leg and loin. Such techniques necessarily effect a change in the method of carcass positioning during postmortem chilling to bring about major improvement in the tenderness rating of these muscles. A method for suspending intact carcasses through the obturator foramen, the so-called "Texas A&M tenderstretch" method has been described and evaluated by Hostetler et al., (1970, 1972); Orts et al., (1972); Quarrier et al., (1972) and Bouton and Harris (1972). Various other methods of carcass suspension have also been designed to place positive stress during rigor in order to optimize sarcomere length and thereby improve tenderness in as many muscles on the intact carcass as possible (Hostetler et al., 1972; Bouton et al., 1974). The application of some pre-rigor mechanical tensioning devices have also been utilized successfully to tenderize the major muscles of the leg and loin (Buege and Stouffer, 1974; Abban et al., 1975).

The present investigation was designed to evaluate the effect of leg-twisting, a traditional Nigerian method of carcass positioning, on the tenderness and fiber characteristics of the major muscles of the leg and loin as well as its effect on the orientation angle of the femur relative to the pelvic axis.

MATERIALS & METHODS

SIX GOOD GRADE West African dwarf ewes ranging in live weight from 27.9–33.2 kg were slaughtered without immobilization and dressed conventionally except that the two hind legs retained their hoofs. One-half from each carcass was randomly assigned to the control (conventional) or to the experimental (leg-twisted) method of suspension. The hoofs of the control group were removed in the conventional manner while the skin on those of the experimental group was removed to the base of the hoofs and the crevices between the hoofs were then carefully cleaned with mild formalin solution, care being taken that the body of each carcass was not contaminated. The leg-twist treatment was achieved by making a slit in the flank at the level of the cranioventral tip of the Rectus abdominis. One hoof of the leg was tucked into this slit such that the thin part of the flank formed a sling in the crevice between the hoofs as seen in Figure 1. Both sides of the carcasses were suspended by the achilles tendon and moved into the cold room maintained at 1°C within 30 min post slaughter.

After a chilling period of 48 hr, the orientation angle of the femur bone relative to the pelvic axis was estimated using the scheme of Abban et al. (1975). The reference points on the femur were the stifle joint distal to the patella and the point of the greater trochanter while those on the pelvic bone were the lateral ischiatic tuber and the tuber coxa.

Chops of 3.75 cm. thickness were cut from some selected muscles as follows: longissimus-rib (LDR), longissimus-loin (LDL) gluteus medius (GM), psoas major (PM), semimembranosus (SM), biceps femoris (BF), semitendinosus (ST), adductor (ADD), vastus lateralis (VL) and rectus femoris (RF). A chop containing the LDR was obtained from the caudal end of the rack. Another chop containing the LDL and PM was obtained from the caudal end of the loin. The chop containing the GM was obtained by taking the section spanning the 3–5 sacral vertebrae. The following muscle pairs were dissected out as a unit respectively, SM and ADD, ST and BF, RF and VL. The respective chops were then obtained from the thickest portion of these muscle masses.

The fascia surrounding each chop was cut vertically as necessary to prevent warping of the chops during broiling. The chops were broiled to medium doneness and cooled to room temperature. Three cores of 1.3 cm thickness were taken in the fiber direction of the LDL, GM and SM. Two cores were taken from the LDR, BF and RF and only one core from the PM, ST, ADD and VL. The shear values estimating the level of tenderness were obtained with a Warner-Bratzler shear device. Each core was sheared midway, and each half was again sheared for a total of three shear values per core.

A section approximately 1 cm thick was obtained from each raw muscle immediately adjacent to the section from which the chop for tenderness was taken. A cube 1 × 1 × 1 cm was obtained at the center of this section and fixed in 10% formalin solution for at least 1 wk at room temperature. It is possible that slight distortion of the muscle as a result of formalin fixation may give different values for the sarcomere lengths, fiber diameter and fragmentation values when compared with values obtained through other methods. A thin strip of muscle was removed in the fiber direction from the center of each fixed sample, and homogenized in 50 ml distilled water using the small covered cup of a Mouliner kitchen blender. The suspension of muscle fibers and myofibrils was observed directly in a phase contrast microscope. The fiber diameters of randomly selected fibers were estimated against a calibrated micrometer eye piece while the sarcomere length was determined in the same manner on 20 randomly selected myofibrils. Fragmentation values were estimated independently by counting the number of sarcomeres on 20 randomly selected myofibrils. Means, standard deviations and paired t statistics were computed where appropriate.

RESULTS & DISCUSSION

THE EFFECT of leg-twisting on mean shear values and the corresponding standard deviations for the 10 muscles evaluated are shown in Table 1. The data indicated significant toughening ($P < 0.05$) of the ST by as much as 24.69%. The PM was also toughened but by only 5.49%. Abban et al. (1975) using a mechanical tensioning device observed similar trends in these two muscles while Quarrier et al. (1972) noted slight but nonsignificant toughening of the PM. The shear values of all other muscles were improved to various extents by the treatment. The VL had the least improvement of 3.02% while the GM, RF, SM and ADD were significantly improved ($P < 0.05$) and in the latter muscle by as much as 31.69%.

Although the treatment appeared to put some tension on the LD as reflected in the straightening of the vertebral axis in the lumbar region, the resultant effect was not significantly reflected in the shear values. It seems likely that the slack introduced by curvature of the skeleton in the neck region as a

Author Okubanjo is with the Meat Science Lab., Dept. of Animal Science, University of Ibadan, Ibadan, Nigeria

0022-1147/78/0004-1130$02.25/0
© 1978 Institute of Food Technologists

result of leg-twisting nullified any advantage that may have resulted from rearrangements in the pelvic region. Except for the BF and ST, the treatment reduced the range in shear values for the various muscles to a limit of 0.58 as against 1.73 for the conventional group. Thus the leg-twisted muscles were more uniformly tender, a trait that is highly desirable in the leg and loin chops. Between animal variability was also reduced as shown by the lower standard deviations in most of these muscles.

The effect of leg-twisting on the angle or orientation of the femur relative to the pelvic axis and on the shear values of four muscles from the individual carcasses are presented in Table 2. The treatment effect on the shear values of these muscles was significant at the 5% level while its effect on the orientation angle was significant at the 1% level. For uniformity of treatment, the carcasses were hung by the achilles tendon rather than by the flexor tendons. This and the fact that the present carcasses have less muscle mass than the temperate breeds may account for the increase in the orientation angle of the conventionally hung carcasses when compared with similar values reported by Abban et al. (1975).

The present treatment achieved approximately a 50% reduction in the orientation angle from a mean value of 129.7° to 65.6°. The reduction may be attributed to the cumulative effect of the movement of the distal end of the femur in a cranial direction and abaxially from the mid-ventral region as well as a distal rotation of the major trochanter of the femur. There was also a rearrangement of the pelvic bone relative to the axial skeleton resulting in straightening of the lumbar region. Thus the conformation achieved following leg-twisting approached that of the live animal in the resting prostrate form in which the leg and loin muscles may be expected to be more relaxed. The movement of the femur as a result of leg-twisting tended to elongate the GM and the SM–ADD muscle mass whereas in the conventionally hung carcass, these muscles remained short and robust. The treatment also introduced a marked slack in the ST resulting in appreciable shortening and significant increase in toughness.

The mean sarcomere lengths and fiber diameters of the muscles resulting from the two methods of suspension are shown in Table 3. These results indicate that leg-twisting significantly increased the sarcomere lengths of the GM, SM, BF, RF ($P < 0.01$) and LDR ($P < 0.05$) and to a smaller extent, those of the LDL, ADD and VL. The slack introduced into the ST by leg-twisting was significantly reflected in its shorter sarcomere length ($P < 0.01$) while the PM showed only a nonsignificant reduction in sarcomere length. These results appear to be in line with results from previous workers (Quarrier et al., 1972; Hostetler et al., 1972; Abban et al., 1975). The latter group of workers noted that their mechanical device which rotated the leg so as to approach the live animal posture had the effect of relieving the tension imposed on the ST and PM by the conventional method thereby decreasing the sarcomere lengths and increasing toughness. Hostetler et al. (1972) and Quarrier et al. (1972) using the tenderstretch method on beef and lamb respectively noted a decrease in sarcomere length only in the PM and not in the ST. The above studies and the present one suggest two things. Firstly, the ST is more critically affected than the other muscles of the thigh by the effective angle between the femur and the pelvic axis on one hand and the femur and the tibia/patella on the other. The orientation of these bone pairs is responsible for introducing slack or tension in some of these muscles and the ST is the only leg muscle of those considered that has no attachment with the femur (Sisson and Grossman, 1953). Secondly, perhaps the ST shows a greater inclination among these muscles to shorten.

Table 1—Effect of leg-twisting on shear force (kg) of selected ewe muscles

Muscle	Conventional Mean ± S.D.[a]	Leg-twisted Mean ± S.D.[a]	% Improvement[b]
LDR	4.58a ± 1.43	4.05a ± 1.13	11.57
LDL	4.33a ± 1.12	3.78a ± 1.34	12.70
GM	4.80a ± 1.18	3.96b ± 0.59	17.50
PM	3.44a ± 0.78	3.64a ± 0.83	–5.49
SM	5.17a ± 1.71	3.81b ± 0.52	26.31
BF	6.08a ± 0.94	5.77a ± 0.75	5.10
ST	4.82a ± 1.40	6.01b ± 1.11	–24.69
ADD	5.08a ± 1.98	3.47b ± 0.49	31.69
VL	3.97a ± 0.66	3.85a ± 1.00	3.02
RF	4.42a ± 1.69	3.53b ± 0.52	20.14

[a] Means on same line with a common letter are not different ($P > 0.05$)

[b] % Improvement = $100 \left(\frac{\text{Reduction in shear value due to leg twisting}}{\text{Shear value due to conventional treatment}}\right)$

The change in sarcomere length of the BF was not significantly reflected in the shear values presumably due to its high connective tissue content. The large change in the shear values of the ADD could also not be matched by a corresponding magnitude in increase in the sarcomere length. The treatment increased the sarcomere lengths of all the muscles except the ST to between 2 and 2.5 which has been demonstrated, at least in beef muscles, to be the range at which tenderness improvement is maximized (Hostetler et al., 1972).

An inverse relationship was noted between the sarcomere length and fiber diameter with the SM, ADD and VL showing a significant decrease in diameter while the ST showed a significant increase in diameter. When all the 10 muscles are considered, the order of magnitude of sarcomere length was not reflected in the inverse order of fiber diameter probably due to the inherent variations in the diameter of fibers of the different muscles.

The fragmentation values are shown in Table 4. The number of sarcomeres per myofibrillar fragment significantly decreased as a result of leg-twisting in the GM, SM, RF ($P < 0.01$) while it increased in the PM ($P < 0.05$). All the other muscles except the ST also exhibited decreased but nonsignificant fragmentation values. A comparison of the change in shear force of the PM and ST which were both adversely

Table 2—Effect of leg-twisting on the orientation angle of femur bone and shear force of selected ewe muscles

Carcass no	Conventional					Leg-twisted				
	Angle (degrees)	Shear force kg				Angle (degrees)	Shear force kg			
		GM	ADD	SM	ST		GM	ADD	SM	ST
1	130.0	4.34	5.33	5.17	6.03	64.0	4.07	3.11	4.15	7.61
2	132.0	5.58	7.03	5.76	4.93	65.0	4.60	4.03	4.08	6.85
3	137.0	6.12	7.20	8.16	6.83	68.5	4.46	3.12	4.06	5.75
4	130.0	5.78	4.22	4.67	3.17	67.0	4.04	4.03	3.97	6.20
5	125.5	3.62	3.10	4.09	4.28	64.3	3.56	3.62	3.80	4.93
6	123.5	3.37	3.48	3.19	3.70	64.5	3.02	2.93	2.77	4.72
Mean	129.7	4.80	5.08	5.17	4.82	65.6	3.96	3.47	3.81	6.01

Table 3—Comparison of sarcomere lengths and fiber diameter of nine muscles from conventional and leg-twisted ovine carcasses

Muscles	Sarcomere Length (um)		Fiber diameter (um)	
	Conventional Mean ± S.D.[a]	Leg-twisted Mean ± S.D.[a]	Conventional Mean ± S.D.[a]	Leg-twisted Mean ± S.D.[a]
LDR	1.87a ± 0.30	2.07b ± 0.15	38.87a ± 2.27	36.24a ± 3.40
LDL	2.09a ± 0.20	2.13a ± 0.28	38.37a ± 2.21	34.97a ± 5.93
GM	1.92a ± 0.13	2.59c ± 0.29	41.03a ± 4.22	36.60a ± 9.18
PM	2.51c ± 0.27	2.33a ± 0.19	35.33a ± 3.32	36.33a ± 3.30
SM	1.93a ± 0.18	2.43c ± 0.27	40.93a ± 3.69	34.80b ± 6.85
BF	1.94a ± 0.11	2.40c ± 0.25	41.25a ± 4.93	37.00a ± 3.56
ST	2.26a ± 0.11	1.86c ± 0.15	42.59a ± 7.10	47.18b ± 6.80
ADD	2.16a ± 0.30	2.28a ± 0.30	36.83a ± 3.16	34.29b ± 5.42
VL	2.06a ± 0.13	2.42a ± 0.41	41.90a ± 2.94	35.89b ± 2.19
RF	2.12a ± 0.12	2.56c ± 0.18	41.03a ± 3.73	39.59a ± 3.74

[a] Means on same line with a common letter are not different ($P > 0.05$)

affected by the treatment indicates that a slight change in the shear force of the PM was more liable to be accompanied by a significant reduction in the ease of fragmentation while in the ST, a similar change in the shear force was not accompanied by a significant effect on the fragmentation value. This seems to support the contention of Sayre (1970) that although muscle fragmentation takes place at the same time as tenderization, they may not have a cause and effect relationship. Phase contrast microscopic observations in this instance also indicated that increases in tension in most of the muscles as a result of the treatment enhanced a higher degree of fragmentation as reflected in the lower fragmentation values through breakages at the level of the Z–I junction (Fukazawa et al., 1963, Takahashi et al., 1967) rather than through a dissolution of the Z line (Davey and Gilbert, 1967; Goll et al., 1970) which would be associated more with aged muscles.

The present method does not involve any change in the conventional on-rail handling of the carcass but rather improves floor clearance under the leg-twisted carcasses. Fast cooling may also be used with minimum cold shortening (Bouton et al., 1973) since most of the major muscles are restrained from shortening or slightly stretched by the hanging posture. Retaining the hoofs may pose microbiological problems and may require designing easily cleaned mechanical devices to perform the same function as the foot now serves. On the other hand, it appears that the hoofs may also be removed following a short immersion in boiling water. Fabrication into primal cuts may be a problem although the same methods presently in use for tenderstretch carcasses should be adequate.

REFERENCES

Abban, A.R., Stouffer, J.R. and Westervelt, R.G. 1975. Pre-rigor mechanical tensioning of lamb carcasses to improve tenderness. J. Food Sci. 40: 1214.

Bouton, P.E. and Harris, P.V. 1972. The effects of some post-slaughter treatments on the mechanical properties of bovine and ovine muscles. J. Food Sci. 37: 539.

Bouton, P.E., Harris, P.V., Shorthouse, W.R. and Baxter, R.I. 1973. A comparison of the effects of aging, conditioning and skeletal restraint on the tenderness of mutton. J. Food Sci. 38: 932.

Bouton, P.E., Harris, P.V., Shorthouse, W.R. and Smith, M.G. 1974. Evaluation of methods affecting mutton tenderness. J. Food Tech. 9:31.

Buege, D.R. and Stouffer, J.R. 1974. Effects of pre-rigor tension on tenderness of intact bovine and ovine muscle. J. Food Sci. 39: 396.

Davey, C.L. and Gilbert, K.V. 1967. Structural changes in meat during ageing. J. Food Tech. 2: 57.

Fukazawa, T., Hashimoto, Y., and Tonomura, Y. 1963. Isolation of single sarcomere and its contraction on addition of adenosine triphosphate. Biochem. Biophys. Acta 75: 234.

Goll, D.E., Arakawa, N., Stromer, M.H., Busch, W.A. and Robson, R.M. 1970. Chemistry of muscle proteins as a food. In "Physiology and Biochemistry of Muscle as a Food." University of Wisconsin Press, Madison, WI.

Hostetler, R.L., Link, B.A., Landmann, W.A. and Fitzhugh, H.A. 1972. Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles. J. Food Sci. 37: 132.

Table 4—Fragmentation value for selected muscles from conventional and leg-twisted carcasses

Muscles	Conventional Mean ± S.D.[a]	Leg - Twisted Mean ± S.D.[a]
LDR	43.17a ± 6.53	40.77a ± 1.88
LDL	43.49a ± 4.74	42.83a ± 2.60
GM	45.04a ± 0.51	35.99c ± 4.35
PM	38.35a ± 4.93	41.28b ± 2.55
SM	45.68a ± 3.90	39.91c ± 1.56
BF	43.30a ± 4.91	39.28b ± 4.72
ST	40.72a ± 3.62	42.33a ± 2.26
ADD	41.68a ± 5.05	40.12a ± 2.92
VL	43.26a ± 2.77	38.72a ± 4.31
RF	47.20a ± 6.32	38.27c ± 5.02

[a] Means on same line with a common letter are not different ($P > 0.05$).

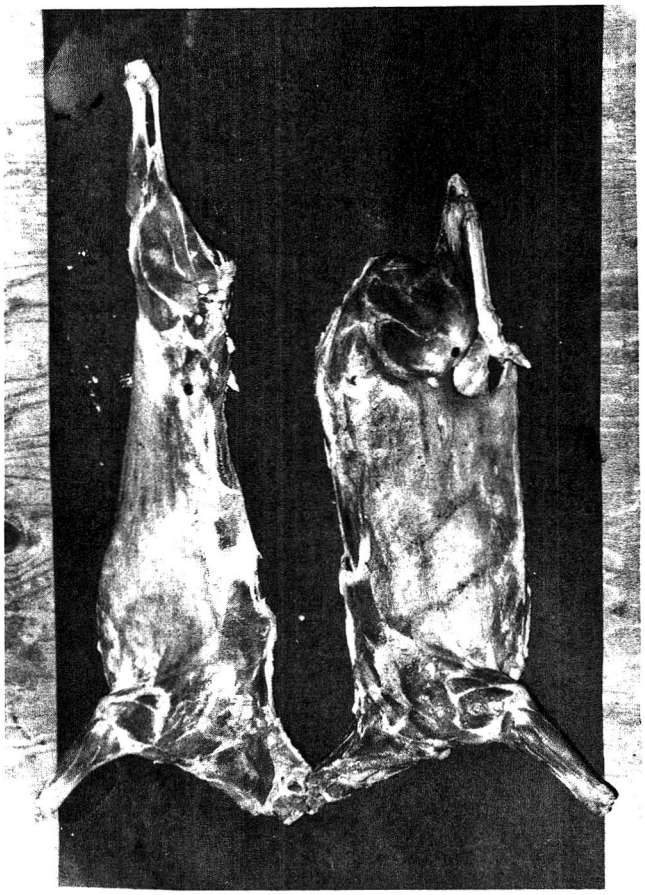


Fig. 1—Conventional and leg-twisted mutton carcass sides.

Hostetler, R.L., Link, B.A., Landmann, W.A., Fitzhugh, H.A. Jr., Carpenter, Z.L. and Orts, F.A. 1970. Texas A&M tenderstretch beef. A method of carcass suspension for improving tenderness. MP943 Texas Agric. Expt. Sta. College Station, Texas.

Orts, F.A., Smith, G.C. and Hostetler, R.L. 1971. Texas A&M tenderstretch. Tex. Agric. Ext. Service Bull. L-1003.

Quarrier, E., Carpenter, Z.L. and Smith, G.C. 1972. A physical method to increase tenderness in lamb carcasses. J. Food Sci. 37: 130.

Sayre, R.N. 1970. Chicken myofibril fragmentation in relation to factors influencing tenderness. J. Food Sci. 35: 7.

Sisson, S.B. and Grossman, J.D. 1953. "The Anatomy of the Domestic Animal." W.B. Saunders Co. Philadelphia.

Takahashi, K., Fukazawa, T. and Yasui, T. 1967. Formation of myofibrillar fragments and reversible contraction of sarcomeres in chicken pectoral muscle. J. Food Sci. 32: 409.

Ms received 6/6/77; revised 10/15/77; accepted 10/20/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA., June 5–8, 1977.

This study was funded through the Senate Research Grant (6.302.26) University of Ibadan.

The author gratefully acknowledges the technical assistance of J. Kolade and O. Bolarinwa.

SOME EFFECTS OF SELECTED PLASTICIZING AGENTS ON THE REVERSIBLE COMPRESSION OF FREEZE-DRIED COOKED BEEF CUBES

J. P. MORGAN and D. F. FARKAS

ABSTRACT

Ethanol (95%) and glycerin were found to plasticize freeze-dried cubes of beef for reversible compression. Both are easily applied and plasticization appears to be rapid and effective. Appearance and texture of the reconstituted, compressed product compared favorably with that of noncompressed product and product plasticized by partial rehydration to 10% moisture; however, the glycerin and ethanol treatments gave a slight off-flavor. Vegetable oil and trichlorofluoromethane did not provide adequate plasticization. Polar liquids appear to be required for plasticization of dried beef for compression.

INTRODUCTION

IT HAS BEEN FOUND that, properly plasticized, most foods capable of being freeze dried can be reversibly compressed, i.e., rehydrated to virtually its original form (Brockman, 1969). Compression has created a new class of foods (Anonymous, 1973); however, current procedures for plasticizing for reversible compression are not commercially attractive.

The objective of this work has been to develop a more economical method of plasticizing freeze-dried, cooked beef cubes so that a densified beef product having good reconstitutive values can be obtained by compression.

Freeze-dried beef is a quality product in terms of flavor and texture, and compression readily reduces the volume. However, since fully freeze-dried beef contains only about 2% moisture, it is extremely friable and compression at this moisture level physically damages the structural components, causing the food to disintegrate when reconstituted.

Freeze-dried beef can be reversibly compressed if a uniform moisture level in the range of 5–13% is maintained (Ishler, 1965; Brockman, 1966). A forming pressure of 3000 psi is suitable for some lots of beef; high forming pressures should be avoided since they generally form compressed products that rehydrate poorly (Pilsworth and Hoge, 1973).

Fully freeze-dried foods can be adequately plasticized with water if sufficient time is allowed for equilibration of the predetermined amount of water added. Four to five hours at 25°C are required to accomplish equilibration; thus there is risk of damage to food quality through chemical and microbial action. Following compression the compressed product must be redried to about a 2% moisture level, an added cost.

An interruption of the freeze drying operation at the desired water content would seem to be a logical solution to the problem. However, this is impractical since there is a nonsublimated ice core within the product which does not disappear until the water content is reduced to 1% of the original weight (4% of dry weight) (Pilsworth et al., 1972). Consequently, stopping the freeze drying operation at the mean plasticizing moisture level yields a soggy wet core and a dry, friable exterior; which on equilibration does not yield a product that can be reversibly compressed.

Mackenzie and Luyet (1969) developed a method of leaving the desired water content uniformly distributed in freeze-dried food through maintenance of a predetermined relative humidity at the surface and within the product during the freeze drying operation. The method, which the authors named "limited" freeze drying, provides good, reliable control of the moisture content, and the product possesses adequate pliability during compression. Unfortunately, lengthy drying times are required.

King et al. (1976) have recently described two processing approaches to limited freeze drying. One is a modification of a normal freeze dryer to control the operating conditions and the second uses a hydrating salt in equilibrium with a circulating gas apparatus to regulate the water vapor pressure and thus, the moisture level within the freeze-dried product. As yet neither of these methods has been commercialized.

This paper reports the results of investigations to obtain easily applied plasticizers, other than water, which can be added to pre-cooked and fully freeze-dried beef cubes to allow immediate compression with or without finish drying. The results were qualitatively evaluated to determine possible directions for future work.

MATERIALS & METHODS

PLASTICIZERS were selected from food grade chemicals and were applied directly to the freeze-dried beef after drying. Plasticizers were evaluated for effectiveness during compression by comparing the reconstituted appearance and texture of the beef cubes with (1) similarly reconstituted noncompressed freeze-dried beef, (2) beef dried to 2% moisture and compressed, and (3) dried samples equilibrated to 10% moisture prior to compression.

Plasticizers were selected as follows. Vegetable oil (Wesson brand) was tested on the assumption that it would provide a lubricating and plasticizing effect to the dry beef during compression. Glycerin was used because of its ability ot permeate tissue and to form hydrogen bonds. Ethanol (95%) was used because of its ability to form hydrogen bonds and its higher volatility. Trichlorofluoromethane (Freon 11), although not a food additive (The Merck Index, 1976), was tested as a nonpolar liquid because of its ease of removal after compression. Mixtures of glycerin and ethanol consisting of 5% glycerin and 50% glycerin by volume were also used.

Freeze-dried beef cubes were prepared by deboning and removing the excess fat from top round steak and dicing into ½-in cubes. The beef cubes were simmered for 25 min in water containing 1.5% salt based on the meat weight. The cooked beef was frozen overnight in a refrigerated box at −29°C. The frozen cubes were freeze dried at a shelf temperature of 21°C and vacuum of about 0.2 mm Hg to a final moisture content of about 2%. For the experiments, sufficient freeze-dried cubes were assembled to give a sample weighing 10 ± 0.2g (about 10–12 cubes).

Three methods of plasticizer application were tested in an attempt to obtain a uniform plasticizer distribution in the dry cubes and to obtain a minimum holding time between application and compression. These were: vacuum infusion at 1.0 mm Hg for 5 min at 30°C; vacuum infusion at 125 mm Hg for 5 min at 25°C for ethanol and 60°C for vegetable oil and glycerin; and needleless jet (Syrijet injector) injection using a pressure of approximately 100 kg/cm². Plasticized cubes were equilibrated as shown in Table 1 to facilitate distribution of the plasticizing agent prior to compression. Treated cubes were weighed to determine plasticizer content before and after compression.

Cubes were compressed on a Carver laboratory hydraulic press. A maximum pressure of 70 kg/cm² was applied for 3 min against the material inside a cylindrical mold, 2.9 cm diam by 6.4 cm deep. An effort was made to produce the same pressure-distance pattern for each treatment by programming the distance the piston traveled during set time intervals.

The compressed wafer, Figure 1, was ejected and weighed to deter-

Authors Morgan and Farkas are with the USDA Western Regional Research Center, Agricultural Research Service, Berkeley, CA 94710.

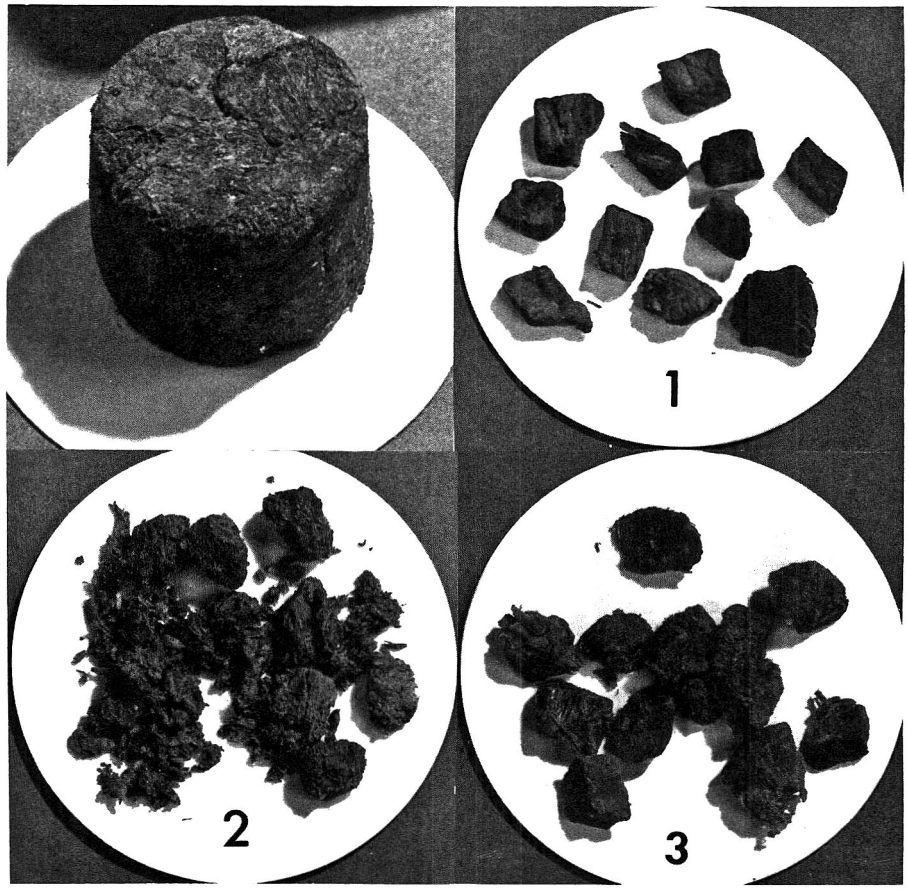


Fig. 1—Representative compressed wafer prior to rehydration and control wafers after rehydration at 88°C for 10 min in 80 ml water without stirring: (1) 2% moisture non-compressed; (2) 2% moisture, compressed; and (3) 10% moisture, compressed.

mine agent uptake after compression. The length of the wafer was measured to determine density. Wafer strength was checked by noting the difficulty with which the compressed wafer could be broken by hand.

Wafers were reconstituted by immersing in 80 ml of water at 88°C for 10 min without stirring (Pilsworth and Hoge, 1973). After decanting the excess water and draining for 1 min, samples were weighed to determine the rehydration ratio, which was calculated as the weight of the rehydrated product divided by its dry weight.

The reconstituted material was evaluated for texture and appearance; off-flavors were evaluated by a single judge acting as an expert taster. Photographs of the reconstituted materials were made immediately after rehydration, and these together with visual observations were used to compare the appearance of samples from various treatments with the uncompressed control. Hamdy (1961) considered visual comparison a satisfactory technique.

The texture parameters, hardness and chewiness, were determined with an Instron Universal Testing machine (model TM) fitted with a 0.87 cm diameter piston. Cross-head speed was 0.5 cm per minute and a fixed stroke length of 0.43 cm was used to produce a maximum sample compression of about 35%.

Two to five cubes of reconstituted beef were tested. The force was applied in two cycles in a direction perpendicular to the fiber orientation. Results were analyzed using the modified texture profile of Reidy and Heldman (1972). Equations for the calculation of the parameters are:

$$\text{Hardness} = L_1$$
$$\text{Chewiness} = L_1 \times A_2/A_1 \times B_2$$

where: L_1 = Maximum compression force in kilograms applied on the first cycle at 0.43 cm stroke length; A_1 = Total work of compression on first cycle; A_2 = Total work of compression on second cycle; and B_2 = Total deformation in mm of the reconstituted cube during the second compression cycle.

RESULTS

THE DENSITY of the freeze-dried beef after compression

Table 1—Summary of pre-compression treatment variables for the freeze-dried beef cubes

Treating agent	Application method	Holding time and temp	% Uptake of plasticizer (based on original wt)	
			Before compression	Remaining after compression
Water	Injection	6 hr @ 25°C	10	9.5
Vegetable oil	Vac. infusion 1.0 mm Hg	30 min @ 82°C	77	12
Vegetable oil	Vac. infusion 125 mm Hg	30 min @ 82°C	67	8
Vegetable oil	Injection	30 min @ 82°C	16	7
Glycerin	Vac. infusion 125 mm Hg	30 min @ 82°C	102	66
Glycerin	Injection	90 min @ 25°C	18	16
Ethanol (95%)	Vac. infusion 125 mm Hg	None	63	11
Ethanol (95%)	Injection	60 min @ 25°C	52	21
Ethanol, 5%; Glycerin, 95%	Injection	None	39	15
Ethanol, 50%; Glycerin, 50%	Injection	None	68	41
Trichlorofluoromethane	Injection	None	14	7
Trichlorofluoromethane	Vac. infusion 1.0 mm Hg	None	114	15

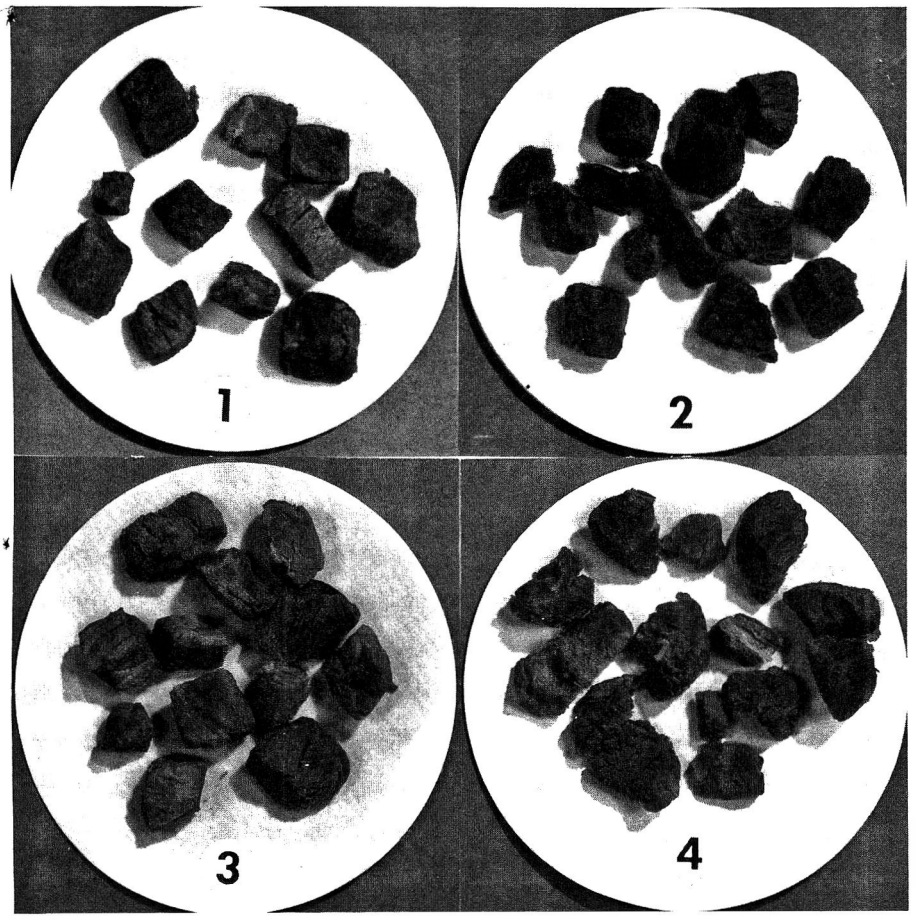


Fig. 2—Compressed wafers of chemically treated freeze-dried beef after rehydration: (1) ethanol treated; (2) glycerin treated; (3) 5% glycerin in ethanol treated; and (4) 50% glycerin in ethanol treated.

ranged from about 690–960 kg/m^3. The density of the freeze-dried beef prior to compression was approximately 240 kg/m^3. Wafer strength was deemed to be low for most of the plasticized samples.

The plasticized, compressed freeze-dried beef absorbed more water and had a higher rehydration ratio than the non-compressed freeze-dried control (Table 2). This may be partly the result of fragmentation, but unknown factors could be involved since the rehydration ratio showed no apparent correlation with the visual appearance of the rehydrated beef.

Glycerin, vegetable oil, and alcohol imparted an off-flavor to the reconstituted product; and, of the three, glycerin was the most noticeable. On reconstitution, all compressed samples produced an effect of mouth dryness.

The rehydrated control samples are shown in Figure 1, and the rehydrated treated samples in Figure 2.

Ethanol- (vacuum and injection) and 5% glycerin in ethanol (injection)-treated samples were comparable in appearance to the freeze-dried noncompressed control.

Glycerin-treated (vacuum and injection) and the 50% glycerin in ethanol-treated material (injection) were comparable in appearance to that of the control prepared by reconstituting the 10% moisture equilibrated and compressed sample.

The reconstituted appearances of the vegetable oil and the trichlorofluoromethane-treated materials were comparable to that of the control compressed at 2% moisture. These products were unacceptable.

Texture parameters shown in Table 2 correlate well with

Table 2—Properties, rehydration ratios and textural parameters of freeze-dried and compressed beef cubes

Treatment	Wafer strength	Wafer density (based on wt of F-D beef) kg/m^3	Rehydration ratio	Textural parameters	
				Hardness (kg)	Chewiness (kg-mm)
Not compressed–2% moisture	–	240	1.8	2.1	2.62
Compressed–2% moisture	weak	865	2.2	0.4	0.28
Compressed–10% moisture	strong	960	2.2	0.8	1.10
Vegetable oil–Vac. Inf., 1.0 mm Hg	weak	945	2.1	0.4	0.29
Vegetable oil–Vac. Inf., 125 mm Hg	weak	880	2.1	0.6	0.69
Vegetable oil–Injection	weak	900	2.3	0.7	0.64
Glycerin–Vac. Inf., 125 mm Hg	v. strong	690	2.4	1.8	2.78
Glycerin–Injection	fair	900	2.5	0.5	0.45
Ethanol (95%)–Vac., Inf., 125 mm Hg	weak	815	2.3	1.1	1.67
Ethanol (95%)–Injection	v. weak	755	2.3	2.7	4.60
5% Ethanol, 95% Glycerin–Injection	weak	900	2.4	1.1	1.93
50% Ethanol, 50% Glycerin–Injection	weak	755	2.3	1.0	1.73
Trichlorofluoromethane–Injection	weak	850	2.6	0.4	0.33
Trichlorofluoromethane–Vac. Inf., 1.0 mm Hg.	v. weak	900	2.5	0.1	0.02

the product's appearance. Both hardness and chewiness of the vacuum infusion glycerin-treated samples are comparable to those of the noncompressed control. The ethanol-injected and held sample showed greater compressive strength. The texture parameters for the vegetable oil, glycerin injected, and trichlorofluoromethane samples were comparable to those of the unplasticized compressed control.

Representative force-distance curves for three controls and four treatments are shown in Figure 3. The force-distance curve for the ethanol-treated cubes are generally comparable to the noncompressed control, the glycerin-treated cubes are generally comparable to the partially rehydrated (10% moisture) control, and the vegetable oil and trichlorofluoromethane-treated cubes are generally comparable to the 2% moisture (fully freeze-dried) control.

CONCLUSIONS

THE APPEARANCE AND TEXTURE of the reconstituted beef pretreated with ethanol or glycerin prior to compression is equal to or better than freeze-dried beef (2% moisture) plasticized by rehydration and equilibrated to 10% moisture, the currently acceptable procedure. In some instances, the appearance and texture approached the quality of the noncompressed reconstituted freeze-dried beef control.

While ethanol has been judged (Pilsworth and Hoge, 1973) to be somewhat inferior to water as a plasticizer for beef, its use as a plasticizer appeared satisfactory based on the reconstituted appearance and calculated textile parameters of the reconstituted compressed freeze-dried beef. Ethanol, glycerin and mixtures of these materials were easily applied using vacuum infusion and high pressure injection, and plasticization appeared to be rapid and effective. Both agents are relatively inexpensive and may be safely added to food.

Vegetable oil and trichlorofluoromethane were unsatisfactory as plasticizing agents for freeze-dried beef.

Results indicate that liquids other than water can plasticize fully freeze-dried beef more rapidly, and more economically than the rehydration and equilibration method in present use. Additional work is needed to determine the effects of nonaqueous plasticizers on the organoleptic properties and long term storage stability of the reconstituted product. Additional information is needed on the best method of application and optimum amount of plasticizers.

The polar liquids tested in this work were better plasticizers than the nonpolar liquids. Benson and Seehof (1951) have noted that only polar sorbates seem to be strongly adsorbed on substances having polar functional groups such as protein. This was confirmed by Bushuk and Winkler (1957a, b) who also demonstrated that the adsorption rate is dependent on the sorbate's molar volume. If the observation that polar solvents are better plasticizers is found to be generally applicable, it could help in elucidating the mechanism allowing good plasticity in freeze-dried foods during compression at low water activity. An understanding of the mechanism would help in selecting the best plasticizer and conditions for use.

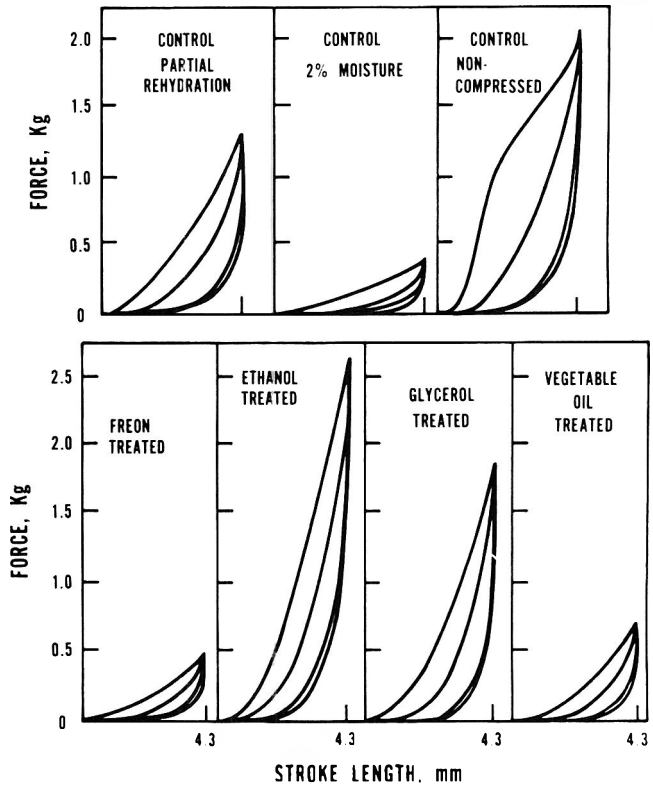


Fig. 3—Representative force-distance curves for the three control wafers and four treated wafers after rehydration.

REFERENCES

Anonymous 1973. Foods of tomorrow. Food Processing: Winter issue.

Benson, S.W. and Seehof, J.M. 1951. The surface areas of proteins. 4. Sorption of polar gases. J. Am. Chem. Soc. 73: 5053.

Brockman, M.C. 1966. Compression of foods. Activities Report 18(2): 173.

Brockman, M.C. 1969. Compaction of military foods. Activities Report 21(2): 83.

Bushuk, W. and Winkler, C.A. 1957a. Sorption of water vapor on wheat flour, starch, and gluten. Cereal Chem. 34(2): 73.

Bushuk, W. and Winkler, C.A. 1957b. Sorption of organic vapors on wheat flour at 27°C. Cereal Chem. 34(2): 87.

Hamdy, M.M. 1961. Compression of dehydrated foods, review of literature. Bulletin No. 5. Quartermaster Food & Container Institute for the Armed Forces, Quartermaster Research & Engineering Command, U.S. Army, Chicago, IL.

Ishler, N.I. 1965. Methods for controlling fragmentation of dried foods during compression. Tech. Report FD-13. U.S. Army Natick Laboratories, Natick, MA, AD 623712.

King, C.J., Carn, R.M. and Jones, R.L. 1976. Processing approaches for limited freeze drying. J. Food Sci. 41: 612.

Mackenzie, A.P. and Luyet, B.J. 1969. Recovery of compressed dehydrated foods. Tech. Report 70-16, FL-90. U.S. Army Natick Laboratories, Natick, MA, AD 703901.

Pilsworth, M.N. Jr., Segars, R.A. and Hoge, H.J. 1972. An experimental study of the freeze drying of raw beef. Tech. Report 73-12-PR. U.S. Army Natick Laboratories, Natick, MA, AD 769592.

Pilsworth, M.N. Jr., and Hoge, H.J. 1973. The compression of freeze-dried beef to form bars; plasticizing with water transferred as vapor. Tech. REport 73-56-PR. U.S. Army Natick Laboratories, Natick, MA, AD 766709.

Reidy, G.A. and Heldman, D.R. 1972. Measurement of texture parameters of freeze-dried beef. J. Texture Studies 3: 218.

Windholz, M. 1976. "The Merck Index," 9th ed, p. 1238. Merck and Company, Inc., Rahway, NJ.

Ms received 9/29/77; revised 12/26/77; accepted 1/4/78.

This research was supported through a grant from the U.S. Army Natick Laboratories to the USDA Western Regional Research Center.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

EFFECTS OF PREPROCESSING BELLY TEMPERATURE AND PUMPING PRESSURE ON BACON YIELDS, SALT RESIDUAL AND ORGANOLEPTIC QUALITY

R. P. NUSBAUM and R. E. RUST

ABSTRACT

Forty fresh pork bellies similar in weight and chemical composition were randomly assigned to four preprocessing belly temperature groups, 4.4, 10.0, 15.6, 21.1°C before being injected with brine at either 50 or 70 psi (344.7 or 482.6 kPa) pumping pressure. Increasing belly temperature and pumping pressure significantly increased pumped and final yields but similarly increased processing loss. Pumping pressure was significantly correlated with total salt residual, salt in lean, salt in fat and taste-panel ratings for flavor, saltiness and overall acceptability. Temperature had no significant effect on taste-panel evaluations but showed some significance on salt residual at the higher two belly temperatures.

INTRODUCTION

TOTAL BACON PRODUCTION in the United States for 1976 was in excess of 1,528,774,000 pounds (USDA, 1977). It is evident why bacon processors are appreciably concerned with various curing procedures and the influence they have on finished product yield and quality.

Relatively few studies relating tissue temperature to brine diffusion and retention have been conducted. Wistreich et al. (1959) reported that residual salt increased proportionately with the concentration of salt in the brine and nonlinearly with temperature. According to Wood (1966), rate of salt diffusion is largely governed by the concentration gradient and the temperature of the tissue being processed.

Processing comparisons of pre-and post-rigor meat have been more numerous. Although prerigor tissue has a chemical nature different from high-temperature, postrigor tissue, several prerigor studies indicate temperature influence on finished product yield and, particularly, salt diffusion. Mandigo and Henrickson (1967) found essentially no difference in the yield of bacon between pre-and post-chill processed bellies. A similar study with hams (Mandigo and Henrickson, 1966) also revealed no significant difference in the yield of finished hams between hot and cold processing treatments. Arganosa and Henrickson (1969), however, reported a significant difference in diffusion distance for cure in hams injected before chilling than in those injected after chilling.

Bacon composition, salt residual and bacon quality were related in a study by Chant et al. (1974). They noted that subjective scores of saltiness and flavor increased as percentage lean in the bacon increased. They also found that an objective measure of precentage salt residual in the slice was significantly correlated with the percentage of lean.

Many variables have been shown to affect the process of converting green bellies into bacon. This study was designed to determine the influence of preprocessing belly tissue temperature and pumping pressure on yields, residual salt and organoleptic quality of processed bacon.

Author Nusbaum is with the Dept. of Animal Science, Iowa State University, Ames IA 50011. Author Rust is with the Dept. of Animal Science and Dept. of Food Technology, Iowa State Univ., Ames, IA 50011.

0022-1147/78/0004-1137$02.25/0
© 1978 Institute of Food Technologists

EXPERIMENTAL

FORTY BELLIES ranging in weight from 5.5–6.4 kg with similar chemical composition (moisture, fat and protein) were used as experimental units. Previous work by Schroder and Rust (1974 a, b) indicated that carcasses similar in weight and backfat thickness would yield compositionally uniform bellies. The 40 bellies used in this study were selected on this premise so as to minimize variation among experimental units.

Twenty chilled carcasses having an average backfat thickness of 4.1–4.6 cm and carcass weight of 72.7–81.8 kg were selected. The 40 bellies from these carcasses were collected from the cutting line and then skinned and trimmed, following normal packing plant procedure.

The 40 bellies were randomly assigned to four preprocessing temperature groups: 4.4, 10.0, 15.6 and 21.1°C. Each group of 10 bellies was placed in a separate temperature-controlled chamber until the internal belly temperatures equilibrated to the respective chamber temperature.

After temperature equilibration, the 10 bellies from each temperature group were randomly assigned to two pumping pressures, either 50 or 70 psi (344.7 or 482.6 kPa) and injected with curing brine containing 10% salt (NaCl). All bellies were injected with a commercial brine injection machine (a prototype of the Townsend Injectomatic) using needles with a 0.076-cm orifice. Belly weight was recorded preceding brine injection (green weight) and after a 3-min dripping period after injection (recorded as pumped weight). Pumped yield was calculated as a percentage of green weight.

Bellies were heat processed (with smoke added) to an internal temperature of 53.3°C, then chilled. A final processed weight was recorded before pressing and slicing, with final yield calculated as a percentage of green weight.

A U.S. Berkel Model 170 GS bacon slicer was used to obtain uniform slices 4.0 mm in thickness. Twelve slices representative of the total bacon composition (Nusbaum et al., 1976) were removed for subsequent moisture, fat, protein and residual salt determinations.

The 12 sample slices from each bacon slab were physically separated into fat and lean portions. Exactly one-half of the lean and one-half of the fat portions (by weight) were recombined to represent the original compostion of the whole bacon slab for subsequent moisture, fat and protein analyses according to AOAC (1970) procedures. Residual salt concentrations of this recombined sample and of the remaining fat and lean portions were determined by using a modified procedure of Kirton and Pearson (1963).

An experienced, 10-member sensory panel evaluated the bacon for flavor, saltiness and overall acceptability using an 8-point hedonic scale, with 8 being the most desirable. Six sample slices (adjacent to the previous 12 sample slices) from each bacon slab were oven cooked at 204°C for about 15 min until golden brown and uniform in crispness (Olson et al., 1973). Cooking loss percentage was determined by the ratio of weight loss to the weight before cooking.

The data were analyzed by use of the Statistical Analysis System of Barr et al. (1976) and by methods described by Snedecor and Cochran (1967). The analysis of variance and t-test were used to determine the significance of variability of the means for belly chemical components, tissue salt residuals, bacon yields and taste-panel evaluations.

RESULTS & DISCUSSION

NO SIGNIFICANT DIFFERENCES were found among the bellies for moisture, fat or protein, indicating the reliability of average backfat thickness and carcass weight as selection criteria for obtaining uniform bellies (Table 1).

Initial simple correlations (Table 2) indicated that both preprocessing belly temperature and pumping pressure significantly affect the pumped yield, final yield and total residual salt of the processed bacon. As expected, pumped yield, final yield and total residual salt were significantly intercorrelated.

—Text continued on page 1138

Table 1—Means, standard errors, ranges and standard deviations for green weight and compositional components of the processed bacon

Variables	Observations	Overall Mean	S.E.[a]	Ranges of individual observations Low	High	S.D.
Green wt (kg)	40	5.78	0.06	5.07 –	6.48	0.41
Moisture (%)	40	33.52	0.69	21.38 –	41.64	4.25
Extractable fat (%)	40	53.12	0.98	39.07 –	65.98	6.06
Protein (%)	40	7.70	0.17	5.44 –	10.14	1.03

[a] Standard error of the eight treatment means

Table 2—Correlation coefficients between preprocessing belly temperature, pumping pressure, pumped yield, final yield and total residual salt

	Pumped yield (%)	Final yield (%)	Total salt (%)	Pressure (psi)
Final yield	0.89**			
Total salt	0.87**	0.75**		
Pumping pressure	0.52**	0.48**	0.41**	
Temperature	0.53**	0.52**	0.32**	0.00

** $P < 0.01$

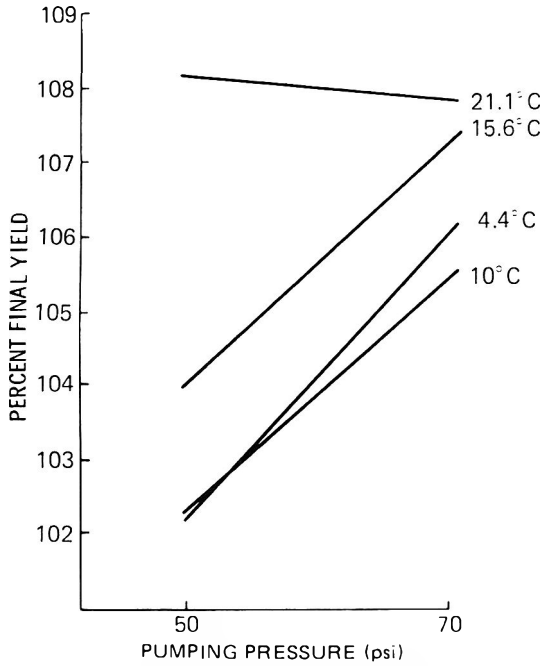


Fig. 1—Interaction between preprocessing belly temperature and pumping pressure on final bacon yield.

Table 3—Treatment means for pumped yield, final yield and processing loss[a]

Temp °C	psi	Observations	Pumped yield (%)	Final yield (%)	Processing loss (%)
4.4		10	113.45a	104.24ab	9.21a
10		10	112.68a	103.96a	8.72a
15.6		10	116.59b	105.67b	10.92a
21.1		10	122.38c	107.93c	14.45b
	50	20	112.90**	104.12**	8.79**
	70	20	119.65**	106.78**	12.86**

[a] Means bearing different letters in the same column are different ($P < 0.05$)
** $P < 0.01$

A significant interaction ($P < 0.05$) between preprocessing belly temperature and pumping pressure was apparent for final yield (Fig. 1). This interaction is graphically depicted by the obvious dissimilar values for 21.1°C temperature group. Differences in final yield between the lower two temperatures, 4.4° and 10°C, and the 15.6° or 21.1°C groups were much greater at the lower pumping pressure. The total range in final yields also was much greater at 50 psi. Significant increases ($P < 0.01$) in final yields when increasing the pumping pressure, within temperature groups, from 50–70 psi were clearly apparent at the lower three temperatures. The 21.1°C temperature group, however, had similar final yields regardless of the pumping pressure used, indicating that the bacon processor can attain similar final yields at lower pumping pressures by increasing the belly temperature before injection, as in this situation, to 21.1°C

The influence of preprocessing belly temperature and pumping pressure on bacon yields is further clarified by a comparison of treatment means (Table 3). Significant differences among belly temperature groups for final yield were primarily due to the 21.1°C temperature group. There were essentially no significant differences revealed among final yields for the lower three temperature groups. In comparisons of pumping pressure, regardless of temperature, bellies pumped at 70 psi retained greater amounts of brine before and after thermal processing, resulting in significantly greater pumped and final yields. However, these bellies pumped at 70 psi also sustained greater losses during thermal processing than did bellies pumped at 50 psi. Therefore, it seems that higher pumping pressures can significantly increase final yield at lower preprocessing belly temperatures. These data also suggest that only preprocessing belly temperatures approaching 21.1°C significantly affect final yield when pumping pressure is not considered.

Treatment means for salt deposition in the separable lean, separable fat and for the bacon as a whole were compared (Table 4). Although preprocessing belly temperature had little effect on residual salt values at the lower three temperatures, the 21.1°C temperature group had greater total residual salt in all tissues. Also, when pumping pressure was increased from 50 to 70 psi, all three tissue samples had highly significant increases in residual salt. These values indicate the influence of higher tissue injection temperatures on salt deposition in the whole bacon as well as individually in the fat and lean tissues. It seems that elevated preprocessing belly temperatures allow the belly to retain more cure after injection and thermal processing and ultimately result in a higher percentage of residual salt. This suggests that the elevated preprocessing belly temperature alters the physical properties of the tissue to enhance salt diffusion and retention.

Saltiness as detected by an experienced taste panel was significantly correlated with both pumped and final yield (Table

Table 4—Treatment means for total salt deposition in the bacon slab and individually in the fat and lean tissues[a]

Temp °C	psi	Observations	Percentages Total salt	Lean salt	Fat salt
4.4		10	0.73a	2.40a	0.64a
10		10	0.45b	1.96b	0.46b
15.6		10	0.83a	2.36a	0.74a
21.1		10	1.07c	2.80	0.89c
	50	20	0.58**	2.10**	0.58**
	70	20	0.96**	2.69**	0.79**

[a] Means bearing different letters in the same column are different ($P < 0.05$)
** $P < 0.01$

Table 5—Correlation coefficients between processing variables and taste panel values for flavor, saltiness and overall acceptability

Final yield percent	Flavor	Saltiness	Overall acceptability
Pumped yield (%)	0.32*	0.63**	0.35*
Final yield (%)	0.37*	0.66**	0.38*
Total residual salt (%)	0.36*	0.59**	0.40**
Pumping pressure (psi)	0.44**	0.48**	0.44**
Temperature (°C)	−0.17	0.17	−0.13

* $P < 0.05$
** $P < 0.01$

5). Both flavor and overall acceptability also were significantly related to pumped and final yield. Simple correlation coefficients between pumping pressure and all taste-panel variables were significant while preprocessing belly temperature showed no significant relationship to any taste-panel considerations. Taste-panel members' detection of saltiness was significantly correlated with the actual amount of total residual salt obtained by objective measurement.

Mean taste-panel values for flavor, saltiness and overall acceptability as based on the hedonic scale are shown in Table 6. No preference for any of the four temperature groups was evident, but there was a definite and significant preference for the 70 psi pressure group for flavor and saltiness, which together contribute substantially to the overall acceptability rating. Increased pumping pressure clearly results in greater salt deposition in all tissues and ultimately increases flavor, saltiness and overall acceptability scores as determined by taste-panel evaluation.

Regression equations were formulated to predict total residual salt and percentage final yield by using preprocessing belly temperature and pumping pressure as dependent variables (Table 7). Because of small r-squared values, however, the reliability of these equations as predictors of final yield and total residual salt is questionable. These regression equations have potential as guides for the bacon processor, but must be further tested to determine the degree of accuracy.

Table 6—Treatment means for taste-panel variables flavor, saltiness and overall acceptability[a]

Temp °C	psi	Observations	Flavor	Saltiness	Overall acceptability
4.4		10	5.19	4.27	5.15
10		10	4.93	4.04	4.72
15.6		10	4.80	4.15	4.70
21.1		10	4.90	4.60	4.89
	50	20	4.68**	3.91**	4.56**
	70	20	5.24**	4.62**	5.16**

[a] Hedonic scale: 1 = extremely undesirable or imperceptible; 8 = extremely desirable or pronounced.
** $P < 0.01$

Table 7—Regression equations for the use of preprocessing belly temperature and pumping pressure in predicting residual salt and final yield

Predicted value	Intercept	Regression coefficients and dependent variables	r^2
% Residual salt =	−0.19	+ 0.15 (temp)[a] + 0.41 (pressure)[b]	0.29
% Final yield =	98.25	+ 1.28 (temp)[a] + 2.67 (pressure)[b]	0.50

[a] Temperature values for predicting equation: 1 = 4.4°; 2 = 10°; 3 = 15.6°; 4 = 21.1°.
[b] Pressure values for predicting equation: 1 = 50 psi; 2 = 70 psi.

REFERENCES

AOAC 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.
Arganosa, F.C. and Henrickson, R.L. 1969. Cure diffusion through pre- and post-chilled porcine muscle. Food Technol. 23: 1061.
Barr, A.J., Goodnight, J.H., Sall, J.P. and Helwig, J.T. 1976. A user's guide to the statistical analysis system. North Carolina State University Press, Raleigh, NC.
Chant, J.L., Kinsman, D.M., Stiffler, D.M. and Kotula, A.W. 1974. Physical, chemical and sensory evaluation of bacon. (abstract) J. Anim. Sci. 39: 167.
Kirton, A.H. and Pearson, A.M. 1963. Comparison of methods of measuring potassium in pork and lamb and prediction of their composition from sodium and potassium. J. Anim. Sci. 22: 125.
Mandigo, R.W. and Henrickson, R.L. 1966. Influence of hot processing pork carcasses on cured ham. Food Technol. 20: 186.
Mandigo, R.W. and Henrickson, R.L. 1967. The influence of pre-chill processing techniques on bacon. Food Technol. 21: 1262.
Nusbaum, R.P., Rust, R.E. and Topel, D.G. 1976. Predicting chemical compostion of pork bellies. (abstract) J. Anim. Sci. 42: 1348.
Olson, D.G., Parrish, F.C. Jr., Rust, R.E. and Miner, B.E. 1973. Effect of feeding roasted soybeans on cured pork palatability. J. Anim. Sci. 37: 49.
Schroder, B.G. and Rust, R.E. 1974a. Composition of pork bellies 1. Specific gravity as a predictor of chemical composition. J. Anim. Sci. 39: 1032.
Schroder, B.G. and Rust, R.E. 1974b. 2. Compositional variation between and within animals and the relationship of various carcass measurements with the chemical components of the belly. J. Anim. Sci. 39: 1037.
Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. Iowa State University Press, Ames, IA.
USDA 1977. Annual Statistical Summary. USDA-FSQS, May.
Wistreich, H.E., Morse, R.E. and Kenyon, L.J. 1959. Curing of ham: 1. A study of sodim chloride accumulation. Food Technol. 13: 441.
Wood, F.W. 1966. The diffusion of salt in pork muscle and fat tissue. J. Sci. Food Agric. 17: 138.

Ms received 9/19/77; revised 12/23/77; accepted 12/30/77.

Journal Paper No. J-8971 of the Iowa Agriculture & Home Economics Experiment Station, Ames, IA. Project No. 2012.

COMPUTERIZED COOKING SIMULATION OF MEAT PRODUCTS

RONALD J. SARKIN

ABSTRACT

A simulation technique for the processing of cooked meat products has been established utilizing computer solutions of empirical mathematical formulae. Parameters used to describe the process are center temperature, degree of pasteurization, extent of organoleptic change (cooking value) and product yields. Results permitted optimization of processing cycles, equipment design, and economic feasibility studies.

INTRODUCTION

ALTHOUGH THERMAL PROCESSING techniques have been extensively investigated in canning (Stumbo, 1973), nonsterile foods such as cooked meats which are processed below 212°F have not been subjected to such intense research. Cooked meats are expected to spoil and the absence of the sterility responsibility has generally imparted on these products a lower research priority.

This work attempts to quantify parameters significant in this temperature region and develops mathematical models of the thermal process which were simulated on a computer. These techniques were based on product results and permitted production yield optimization, explained equipment performance variations, defined quality parameters more accurately, and assisted in trouble shooting.

EXPERIMENTAL

UNCOOKED SKIN-OFF HAMS weighing 13¾ lb each and potted in containers of the traditional D-shaped loaf were processed in high humidity batch cookers (Cox, 1969) at various times and temperatures. Center temperatures were measured continuously with multipoint recorders and the processed weights compared to the uncooked weights to determine the cooking yield (η). Data were also obtained from a continuous cooker using temperature telemetry equipment (Steele, 1972).

RESULTS

A TYPICAL center temperature curve was determined to represent the standard against which future experimental parameters could be compared (Fig. 1). The three parameters found to adequately describe the performance of meat products during a cooking cycle were the microbiological status, cooking (organoleptic) effect, and yield.

In the temperature regions experienced in practice, Frazier (1967) suggests a minimum attained center temperature of 149°F. Using the F concept in canning (Stumbo, 1973) to establish a pasteurization value P, Jarvis (1975) recommended that a suitable indicator would be *Streptococcus faecalis* being more thermoduric than the more common meat pathenogens, *Salmonella spp.* and *Trichinella spiralis. S. faecalis* has a D value of 30 min at 140°F and a z of 14.6°F. A standard value

Author Sarkin is associated with Irvin & Johnson Ltd., P.O. Box 479, Johannesburg 2000, South Africa.

0022-1147/78/0004-1140$02.25/0
© 1978 Institute of Food Technologists

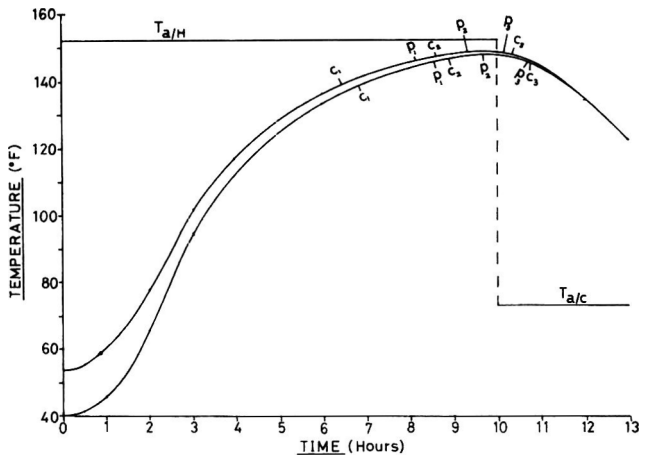


Fig. 1—Center temperature of a standard cooked ham during processing compared to one where the temperature lagged throughout the cycle. The three quartiles of 25, 50 and 75% of the standard P and C values have been marked. The final P and C values in the lagging example were below the standard at 38.3 and 33.7 min respectively.

for P of 43.4 min was obtained using the center temperature curve in Figure 1, and the relationships:

$$P = \Sigma P_i \cdot \Delta t \quad (1)$$

$$P_i = 10^{-12.48+0.08T_i} \quad (2)$$

Eq (2) is obtained from the slope of a semilog plot of the D and z values of P given above.)

Similarly a suitable cooking value (C) was found (Eisner, 1972) having a value for D of 1 min at 100°F and z of 15.9°F. The equations,

$$C = \Sigma C_i \cdot \Delta t \quad (3)$$

$$C_i = 10^{-3.51+0.0165T_i} \quad (4)$$

yielded a standard value for C (representing the cooking effect) of 36.3 min.

Future cooking results and simulation models were then required to have their P and C values equal to or greater than these standard values. To simulate processing cycles under any set of conditions (including the ambient heating and cooling temperatures, initial and final center temperatures, and duration of the heating and cooling cycles), equations relating the center temperature to the cooking time were obtained from a graph of dimensionless center temperature (τ) versus dimensionless time (θ) (Dickerson and Ralston, 1968).

$$\theta = \frac{T_a - T}{T_a - T_o} \quad (5)$$

$$\tau = \frac{\alpha}{r^2} t \quad (6)$$

Table 1—Recommended cooking times (t_h) at increasing cooker temperatures ($T_{a/h}$) required to satisfy pasteurization (P) and organoleptic (C) standards. The average batch cooked weights ($\bar{w}$) from a starting weight of 13¾ lb and the resultant yield percent (η) are also tabulated.

$T_{a/h}$ (°F)	t_h (hr)	P (min)	C (min)	$\bar{w}$ (lb)	η (%)
152	10.33	44.69	36.72	13.0	94.5
154	9.83	51.64	36.14	12.8	93.4
158	9.17	74.60	36.89	12.7	92.2
160	8.83	87.19	36.82	12.6	91.6
162	8.50	99.73	36.96	12.5	90.6

[Fig. 1 has been normalized to curves illustrated in Fig. 2 using Eq (5).] The linear portion of the curve yielded equations suitable for programming in the form:

$$\text{heating } T = T_a - (T_a - T_o)\cdot 10^{a+bt} \tag{7}$$

$$\text{cooling } T = T_a + (T_o - T_a)\cdot 10^{c+ft} \tag{8}$$

In practice, the constants a, b, c and f in Eq (7) and (8) were found using regression analysis as part of the simulation program to obviate the need of drawing curves to recover these data (Table 3).

To obtain a best fit to the data of the linear portion of the heating curve;

$$\log \theta = a + bt \tag{9}$$

Regression analysis gives the constants a and b as:

$$a = \frac{\Sigma \log\theta \cdot \Sigma t^2 - \Sigma(t\cdot \log\theta)\cdot \Sigma t}{N\Sigma t^2 - (\Sigma t)^2} \tag{10}$$

$$b = \frac{N\Sigma t\cdot \log\theta - \Sigma t\cdot \Sigma \log\theta}{N\Sigma t^2 - (\Sigma t)^2} \tag{11}$$

Alternatively, given any two points (T_1, t_1) and (T_2, t_2), the constants a and b may be found from the expressions:

$$a = \log\left(\frac{T_a - T_1}{T_a - T_o}\right) - \log\left(\frac{T_a - T_1}{T_a - T_2}\right)^{\frac{t_1}{(t_1 - t_2)}} \tag{12}$$

$$b = \log\left(\frac{T_a - T_1}{T_a - T_2}\right)^{\frac{1}{(t_1 - t_2)}} \tag{13}$$

As the contribution to the values of P and C became insignificant below about 130°F, the curvilinear portion of the heating curve was not significant; however, the reverse was true for the cooling curve. Hayakawa (1970) suggested the following relationship for the curvilinear portion of Figure 2:

$$T = T_a + (T_o - T_a)^{\cos Bt} \tag{14}$$

The thermal processing of meat products could then be quantified or simulated using the above equations. A variety of results were then obtained by either determining processing conditions through simulation and measuring results such as yields (η) or obtaining data from cooking cycles and evaluating their P, C and predicting η values.

Table 1 indicates the yield obtained at different cooker temperatures where the process time necessary to satisfy the P and C standards was determined by iterative simulation runs using the above defining equations.

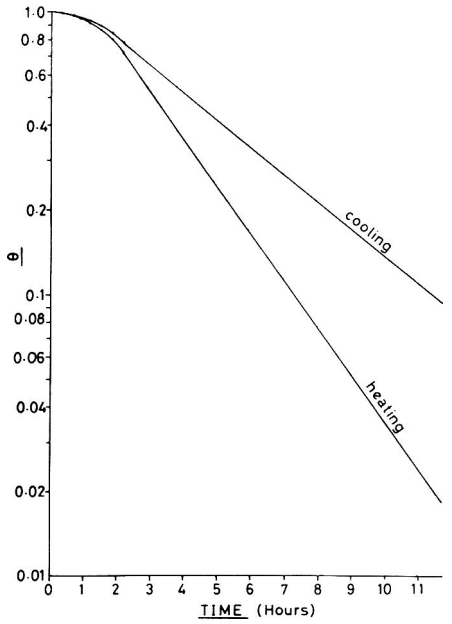


Fig. 2—Normalized center temperature (θ) versus processing time (t) during heating and cooling where

$$\theta_h = \frac{T_a - T}{T_a - T_o} \text{ and } \theta_c = \frac{T_o - T}{T_o - T_a}$$

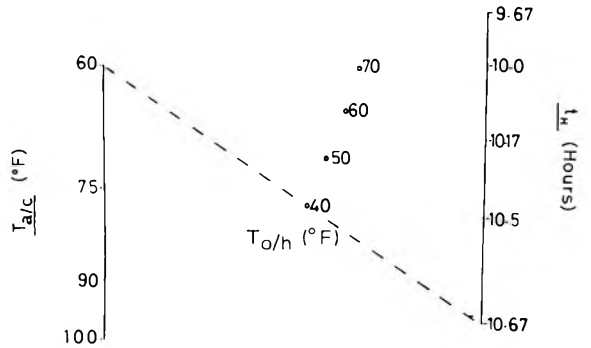


Fig. 3—Nomograph correcting cooking time (t_h) for various initial center temperatures ($T_{o/h}$) and ambient cooling temperature ($T_{a/c}$) at a given cooking temperature of 152.6° F.

In an industrial situation quality variations stem from a variety of causes including biological factors (pH, meat age, fat content), and physical factors such as initial meat temperature and ambient cooling temperature. Having defined a standard for P and C, simulation runs determined for example how to adjust the processing time at a cooker temperature of 152.6°F if the initial meat temperature were in the range 40°–60°F (as found in practice), and the ambient cooking conditions between 60°–90°F. Results following a relationship suitable for transposing onto a nomograph (Fig. 3) were obtained. This permitted an easy means of compensating for physical variations in a production situation which would then help reduce subsequent quality variations in the cooked product.

A technique to bulk handle pots of cooked meat into and out of cookers rather than loading each pot by hand was developed by fork-lifting cages into which ham pots had been "brick stacked" one on top of each other. The product quality obtained by this method was inferior to the original method and the results have been listed in Table 3. Another source of quality difference was obtained in a continuous cooker which accepted a wide range of products, but was forced to process

Table 2—Values of five different products cooked under identical conditions in a continuous cooker. Cooker temperature = 152.6° F; cooking time = 10 hr; ambient cooling temperature = 64.4° F; P = pasteurization value; C = cook (organoleptic) value; a,b = constants defined by Eq (7).

	Prime shoulder		De-fatted ham		Gammon steak		Forend		Brand name	
$T_{o/H}$	53.6°		53.6°F		50.9°F		40.0°F		40.0°F	
(hr)	Actual	Calculated	Actual	Calculated	Actual	Calculated	Actual	Calculated	Actual	Calculated
1	68.0	–	88.7	–	78.8	–	81.5	–	70.7	–
2	95.0	–	106.9	–	102.2	–	107.4	–	95.0	–
3	113.0	113.2	118.4	121.2	118.0	118.3	123.8	123.9	111.4	111.8
4	125.6	125.7	130.1	131.2	129.2	129.3	134.4	134.2	123.8	123.5
5	134.6	134.3	138.0	138.1	136.6	136.8	140.9	140.8	132.1	131.9
6	140.0	140.1	143.4	142.7	141.8	141.9	145.2	145.0	138.2	137.9
7	143.8	144.1	146.1	145.9	145.4	145.4	147.9	147.8	142.5	142.1
8	147.0	146.8	148.1	148.0	147.9	147.7	149.5	149.5	145.0	145.1
9	148.6	148.6	149.2	149.5	149.2	149.3	150.6	150.6	147.2	147.3
10	149.9	149.9	150.6	150.5	150.4	150.4	151.3	151.3	148.8	148.8
P	54.9		66.53		62.65		81.27		42.09	
C	38.58		41.87		40.72		43.70		36.42	
a	0.0979		0.0038		0.0353		–0.0141		0.0020	
b	–0.1660		–0.1675		–0.1691		–0.1932		–0.1475	

Table 3—Comparison of center temperatures during heating between the conventional and a "brick-stacking" method. The initial center temperature ($T_{o/h}$), cooker temperature ($T_{a/h}$), pasteurization and cook values (P, C), and constants (a, b) in Eq (7) have also been tabulated.

	Standard ham	Brick-stacked ham
$T_{o/h}$	53°F	53°F
$T_{a/h}$	152.6°F	152.6°F
Hour		
1	70.3	62.3
2	99.4	88.5
3	116.0	107.0
4	125.8	120.3
5	133.6	129.6
6	139.1	136.3
7	143.1	141.0
8	145.8	144.4
9	147.8	146.8
10	149.2	148.5
P	46.95	38.87
C	37.80	35.56
a	0.0285	0.1076
b	–0.1496	–0.1491

all of them for 10 hr at 152.6°F. The wide range in quality has been quantified in Table 3 which indicates parameter values such as P and C for different products.

DISCUSSION

PREVIOUS MEAT PROCESSING techniques were essentially established by trial and error and accumulated experience. Deviations from the norm were therefore generally inexplicable and the results of deliberate departures from standard conditions could only be guessed at. The techniques illustrated here were developed out of a need to explicitly detail the behavior of meat products during processing by either quantifying measured parameters or predicting important characteristics using mathematical models. [This simulation technique need not be restricted to meat products. Eisner (1972) includes in the concept of C values the effect of thermal processing on foodstuffs such as the gelling and liquefaction of starch, caramelization, enzyme inactivation, vitamin destruction, chlorophyll conversion to pheophytin, and others.]

Production control

In view of the limited shelf-life of cooked meats (Brown and Twedt, 1972), product could not be stored for substantial periods of time and orders were often not met during peak seasons. To boost production, cooking times could be reduced by increasing the cooker temperature which in turn led to a decrease in cooking yield (η) and the concomitant loss of profit. The relationship between cooker temperature (being a function of the P and C standards) and yield (η) was found to be linear in the cooking region considered. An acceptable balance between boosted production and increased cooking losses while maintaining a predefined standard was therefore possible by utilizing the simulation model to predict the relevant results.

Quality assurance

In-line control was possible in minimizing variations in the final product, given a range of initial center temperatures and ambient cooling temperatures commonly experience in a production environment (Fig. 3). These temperature variations were a result of production delays, seasonal temperature fluctuations, and ambient differences between cold Monday start-up and accumulated heat in the factory by Fridays.

An example of a lower initial center temperature subsequently lagging through the cooking cycle has been superimposed in Fig. 1. The later times were the quartile values of P and C developed along the lagging curve relative to the standard curve (25, 50 and 75% of the standard P and C values) have been marked to illustrate this effect.

Although taste panels accepted the range of product quality for the different cooking yields, as the yield increased so did the wetness and rubbery texture of the meat.

Equipment design

The original batch cooker design (Cox, 1969) was a high humidity cooker with shelves on which each ham pot stood separated from its neighbors exposed to turbulent moist air. Various configurations and deviations from the original design had been constructed with mixed successes. Measured and calculated parameters (T, P, C and η) often indicated reasons for differences exhibited by these cookers. Improvement modifications could then be effected with confidence, for example removing a steam-heated heat exchanger and sparging low pressure steam directly into a cooker. The steam then became the heat and humidity source simultaneously, and energy and maintenance costs were dramatically reduced.

Equipment performance should therefore be examined utilizing these parameters to indicate reasons for differences in cooking results.

Trouble shooting

A system to bulk handle ham pots into and out of cookers was devised by stacking pots "brick" fashion into a cage and handling the cage with a fork-lift. The high surface heat transfer coefficient resulting from the pots originally being individually exposed on shelves was substantially reduced and quality fell dramatically. As the center temperature lagged marginally behind the original cooking method, and as the two temperature profiles converged as they asymptotically approached the ambient cooking temperature (Fig. 1) this bulk handling procedure was not considered to be the direct cause of the quality failure of the product. Much energy was wasted examining other steps in production until the results were examined by this technique and noticably lower P and C values were derived from the bulk method center temperatures. As the most significant P_i and C_i values were obviously contributed to P and C at the terminal center temperature, even a small time lag in attaining the elevated temperature region exhibited a drop in the final P and C values. The two most serious effects were a decrease in shelf-life and increased texture wetness (Table 3).

Product comparisons

Although the various meat products were all essentially 'meat products', different physical qualities were emphasized in results obtained by processing a range of products under identical conditions in the same cooker. A range of differing values experienced in normal production was compiled in Table 2 for five products. Differences in initial center temperatures ranged from 40°–53.6°F, and final center temperatures from 148.8°–151.3°F, all products having been cooked for 10 hr at 152.6°F. (The biggest difference in final center temperatures was exhibited by products at the same initial center temperature of 40°F).

All the products in Table 2 exceeded the standard value of C (36.3 min), but the standard P value of 43.4 min was not attained in one case and exceeded by 87% in another. Variations in the a and b values of Eq (7) between products have been listed, as well as a comparison between actual center temperatures and those calculated by the linear regression model. (Temperatures should not be calculated during the first few hours as these lie on the curvilinear portion of the heating curve and fortunately are not significant contributors to P and C. As indicated above, this is not the case during initial cooling period).

These differences between products highlight the potential for a tighter product quality description and ability to maintain a closer quality control.

CONCLUSION

THE P AND C PARAMETERS chosen were found to accurately predict the bacteriological, organoleptic and yield status of products processed in different types of cookers and under varying conditions. The results were also sufficiently sensitive to indicate a warning when P or C values were marginally short of predetermined values. With further refinements to the system, in-line process control would be possible as in canning with in-line F_o simulating/controlling equipment.

The time/center-temperature relationship for any product in each type of cooker needs to be separately established as the curve varies according to the surface heat transfer coefficient and overall heat transfer coefficient if different types of containers are used for the same product. Normalizing the axes to dimensionless center temperature and time (Dickerson and Ralston, 1968) permits a certain amount of flexibility between different products and geometric shapes.

SYMBOLS USED

a, b, B, c, f, = constants
C = cook value (minutes)
C_p = heat caoacity (Btu/lb °F)
k = thermal conductivity (Btu/hr (ft^2/ft) °F)
N = total number of values
P = pasteurization value (minutes)
r = product radius (feet)
t = time (hours)
T = temperature (°F)
z = time to produce a decade change in the ordinate (minutes)

Subscripts

a = ambient
c = cooling
h = heating
i = ith value in a set of N values
o = initial

Greek symbols

α = thermal diffusivity $= \frac{k}{\rho \cdot C_p}$
$\triangle$ = difference (Δt = time interval between values of T. Here Δt = 10 minutes)
η = yield percent
θ = dimensionless temperature
ρ = density (lbs/ft^3)
Σ = sum of
τ = dimensionless time

REFERENCES

Brown, D.F., Twedt, R.M. 1972. Assessment of the Sanitary Effectiveness of Holding Temperatures on Beef Cooked at Low Temperature. Applied Microbiology. 24(4): 599.
Cox, J.A. 1969. Improvements in the cooking of foodstuffs. British Patent 1,173,021.
Dickerson, R.W. Jr., Ralston, B.R. Jr. 1968. Calculation and measurement of heat transfer in food. Food Technol. 22(12): 37.
Eisner, M. 1972. Introduction into the Technique and Technology of Rotary Sterilisation. Verlag Gunter Hempel. Braunschweig, Germany.
Frazier, W.C. 1967. "Food Microbiology." McGraw-Hill, New York.
Hayakawa, Kan-ichi. 1970. Experimental Formulas for Accurate Estimation of Transient Temperature of Food and Their Application to Thermal Process Evaluation. Food Technol. 24(12): 89.
Jarvis, B. 1975. Private communication. Department of Microbiology, Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, England.
MRI. Meat Research Institute. 1972. Special Report No. 1. Thermal Properties of Meat: Tabulated Data. Langford, Bristol BS18 7DY, England.
Steele, D.J. 1972. Radio Techniques Monitor Temperature. Food Manufacture. 47(11): 35.
Stumbo, C.R. 1973. Thermobacteriology in Food Processing. Academic Press, New York.
Ms received 6/21/77; revised 11/18/77; accepted 11/23/77.

YIELD CHARACTERISTICS, MICROBIAL LOAD AND CHILLING RATES OF SKINNED PORK CARCASSES

C. P. SALM, F. K. RAY, E. D. ABERLE, M. R. OKOS and M. D. JUDGE

ABSTRACT

Three groups of 25 hogs were selected and slaughtered using a vertical drum skinner. Weights were observed on the live animals, skins, feet, warm carcasses, and the trimmed bellies. The extent of belly damage was measured subjectively. Mean values and standard errors for the combined groups were as follows: live wt, 100.91 kg ± 16.82; percent skin (of live wt), 6.91% ± 0.24; percent feet (of live wt), 1.59% ± 0.01; dressing percentage (warm wt) 69.55% ± 0.42; cooler shrinkage (18 hr), 1.16% ± 0.64; percent of carcasses with exposed cutaneus trunci muscle, 14.67% ± 21.33. Microbial counts on the exterior surface of the hams, bellies and shoulders of 40 skinned and 40 scalded carcasses were determined. Counts were significantly greater over the hams of scalded than skinned carcasses but the shoulders of skinned carcasses had significantly higher counts than those of scalded carcasses. Although the skinning process resulted in significantly less shrinkage than the scalding process, the chilling rates for the deep ham, longissimus muscle and belly were slightly retarded for 17 skinned carcasses (pulled skins) as compared to 17 scalded carcasses. Prediction equations and resultant chilling curves are shown for skinned and scalded carcasses at each of the above three locations.

INTRODUCTION

THE CONVENTIONAL PROCESS of hair removal from slaughtered hogs utilizes large quantities of hot water for scalding and natural gas for singeing. Since the energy shortage is likely to continue, the process of skinning hogs may serve as an effective alternative.

Wang et al. (1954) and Mowafy and Cassens (1975) suggested that the scalding times and temperatures required for an effective subsequent removal of hair were, in most cases, detrimental to the quality of the skin. They reported a noticeable loss of grain layer when the conventional 4 min scald at 60°C was used. The skinning process thus keeps the skins suitable for production of high quality leather.

Snidjers (1973) and Ayres (1955) suggested skinning as a method of decreasing the microbial contamination of the pork carcass. They found the scalding tank to be an abundant source of contamination since the water is not hot enough to kill all microbes and spores. The numbers of microbes contributed to the scalding tank from bits of soil, fecal material, blood, and other foreign materials are very high. Since the heart of the hog pumps from 2–9 min after sticking (Jensen and Hess, 1941), and the hogs are required by U.S. inspection laws to hang on a rail for only 6 min after sticking, there is a possibility for the scalding water to enter the circulatory system. The still functioning circulatory system could then spread contamination throughout the carcass. On the other hand, the contamination resulting from the skinning process is largely limited to the exposed surfaces of the carcass.

Authors Salm, Ray, Aberle and Judge are with the Dept. of Animal Sciences, Purdue Agricultural Experiment Station, West Lafayette, IN 47907. Author Okos is with the Dept. of Agricultural Engineering, Purdue Agricultural Experiment Station, West Lafayette, IN 47907.

0022-1147/78/0004-1144$02.25/0
© 1978 Institute of Food Technologists

Some problems have been associated with the skinning process. Jabaay (1977) mentioned several precautions that should be taken to decrease the incidence of scored bellies and Kolb et al. (1976) pointed out that the process requires more skilled laborers and the throughput of carcasses is limited.

There have been relatively few research projects conducted on the effect of skinning on the carcass and on subsequent products. Romans and Ziegler (1974) reported that skinned pork products take cure faster and keep as well as unskinned cuts. Montgomery et al. (1976) demonstrated that skinned hams could be used in country ham production; however, adjustments in the amount of cure to be used were necessary.

This study was conducted to characterize the yield factors, chilling rate and microbiological quality of the skinned pork carcass.

MATERIALS & METHODS

Phase 1

Three groups of 25 hogs each were selected on the basis of sex, weight and finish. All were selected to be moderately finished barrows with a live weight near 100 kg. Each group of hogs was then slaughtered and skinned in a commercial plant. After stunning with an electrical stunner and sticking, the skin was removed by hand from the upper ham, the head and jowl region and front shanks. The skin from the remainder of the carcass was removed using a vertical drum skinner (Wolverine World Wide, Inc., Rockford, MI). The skin was weighed wet, with the hair on. The feet were removed at a point distal to the carpal and tarsal bones.

Group weights were recorded on the live animals, skins (wet with hair on), feet, warm carcasses (after washing), chilled carcasses (18 hr chill), and trimmed bellies. The extent of belly damage was estimated by subjective evaluation of the frequency of exposure of any portion of the cutaneus trunci muscle on either side of the carcass. Calculations were made of the percentage of carcasses having the muscle exposed on at least one side. The bellies were removed leaving a 2½ rib shoulder and were cut from the ham and loin in the normal industrial manner. The spareribs were removed and the bellies were trimmed and squared.

Phase 2

In the second phase of this study, surface contamination by microorganisms was compared in skinned versus scalded pork carcasses. Forty skinned carcasses and 40 scalded carcasses were selected 18 hr postslaughter at random from their respective chill coolers in commercial plants. The swab-rinse technique of Favero et al. (1968) (10 cm^2 area) was used to determine the total aerobic microbial plate counts for the external surface of the ham, belly (at approximate mid point of dorsal and ventral borders of the carcass) and shoulder regions of the carcasses using nutrient broth (pH 7.0) as diluent. The samples were held on ice for 8 hr and then plated using Standard Methods Agar. The plates were incubated at 32°C for 48 hr after which a total plate count was obtained. Data were analyzed using a two-tailed t-test.

Phase 3

In the third phase of this study, the rate of chill and cooler shrinkage were determined in skinned and scalded carcasses. Thirty-four hogs representing varying weight ranges were selected and slaughtered in the Purdue University Meat Science Laboratory. Seventeen hogs were skinned by pulling the skin from the carcass. The skin over the shanks, jowls, head and shoulders was removed with knives. The remainder of the skin was pulled free of the carcass by pulling in an anterior to posterior direction. The other 17 hogs were scalded in the conventional manner. The warm carcasses were weighed prior to the final pressure spray wash and were placed in a −2°C cooler (no added humidity) with an air velocity of 0.6–1.0 m/sec, for 18 hr beginning at 1.25 hr after

Table 1—Means and standard errors for skinned pork carcasses (Phase 1)[a]

	Mean	S.E.
Live wt (kg)	100.91	16.82
Dressing percentage (%)	69.91	0.42
Cooler shrinkage (%)	1.16	0.64
Skin and hair (% of live wt)	6.91	0.24
Feet (% of live wt)	1.59	0.01
Belly (% of chilled wt)	15.96	0.66
Frequency of exposure of cutaneus trunci (%)	14.67	21.33

[a] n = 3 groups (25/group)

stunning. The chilled carcass weight was obtained and percentage cooler shrink was calculated. Data were analyzed using a 2-tailed t-test.

The rate of temperature decline during chilling was obtained for the deep ham, belly and longissimus muscle. A Honeywell Model Electronik 15 temperature recorder was used to record temperature over the 18 hr period. To determine the rate of temperature decline in the deep ham, a 15 cm long thermocouple probe was inserted through the obturator foramen and along the femur bone (ASHRAE, 1974). Temperature decline in the longissimus muscle was obtained by inserting, via the abdominal cavity, a 3.2 cm long thermocouple into the approximate center of the longissimus muscle between the 14th and 15th ribs. Temperature decline in the belly was measured by inserting a 2 cm long thermocouple into the belly between the 11th and 12th ribs. The temperature recorded for each hour was used to calculate least squares regression equations (Neter and Wasserman, 1974) for the respective rates of temperature decline in the three locations.

RESULTS & DISCUSSION

Phase 1

Means and standard errors of live weight, dressing percentage, percent cooler shrinkage, percent skin (of live weight), percent feet (of live weight), percent belly (of chilled weight), and percent carcasses with at least one exposed cutaneus trunci muscle for carcasses skinned with the vertical drum skinner are shown in Table 1.

The dressing percentage, calculated on a warm weight basis, appears high for skinned hogs with feet removed. Average U.S. No. 1 and 2 barrows have a dressing percentage of 70–71% when scalded and dressed packer style (Forrest et al., 1975). However, many of the animals used for this phase of the study appeared to have been subjected to a very long pre-kill holding period.

The percentage shrink for a pork carcass after an overnight chill has been reported to fall in the range 1.25–2.0% (ASHRAE, 1974) or 1.5–2.1% (Cooper, 1968) for scalded carcasses. The percentage cooler shrink for skinned carcasses shown in Table 1 (1.16%) is slightly low by comparison.

The 6.91% skin (of live weight) appears high when compared to the 5.5% skin estimate of Cooper (1968). However, this value includes the hair and any moisture that was added prior to the skinning process.

The belly yield of skinned hogs (15.69%) agrees with literature values after adjustment for the loss of skin and feet. Fredeen et al. (1975) showed that the yield of belly for scalded barrows would range from 12–15% on a hot carcass basis. Since the yield is based on the weight of the carcass, the removal of the skin and feet should increase belly yield by 1.5–2%.

The incidence of scored bellies is included here since scored bellies have a definite effect on the economics of hog skinning. Deeply scored bellies are often too thin to use for bacon production. However, in this study, few if any of the bellies were damaged enough to preclude their use as bacon. In most instances the exposure of the cutaneus trunci muscle was accompanied by only minor losses of subcutaneous fat.

Table 2—Means[a] *and standard errors of total plate counts for skinned and scalded pork carcasses (Phase 2)*

	Skinned			Scalded	
Carcass location	Mean	S.E.		Mean	S.E.
Ham	254	86	*	969	162
Belly	153	47	N.S.	140	28
Shoulder	522	117	*	102	28

[a] Organisms/cm^2
* $P \leq 0.001$.

Table 3—Means and standard errors for fat depth and cooler shrinkage of skinned and scalded pork carcasses (Phase 3)

	Skinned			Scalded	
	Mean	S.E.		Mean	S.E.
Cooler shrinkage (%)	0.81	0.20	**	2.14	0.20
Fat depth	1.89	0.07	*	2.17	0.09

* Significant ($P \leq 0.05$)
** Significant ($P \leq 0.001$)

Phase 2

Means and standard errors of organisms per cm^2 for skinned and scalded hams, bellies and shoulders are presented in Table 2. The numbers of organisms on both the skinned and scalded carcasses fall within the range reported by other investigators. Cooper (1968) obtained counts ranging from 158 to 851 organisms per cm^2 and Hansen (1962) obtained counts below 1800 organisms per cm^2.

The data also represent the distribution of microorganisms on the carcasses. The severity of contamination probably reflects the amount of handling done by workers on the tested regions of the carcass. The belly went relatively untouched in both the skinning and scalding processes. The shoulder was one of the most handled regions in the skinning process and, therefore, had a high chance of becoming contaminated. In the scalding process, the hogs were conveyed past the workers at a height that exposed the hams to worker contact. The hams also received less washing after the scalding and evisceration processes than other carcass areas.

Phase 3

Table 3 shows the means and standard errors for percent cooler shrinkage and fat depth of carcasses used in phase 3 of this study.

The fat depth for the skinned and scalded carcasses (measured over the loin at the 10th rib; Fahey et al., 1977) was significantly higher ($P \leq 0.05$) for the scalded carcasses than for the skinned carcasses. However, there would be no significant difference between these two values if the 0.25 cm adjustment for the skin was made.

The percentage cooler shrink for the scalded carcasses appears to be in the expected range after an overnight chill (ASHRAE, 1974; Cooper, 1968). However, the shrinkage for scalded carcasses was significantly higher ($P < 0.001$) than for skinned carcasses after receiving the same duration of chilling. The extra shrinkage of the scalded carcasses may have been caused by a loss of the water added to the skin by the scalding process and that in the skin itself. During the time the carcasses were soaking in the 60°C scald water, there was likely to have been an adsorption of water by the skin. The added water plus the moisture present in the skin prior to scalding was evaporated from the skin during chilling. In the skinned carcasses there was little moisture added to the hydrophobic fat surface.

—Text continued on page 1146

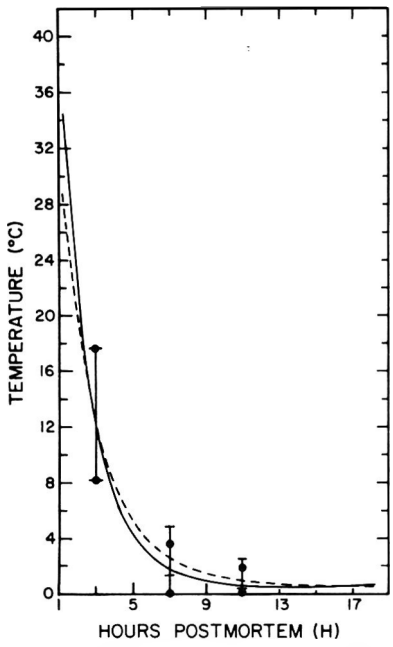


Fig. 1—Regression curves for belly temperatures during chilling of skinned (--) and scalded (—) pork carcasses. Vertical lines show 95% C.I. for skinned (—) and scalded (•) carcasses.

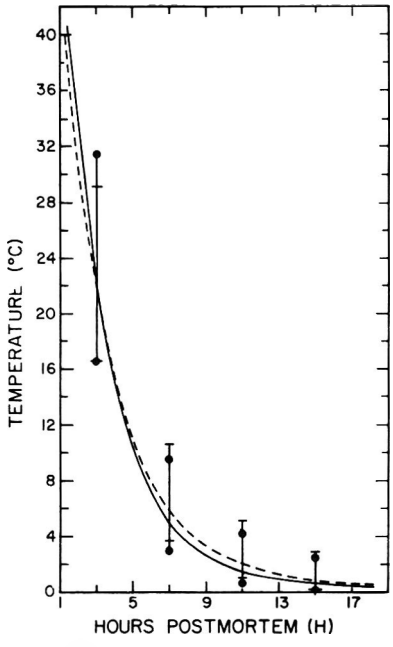


Fig. 2—Regression curves for longissimus muscle temperature during chilling of skinned (--) and scalded (—) pork carcasses. Vertical lines show 95% C.I. for skinned (—) and scalded (•) carcasses.

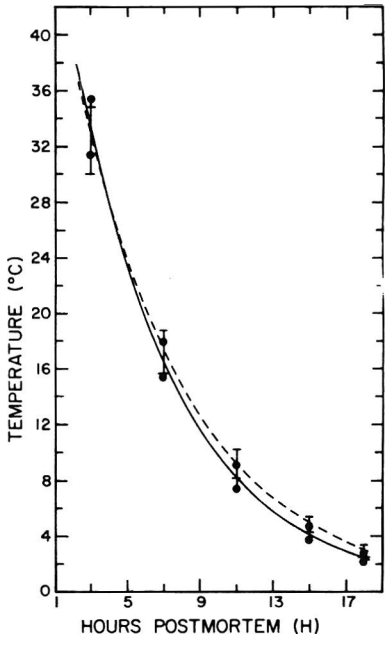


Fig. 3—Regression curves for deep ham temperatures during chilling of skinned (--) and scalded (—) pork carcasses. Vertical lines show 95% C.I. for skinned (—) and scalded (•) carcasses.

Means and confidence intervals for the rates of temperature decline in the belly, loin and ham are shown in Figures 1, 2 and 3, respectively. Least squares regression equations, R^2 values and standard errors for these regressions are listed in Table 4. Carcasses used for this phase weighed 93–125 kg. The initial ambient temperature was not the same for all carcasses since the temperature in the cooler rose with an increase in the load within it. The temperature of the cooler was −2°C when the first carcass entered and approximately 3°C when the sixth carcass was placed in the cooler (the hogs were processed in groups of six).

The chill curves reveal that the scalded carcasses entered the cooler at a higher temperature than the skinned carcasses. Apparently this was caused by the transfer of heat energy from the scald water to the carcass. The belly wall chill curves display the largest difference between the scalded and skinned carcasses at 1.25 hr postmortem and may have been even greater immediately after scalding. This absorption of heat energy was probably enhanced by the presence of a comparatively thin body wall in the belly region. Such heat absorption by scalded carcasses increases the energy requirements for cooling as compared to skinned carcasses.

In spite of the higher initial temperature, the scalded carcasses chilled faster than the skinned carcasses. The faster rate of chill is related directly to the amount of evaporative cooling that occurs on the surface of the carcass (Locker et al., 1975). The high rate of cooling of scalded carcasses was probably due to the high moisture content of the skins whereas the skinned carcasses had a hydrophobic fat surface that suppressed the evaporative cooling process. This does not appear to be a disadvantage of the skinning process since carcass shrinkage was found to be less after such processing than in scalded carcasses.

The regression equations (Table 4) for the belly and loin can be used to predict internal temperature between 1.25 and 18 hr postmortem and that for the ham can be used to predict internal temperature from 2.0 to 18 hr postmortem since observations outside of these ranges were not used to develop the equations.

The widespread application of the skinning process under industrial conditions will be dependent upon relative costs and availability of energy, equipment and labor. This investigation indicates that the process would not be deleterious to carcass and belly yields nor to microbiological quality and the rates of carcass chilling would be consistent with good industrial practice.

Table 4—Regression equations for temperature decline in skinned and scalded pork carcasses during post slaughter chilling

Treatment — position	Equation[a]	S.E.	R^2
Skinned — Belly	$T = 10^{1.75 - .24H + .007H^2}$	1.69	0.86
Scalded — Belly	$T = 10^{1.91 - .31H + .011H^2}$	1.73	0.86
Skinned — Longissimus muscle	$T = 10^{1.80 - .17H + .003H^2}$	1.49	0.91
Scalded — Longissimus muscle	$T = 10^{1.92 - .20H + .004H^2}$	1.51	0.93
Skinned — Deep ham	$T = 10^{1.72 - .069H}$	1.21	0.96
Scalded — Deep ham	$T = 10^{1.75 - .076H}$	1.29	0.92

[a] T = Temperature (°C); H = Hours postmortem

REFERENCES

ASHRAE. 1974. Handbook and Products Directory. Part 2. Hog chilling and tempering: The pork carcass." Amer. Soc. Heat. Refrig. Air Cond. Eng.

Ayres, J.C. 1955. Microbiological implications in the handling, slaughtering, and dressing of meat animals. Adv. Fd. Res. 6: 109.

Cooper, T.J.R. 1968. Chilling of pig carcasses — economic and practical considerations. ASHRAE J. 10(Dec.): 79.

Fahey, T.J., Schaefer, D.M., Kauffman, R.G., Romans, J.R., Epley, R.J., Topel, D.G. and Smith, G.C. 1977. A comparison of pork carcass evaluation systems. J. An. Sci. 44: 8.

Favero, M.S., McDade, J.J., Robertson, J.A., Hoffman, R.K. and Edwards, R.W. 1968. Microbiological sampling of surfaces. J. Appl. Bact. 31: 336.

—Continued on page 1152

A SURVEY OF THE MALONALDEHYDE CONTENT OF RETAIL MEATS AND FISH

G. M. SIU and H. H. DRAPER

ABSTRACT

A survey was made of the malonaldehyde (MA) content of 96 fresh and processed meat and fish samples obtained from supermarkets. MA content ranged from 0.14 μg/g in a cooked ham sample to 10.05 μg/g in a cooked chicken sample. Ninety-two percent of the processed or cured meats and 38% of the fresh meats contained less than 1 μg/g. Sixty percent of the fresh meat samples ranged between 1 μg/g and 6 μg/g. Cooking led to only slight increases in MA in most meat samples, but up to 10-fold increases in roasts cooked for 3 hr.

INTRODUCTION

MALONALDEHYDE (MA) in foods generally has been associated with oxidative rancidity (Sinnhuber and Yu, 1958; Koning and Silk, 1963). However, it has also been found in a number of 'fresh' supermarket products including meats (Bidlack et al., 1972), fish (Kuusi et al., 1975), vegetable oil (Arya and Nirmala, 1971) and orange juice essence (Braddock and Petrus, 1971).

Recently, interest in the possible significance of MA in human health has been stimulated by reports that it is mutagenic (Mukai and Goldstein, 1976) and carcinogenic (Shamberger et al., 1974). Brooks and Klamerth (1968) found evidence that MA reacts with deoxyribonucleic acid (DNA), suggesting a possible rationale for its mutagenic effect. Klamerth and Levinsky (1969) observed a decrease in template activity of liver DNA from rats treated with MA.

In the present study, a survey was conducted on concentrations of MA in fresh and processed meats and fish obtained from supermarket outlets. The effect of cooking on MA content also was determined.

EXPERIMENTAL

MA WAS DETERMINED by either the 'distillation method' of Tarladgis et al. (1960) or by a modification of the 'filtration method' of Tarladgis et al. (1964). In the distillation method, a 10-g meat sample was homogenized with 50 ml of distilled water in a Sorvall homogenizer for 2 min. The homogenate was transferred to a Kjeldahl flask and 47.5 ml of distilled water and 2.5 ml of 4N HCl were added (final pH 1.8). Dow antifoam A was sprayed onto the neck of the Kjeldahl flasks to control foaming. The mixture was heated until about 50 ml of distillate was collected. Five ml of distillate was added to 5 ml of 0.02M thiobarbituric acid (TBA) in H_2O and incubated for 90 min at 80°C. The absorbance of the pink solution was read at 532 nm in a Beckman DB GT Spectrophotometer.

In the modified filtration method, meat was selected from various portions of the cut to make up a representative 10-g sample which then was homogenized with 50 ml of distilled water in a Sorvall homogenizer for 2 min. Fifty ml of 10% trichloroacetic acid (TCA) was added and the homogenate was suction-filtered using Whatman No. 1 filter paper. TCA was used as suggested by Turner et al. (1954) to prevent a red color in the extracts. Eight ml of the clear filtrate was added to 2 ml of 0.06M TBA reagent and incubated for 90 min at 80°C before reading the absorbance at 532 nm. The filtration procedure was used for most of the samples because it yielded higher recoveries of added MA. However, it was necessary to use the distillation method for samples high in fat because of turbidity in the TCA extracts.

A standard curve was constructed using the MA derivative 1,1,3,3-tetramethoxypropane (TMP) (Aldrich Chemical Co.) which hydrolyzes under acid conditions to form free dialdehyde. The recovery of MA was estimated by adding known amounts of TMP (0.1–0.5 μmole) to meat homogenates.

To determine whether MA was generated as an artifact from polyunsaturated fatty acids (PUFA) during the procedure, the effect of adding arachidonic acid or linolenic acid to beef samples on their MA content was determined. One mg of arachidonic or 5 mg of α-linolenic acid was dissolved in hexane and added to beef samples before homogenization. These amounts of PUFA were based on their estimated levels in 10g lean beef (Leverton and Odell, 1958).

The possibility that MA was generated in the course of the assay procedures also was evaluated by comparing the amounts of MA found in the presence and absence of added antioxidants. A mixture of 0.1 mmole dl-α-tocopherol, 0.1 mmole L-ascorbic acid and 1 mmole EDTA was added to the meat homogenates before filtration or distillation.

The identity of the chromogen formed by addition of TBA to the meat extracts was confirmed by spectral analysis and thin-layer chromatography. The TBA reaction products from meats were co-chromatographed with pure TBA-MA complex on silica Gel G thin-layer plates developed with butanol:ethanol:acetic acid:H_2O (2:2:1:5).

Analyses were performed on a total of 96 fresh and processed meat samples representing 34 different products. Three samples of each product were obtained from different supermarkets and analyzed in duplicate before and after cooking. These included 12 beef, 7 pork and 2 veal, lamb, chicken and processed meat samples as well as 7 fresh and frozen fish products. Lean meat was analyzed separately from fat. Cooking methods followed the recipes recommended by the Canada Department of Agriculture (1975). Roasts, steaks, chops and filets from beef, veal, pork, lamb and fish were cooked by the dry heat method for the appropriate time. Whole chickens were cooked in one piece using the dry heat method and white meat was analyzed separately from the dark. Beef and lamb stews were cooked by the 'moist heat method' described in the same publication. Seasonings were omitted from the recipes to avoid possible interference in subsequent MA assays. The effect of cooking also was determined by periodically removing samples at 30-min intervals from beef and pork roasts up to a total cooking time of 3 hr. Cured and processed meat samples were analyzed in the presence of sulfanilamide (Zipser and Watts, 1962) to minimize the effect of nitrate interference with the TBA-MA reaction.

RESULTS & DISCUSSION

THE RECOVERY OF MA added to meat homogenates as TMP was 57 ± 2% and 83 ± 1% (Mean ± SEM, n = 4) for the distillation and TCA filtration procedures, respectively. In the absence of a meat sample, the respective recoveries were 70 ± 3% and 94 ± 3%. These findings indicate that the recovery of MA is somewhat lower in the presence of a meat sample, possibly as a result of binding of MA to meat proteins or other components of the sample.

All values for MA were corrected for 83% recovery when the TCA filtration method was used and for 57% recovery when the distillation procedure was used. When these corrections were applied to results obtained on beef samples assayed by both procedures, the distillation method gave higher values than the filtration method (Table 1). This finding suggests several possibilities: (a) the filtration method gives incomplete extraction of endogenous MA; (b) the distillation procedure causes preferential loss of added MA; (c) the hot conditions associated with the distillation method promote the release or formation of MA. The filtration procedure was employed for analysis of all but the high fat samples on the basis of the

Authors Siu and Draper are with the Dept. of Nutrition, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

0022-1147/78/0004-1147$02.25/0
© 1978 Institute of Food Technologists

higher recovery values obtained and its milder operating conditions.

The results shown in Table 2 suggest that addition of exogenous arachidonic acid to beef samples led to an increase in MA content as measured by the TCA filtration method. This increase was equivalent to 0.5 moles per 100 moles added arachidonic acid. Addition of linolenic acid to the samples produced no change in MA content. The antioxidant/EDTA mixture prevented the formation of MA in the presence of added arachidonic acid.

Addition of antioxidants/EDTA to meat samples not supplemented with exogenous PUFA had no effect on their MA content as determined by either method (Table 3). This finding indicates that MA was not generated by autoxidation of endogenous PUFA in the meat samples even though some increase in MA was observed when exogenous arachidonic acid was added to the samples without added antioxidants/EDTA.

The visible absorption spectrum of the pink solution generated by adding the TBA reagent to meat distillates or filtrates was identical to that of the complex formed with pure TMP. The meat extracts also exhibited a single pink spot corresponding to that of the TBA-MA complex when chromatographed on silica gel G (R_f = 0.83). These findings indicate that the TBA reaction is a valid indicator of the MA present in meats.

The results of the analyses of meat and fish are summarized in Table 4. There were marked variations among samples of the same type of meat obtained from different supermarkets. These variations may be a consequence of differences in methods of handling, storage conditions, age or source of samples. However, when the data from all three outlets are considered, a general pattern is evident. Processed meats as well as cured meats such as wieners, bologna, cooked ham, back bacon and corn beef had consistently low levels of MA (less than 1 μg/g). Among the 'fresh' meats, raw chicken and pork contained relatively less MA than beef, lamb and veal. Thirty-eight percent of all fresh meat samples tested had MA contents less than 1 μg/g while 60% were in the range of 1 μg/g to 6 μg/g. Highest MA values were found among cooked chicken, cooked pork and cooked beef roasts. Among the fish samples analyzed, the fresh samples yielded lower MA levels than the frozen samples, confirming the observations of Kuusi et al. (1975) who observed increases in MA in Baltic herring during storage at −8°C. The susceptibility of fish to autoxidation at low temperatures may be related to the highly unsaturated long chain fatty acids present in these products.

The lower levels of MA found in processed and cured meats suggest that the handling conditions applied to these products are less conducive to lipid peroxidation than those which pertain to fresh meats. Marked variations in MA content of fresh samples presumably are related to differences in storage and handling conditions and rates of movement through the food chain.

In some meats, cooking led to substantial increases in MA content while no consistent changes were observed in others. In general, meats which required a long cooking time, such as beef roasts, pork roasts and chicken showed the greatest increases after cooking. A correlation between cooking time and MA content was confirmed by periodic sampling of a beef and a pork roast at 30 min intervals. The results are plotted in Figure 1. After an induction period of 60–90 min, MA content rose rapidly for the next 60 min after which a plateau appeared to be reached. Thermal oxidation apparently was not promoted in meat samples which required relatively short cooking times (less than 30 min); however, roasting for 3 hr led to significant increases in MA content. Beef contained relatively less PUFAs and exhibited smaller increases in MA during roasting than chicken or pork.

The TBA values found for fresh and cooked meats in this study are in general agreement with those reported by Tims and Watts in 1958. These workers further observed that the TBA values of cooked (but not raw) meats rose during storage at 5–7°C to levels which "surpassed the limit of acceptability." The present values also are in general agreement with those of Kenskinel et al. (1964), but Shamberger et al. (1977) recently have reported substantially higher levels of MA in meat and poultry products purchased in Cleveland supermarkets. The significance for human health of any of these reported concentrations of MA in meats is unknown, but re-

Table 1—Comparative data for the MA content[a] of meat samples obtained by two analytical procedures (μg/g, Mean ± SEM)

Method	Distillation	TCA filtration
Cooked beef	4.75 ± 0.05	3.35 ± 0.25
Raw beef	3.25 ± 0.05	1.90 ± 0.10

[a] Values corrected for 57% recovery of added MA by the distillation method and 83% recovery by the TCA filtration method (see text).

Table 2—Effect of adding arachidonic acid (20:4), α-linolenic acid (18:3) and antioxidants/EDTA on the MA content of beef as determined by the TCA filtration method

Additions		
PUFA[a]	Antioxidants/EDTA[b]	MA (μg/g)[c]
–	–	3.47 ± 0.14
20:4	–	4.12 ± 0.04
20:4	+	3.40 ± 0.07
18:3	–	3.25 ± 0.14
18:3	+	3.33 ± 0.04

[a] 1 mg 20:4 or 5 mg 18:3

[b] 0.1 mmole dl-α-tocopherol, 0.1 mmole L-ascorbic acid and 1 mmole EDTA

[c] Corrected for 83% recovery; Mean ± SEM (n = 4).

Table 3—Effect of adding antioxidants and EDTA on the MA content of beef as measured by the TCA filtration and distillation methods

Assay method	Antioxidants/EDTA[a]	MA (μg/g)[b]
Distillation	–	4.66 ± 0.24
Distillation	+	5.02 ± 0.16
TCA filtration	–	5.17 ± 0.25
TCA filtration	+	5.24 ± 0.14

[a] See footnote to Table 2.

[b] Mean ± SEM (n = 2). Different samples were used in the two assay methods.

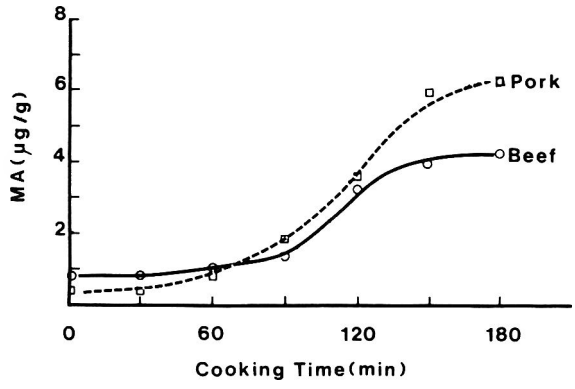


Fig. 1—Effect of cooking time on MA content of beef and pork roasts.

Table 4—Malonaldehyde content of raw and cooked meat and fish samples

Sample		(μg/g) 1	2	3	Avg[a]
Beef					
Roasts:					
Standing rib	– raw	1.66	1.70	0.32	1.23
	– cooked	2.60	2.42	1.81	2.28
Sirloin tip	– raw	1.45	0.80	0.76	1.00
	– cooked	2.17	4.19	2.39	2.92
Rump	– raw	1.34	1.41	1.45	1.43
	– cooked	3.22	3.76	4.70	3.89
Steaks:					
T-bone	– raw	1.41	2.21	1.66	1.76
	– cooked	0.98	1.34	4.58	2.07
Sirloin	– raw	0.90	1.95	2.21	1.69
	– cooked	1.40	1.48	2.67	1.78
Chuck	– raw	1.37	1.95	0.87	1.40
	– cooked	1.05	1.55	1.12	1.24
Round	– raw	1.12	3.32	2.71	2.39
	– cooked	0.94	3.14	2.89	2.32
Ground beef:					
Ground chuck	– raw	0.76	1.44	1.66	1.29
	– cooked	1.19	2.85	1.55	1.87
Hamburger	– raw	1.66	1.12	1.44	1.41
	– cooked	1.63	3.40	3.18	2.73
Stew:					
Beef	– raw	0.76	0.61	0.87	0.75
	– cooked	0.69	0.40	0.94	0.67
Corn beef:					
Corn beef	– raw	0.54	0.29	0.80	0.55
	– cooked	0.50	0.61	2.31	1.14
Fat:					
Beef	– raw	0.44	0.79	0.85	0.69
	– cooked	0.80	0.93	1.20	0.97
Pork					
Pork butt roast	– raw	0.40	0.25	0.58	0.41
	– cooked	2.75	4.34	5.24	4.10
Pork chop	– raw	0.90	0.40	0.21	0.50
	– cooked	0.69	2.75	1.34	1.59
Ground pork	– raw	0.43	1.23	1.30	0.99
	– cooked	1.51	4.99	3.97	3.49
Pork sausage	– raw	0.69	0.61	0.76	0.68
	– cooked	1.16	1.51	0.61	1.09
Back bacon	– raw	0.36	0.29	0.40	0.35
	– cooked	0.32	0.80	0.36	0.49
Ham	– cooked	0.29	0.14	2.75	1.06
Fat	– raw	–	0.77	2.39	1.58
	– cooked	–	2.73	7.38	5.04
Veal					
Veal shoulder chop	– raw	2.24	5.64	0.32	2.73
	– cooked	2.31	4.92	0.94	2.72
Veal sirloin	– raw	3.00	4.92	0.76	2.89
	– cooked	2.96	5.64	1.01	3.21
Lamb					
Chop	– raw	2.68	1.12	1.55	1.78
	– cooked	2.02	1.12	3.11	2.08
Stew	– raw	1.51	1.77	1.12	1.47
	– cooked	1.12	1.77	1.99	1.63
Chicken					
White meat	– raw	0.94	0.47	0.40	0.61
	– cooked	0.79	1.63	5.82	2.75
Dark meat	– raw	0.61	0.54	0.47	0.55
	– cooked	2.02	3.79	10.05	5.29
Processed meats					
Wieners	– raw	0.43	0.40	0.51	0.44
	– cooked	0.47	0.40	0.43	0.43
Bologna	– cooked	0.36	0.61	0.47	0.48
Fish					
Frozen					
Boston Blue fish	– raw	1.10	1.27	0.65	1.20
	– cooked	0.50	1.70	0.98	1.06
Perch	– raw	0.64	1.27	–	0.90
	– cooked	0.24	1.13	–	0.68
Salmon	– raw	2.85	1.55	0.75	1.72
	– cooked	2.07	1.23	0.86	1.39
Rainbow trout	– raw	0.54	1.14	–	0.84
	– cooked	1.14	1.35	–	1.25
Fresh					
Salmon	– raw	0.52	0.43	–	0.47
	– cooked	0.60	1.16	–	0.88
Whitefish	– raw	0.27	0.26	0.27	0.27
	– cooked	0.43	0.43	0.70	0.52

[a] Each sample was obtained from a different retail outlet and analyzed in duplicate before and after cooking. All samples except fat and processed meats were analyzed by the TCA filtration method.

ports that this substance is mutagenic and carcinogenic emphasize the desirability of minimizing its occurrence during storage and marketing.

REFERENCES

Arya, S.S. and Nirmala, N. 1971. Determination of free malonaldehyde in vegetable oils. J. Food Sci. Technol. 8: 144.

Bidlack, W.R., Kwon, T.W. and Snyder, H.E. 1972. Production and binding of malonaldehyde during storage of cooked pork. J. Food Sci. 37: 664.

Bieri, J.G. and Anderson, A.A. 1960. Peroxidation of lipids in tissue homogenates as related to vitamin E. Arch. Biochem. Biophys. 90: 105.

Braddock, R.J. and Petrus, D.R. 1971. Malonaldehyde in aqueous orange juice essences. J. Food Sci. 36: 1095.

Brooks, B.R. and Klamerth, O.L. 1968. Interaction of DNA with bifunctional aldehydes. Eur. J. Biochem. 5: 178.

Canada Department of Agriculture. 1975. Meat, how to buy—How to cook. Information Canada, Ottawa, K1A 0S9.

Kenskinel, A., Ayres, J.C. and Snyder, H.E. 1964. Determination of oxidative changes in raw meats by the 2-thiobarbituric acid method. Food Technol. 18: 223.

Klamerth, O.L. and Levinsky, H. 1969. Template activity in liver DNA from rats fed with malonaldehyde. FEBS letters 3(3): 205.

Koning, A.M. and Silk, M.H. 1963. The 2-thiobarbituric acid reagent for determination of oxidative rancidity in fish oils. J. Am. Oil Chem. Soc. 40: 167.

Kuusi, T., Nikkilä, O.E. and Savolainen, K. 1975. Formation of malonaldehyde in frozen baltic herring and its influence on the changes in proteins. Z. Lebensm. Unters-Forsch. 159: 285.

Leverton, R.M. and Odell, G.V. 1958. The nutritive value of cooked meat. Oklahoma Agricultural Experiment Station, Misc. Pub. No. MP-49.

Mukai, F.H. and Goldstein, B.D. 1976. Mutagenicity of malonaldehyde, a decomposition product of peroxidized polyunsaturated fatty acids. Science 191: 868.

Shamberger, R.J., Andreone, T.L. and Willis, C.E. 1974. Antioxidants and cancer. 4. Initiating activity of malonaldehyde as a carcinogen. J. Natl. Cancer Inst. 53: 1771.

Shamberger, R.J., Shamberger, B.A. and Willis, C.E. 1977. Malonaldehyde content of food. J. Nutrition 107: 1404.

Sinnhuber, R.O. and Yu, T.C. 1958. Characterization of the red pigment in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23: 626.

Tarladgis, B.G., Watts, B.M., Younathan, H.T. and Dugan, L. Jr., 1960. Distillation method for the quantitative determination of malonaldehyde in rancid food. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. 5. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food and Agric. 15: 602.

Tims, M.J. and Watts, B.M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.

Turner, E.W., Paynter, W.D., Montie, E.J., Bessert, M.W., Struck, G.H. and Olson, F.C. 1954. Use of 2-thiobarbituric acid reagent to measure rancidity in frozen pork. Food Technol. 8: 326.

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 9/8/77; revised 12/3/77; accepted 12/8/77.

This work was supported by the Ontario Ministry of Agriculture and Food and the Ontario Ministry of Health. The assistance of Eileen Grogan in analyzing the fish samples is gratefully acknowledged.

CONSUMER DISCRIMINATION OF PORK COLOR AT THE MARKET PLACE

D. WACHHOLZ, R. G. KAUFFMAN, D. HENDERSON and J. V. LOCHNER

ABSTRACT

Pork chops from 96 fresh loins representing PSE, normal and DFD quality were displayed in a supermarket. Price was not altered and equal numbers of the three quality types were presented in a manner disguising any evidence that a survey was in progress. A total of 280 packages were purchased. Over one-half (146) of the purchases included packages of normal chops ($P < 0.001$), whereas the remaining purchases were divided between DFD (72) and PSE (62) packages. These findings suggest that some consumers may be aware of and may actively select for 'normal' pork color, but that others are either unaware of the limitations of, or do actually prefer, PSE and DFD pork.

INTRODUCTION

VARIATIONS in muscle quality of fresh pork have been thoroughly investigated and characterized by numerous workers (Briskey et al., 1966; Beecher et al., 1968; Kauffman et al., 1964). Pale, soft, and exudative (PSE) muscles lose excess weight during storage and processing due to the release of juices, primarily as a result of the rapid production of lactic acid immediately after death and before the onset of rigor mortis. This acidic condition subsequently results in a decreased water-binding capacity of the myofibrillar proteins. The opposite is true for dark, firm and dry (DFD) pork (Sayre et al., 1964). It has also been demonstrated that DFD pork is more susceptable to microbiological growth (Rey et al., 1976).

There is evidence (Topel et al., 1976) that the general appearance of pork may influence consumers' selection habits. Consumers indicated a definite tendency to select normal pinkish red (NPR) pork and to reject both PSE and DFD pork; however, the conditions in which decisions were made did not include a practical marketing exposure in that the participants were only asked to make a comparison. This environment biased the participants by asking for comparisons rather than examining their reactions during an uncontrolled marketing situation in which actual purchases could have been the measures of preference. It is clear that there are several factors influencing the consumer's decision to select pork, price being one of many factors involved. In a study to test the consumer's discrimination for or against the presence or absence of marbling in pork muscle (Kauffman et al., 1961), it was concluded that consumers preferred marbled over unmarbled pork chops when the price was the same, but when price varied, price greatly affected the preference pattern.

Scientists and various segments of the meat industry are aware of and concerned about pork quality as discussed above. They recognize, as has been described, that porcine muscle should be firm, possess a pinkish red color and be free of excess surface juices. They fully realize that PSE pork will shrink more during processing, will discolor more quickly during retail display, and will be less palatable when consumed.

This research was specifically aimed at determining how discriminitive consumers were in selecting for color of fresh pork at the market place. When provided the opportunity, would they select for a particular color trait when purchasing pork under normal market conditions and when price as well as all other physically visible traits (marbling, leanness, size) were similar for all cuts?

Author Wachholz is with the Univ. of Wisconsin-Platteville. Authors Kauffman and Lochner are with the Muscle Biology Lab., Univ. of Wisconsin, 1805 Linden Drive, Madison, WI 53706. Author Henderson is with the Univ. of Wisconsin–River Falls.

0022-1147/78/0004-1150$02.25/0
© 1978 Institute of Food Technologists

EXPERIMENTAL

FOR EACH OF eight consecutive weeks, 12 chilled, trimmed, fresh (approximately 72 hr postmortem) pork loins ranging in weight from 6–9 kg were selected at a Madison, WI packing company. The loins were visually selected to possess similar composition, size and weight characteristics, but to represent three distinctly different muscle quality conditions: PSE, NPR and DFD. A Göfo Meat-Brightness Meter was used to verify color variations. After weighing and preparing the loins for shipment, they were wrapped in paper, boxed, stored at ∿3°C and commerically transported to a supermarket in Platteville, WI. Storage and transit time was ∿24 hr. Approximately 24 hr after arrival, the loins were unwrapped, weighed, and cut into retail cuts (the two ends were left as roasts and the center portion was cut into loin and rib chops approximately 1.2 cm thick). After cutting, the chops were uniformly trimmed and packaged (4/package). Each type of chop (rib or loin) was appropriately priced and placed in separate compartments of the retail display case in such a manner that equal numbers of the three quality conditions were present at all times. Care was also taken to ensure that the three quality conditions were uniformly distributed in the case. The retail case was then kept under constant surveillance (not noticeable by customers), and efforts were made to continually restock the case with packages similar in quality to those purchased by customers. Whenever replacements of any one of the three quality types (stored in the cutting room) had been exhausted, that period of testing was terminated so that, at the end of each test period, there remained in the display case equal numbers of packages representing each of the three quality types.

One package of chops from each of the 96 loins was tested for pH, objective color and expressible juice. pH was measured with a pocket-pH meter (Beckman, model 180). Color intensity was again measured with the Göfo instrument and expressible juice was determined by the technique described by Briskey (1958).

For each of the eight experimental periods, price of the three quality types of chops was held constant. However, a price differential existed between rib and loin chops and among like chops displayed for sale at various time periods. Analysis of variance procedures and Duncan's new multiple range test were used to statistically examine the data.

RESULTS & DISCUSSION

TABLE 1 includes the means and standard deviations for the physical traits of the loins used. As expected, differences ($P < 0.01$) were found among the three quality types for Göfo (color), pH, and expressible juice values. These differences substantiated that the three types of quality subjectively selected for, were of sufficient magnitude to insure visual differences as displayed for consumers, and to provide selection differentials to adequately test consumer discrimination. As anticipated, the data also indicated a difference ($P < 0.01$) in the percentage shipping shrinkage. PSE loins shrank approximately twice the amount of NPR loins and four times that of DFD loins. The management of the supermarket did not comment about the NPR and DFD loins, but displayed concern about

Table 1—Properties of loins selected for each test period

	1	2	3	4	5	6	7	8
Shipping shrink, %[a]								
PSE[c]	2.1 ± 1.4[b]	3.1 ± 1.1	3.2 ± 1.0	3.2 ± 0.6	3.3 ± 1.0	2.7 ± 1.0	3.6 ± 1.0	3.3 ± 0.8
NPR[d]	2.3 ± 0.7	2.7 ± 1.3	1.6 ± 0.8	1.3 ± 0.5	1.9 ± 0.7	0.2 ± 0.4	1.5 ± 0.9	1.3 ± 0.6
DFD[e]	1.5 ± 0.3	0.2 ± 0.3	0.7 ± 0.2	0.5 ± 0.4	0.7 ± 0.7	0.0 ± 0.0	1.0 ± 0.5	0.8 ± 0.8
Gõfo[f]								
PSE	53 ± 8	34 ± 5	35 ± 4	31 ± 11	39 ± 3	35 ± 6	34 ± 2	33 ± 13
NPR	62 ± 11	63 ± 6	55 ± 5	58 ± 6	47 ± 3	52 ± 9	53 ± 10	64 ± 5
DFD	61 ± 13	76 ± 5	75 ± 9	76 ± 5	82 ± 2	76 ± 16	72 ± 3	66 ± 7
pH								
PSE	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.2	5.4 ± 0.1	5.4 ± 0.2	5.6 ± 0.1
NPR	5.8 ± 0.3	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.7 ± 0.2	5.8 ± 0.1
DFD	5.9 ± 0.3	6.1 ± 0.2	6.4 ± 0.2	6.3 ± 0.1	6.3 ± 0	6.3 ± 0.3	6.0 ± 0.2	6.1 ± 0.5
Expressible juice, %[g]								
PSE	37 ± 4	44 ± 2	37 ± 3	42 ± 2	36 ± 1	39 ± 1	35 ± 4	42 ± 2
NPR	27 ± 6	32 ± 2	28 ± 6	31 ± 5	30 ± 5	30 ± 3	35 ± 3	32 ± 4
DFD	28 ± 7	23 ± 3	21 ± 5	23 ± 2	19 ± 4	22 ± 3	24 ± 3	31 ± 6

[a] Expressed on a pre-shipping weight basis
[b] Mean and standard deviation
[c] Pale, soft and exudative loins (N = 3 for each test period)
[d] Normal pinkish red loins (N = 6 for each test period)
[e] Dark, firm and dry loins (N = 3 for each test period)
[f] 0 = light, 100 = dark.
[g] Expressed as % of total water in muscle

Table 2—Properties of loins representing three quality types selected for the study[a]

	24 PSE Loins $\bar{x}$ sd	48 NPR Loins $\bar{x}$ sd	24 DFD Loins $\bar{x}$ sd
Shipping shrink, %[b]	3.1 ± 1	1.6 ± 1	0.7 ± 0.6
Gõfo[c]	36 ± 10	58 ± 9	74 ± 9
pH	5.5 ± 0.1	5.8 ± 0.2	6.2 ± 0.3
Expressible Juice, %[d]	39 ± 4	31 ± 5	24 ± 5
Increase in Gõfo reading during 48 hr storage	17 ± 7	6 ± 5	3 ± 2

[a] All values in each row were different ($P < 0.05$), except NPR and DFD change in Gõfo during storage ($P > 0.05$).
[b] Expressed on a preshipping weight basis
[c] 0 = light, 100 = dark.
[d] Expressed as % of total water in muscle

Table 3—Consumer preference for PSE, NPR and DFD pork chops

Test period	PSE[a]	NPR[b]	DFD[c]	Chi square
A. Distribution of 74 loin chop packages selected by consumers				
1	3	1	2	
2	2	6	2	
3	1	5	3	
4	2	6	2	
5	3	7	2	
6	1	9	2	
7	3	5	2	
8	1	3	1	
	Σ 16	42	16	18.27***
B. Distribution of 206 rib chop packages selected by consumers				
1	7	14	8	
2	6	16	8	
3	4	9	8	
4	7	10	7	
5	6	13	5	
6	6	15	6	
7	7	9	7	
8	3	18	7	
	Σ 46	104	56	28.00***
C. Distribution of 280 pork chop packages selected by consumers (Total of parts A & B)				
1	10	15	10	1.43 NS
2	8	22	10	8.60*
3	5	14	11	4.20 NS
4	9	16	9	2.88 NS
5	9	20	7	8.16*
6	7	24	8	14.00**
7	10	14	9	1.27 NS
8	4	21	8	14.36**
	Σ 62	146	72	45.11***

[a] Pale, soft, exudative
[b] Normal pinkish red
[c] Dark, firm, dry

NS = nonsignificant
* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$

the shrinkage of PSE loins when they were unpacked for cutting. The incidence of shrinkage was also evident in the amount of expressible juice of PSE chops. PSE chops had 8% more juice than NPR and 15% more than DFD chops.

During the 48-hr storage period, all chops darkened in color, but this color change was particularly noticeable for the PSE chops. The PSE chops tended to change from a pale color to a very dull grayish color. After the 48-hr storage period, the PSE chops were considered by the retailer to be objectionably undesirable in color (see Table 2).

Table 3 includes the results of consumer preferences. Highly significant differences were found in the selection of loin chops and rib chops from the three quality groups. Consumers preferred the NPR chops over the PSE and DFD chops at a rate of approximately two to one; however, there was little difference in the selection between PSE and DFD chops, even though there appeared to be a slight trend in favor of DFD chops.

These results agree with those of Topel et al. (1976) and clearly reflect specific preferences for fresh pork by consumers when price variation and other physical characteristics are not factors. When given the choice, about half of the consumers chose normal chops and the other half were nearly equally divided in their selection for PSE and DFD pork. It can be argued statistically that if their preferences were strictly random, then approximately one-third of the population would have chosen each of the three quality types offered. However, the results are not entirely conclusive, because of the following reasons:

1. The number of selection opportunities was small and such parameters as sex, income level, ethnic background, geographical location, age, etc., were ignored.
2. Only extremes in color variation were included—not the entire gradation that is normally encountered, or not one specific color exclusively.
3. Various price differentials among the three quality groups were not studied.
4. Reasons for the selections were not obtained (for fear of biasing the customer's selection during later periods of the survey).

—Text continued on page 1152

In conclusion, the study suggests that a majority of consumers tend to select against extremes in fresh pork color, but that some consumers are either not aware of undesirable traits of, or actually prefer PSE pork. For the three-quarters that may discriminate against PSE, the study made no attempt to ascertain whether the major reason for the selection of NPR or DFD pork was color or if it included other factors such as firmness, freedom from excess surface and package juices, and other possible factors that may not have always been held constant (marbling, muscle size, amount of bone, etc.). However, the results provide some evidence that awareness of pork quality exists at the retail market, and that a significant proportion of the consumers in this study selected the normal pinkish red color. This information should provide additional impetus for (1) swine producers to select genetic strains free of the PSE and DFD conditions if, in fact, price differentials existed, and (2) pork processors to consider muscle quality standards when purchasing pigs and during product preparation for distribution to the retailer.

REFERENCES

Beecher, G.R., Kastenschmidt, L.L., Cassens, R.G., Hoekstra, W.G. and Briskey, E.J. 1968. A comparison of the light and dark portions of a striated muscle. J. Food Sci. 33: 84.

Briskey, E.J. 1958. The chemical and physical characteristics of certain pork ham muscles as associated with various physiological stress factors. Ph.D. thesis, University of Wisconsin, Madison, WI.

Briskey, E.J., Kastenschmidt, L.L., Forrest, J.C., Beecher, G.R., Judge, M.D., Cassens, R.G. and Hoekstra, W.G. 1966. Biochemical aspects of postmortem changes in porcine muscle. J. Agr. Food Chem. 14: 201.

Kauffman, R.G., Bray, R.W. and Schaars, M.A. 1961. Price vs. marbling in the purchase of pork chops. Food Technol. 15: 22.

Kauffman, R.G., Carpenter, Z.L., Bray, R.W. and Hoekstra, W.G. 1964. Biochemical properties of pork and their relationship to quality. 1. pH of chilled, aged and cooked muscle tissue. J. Food Sci. 29: 65.

Rey, C.R., Kraft, A.A., Topel, D.G., Parrish, F.C. Jr. and Hotchkiss, D.K. 1976. Microbiology of pale, dark and normal pork. J. Food Sci. 41: 111.

Sayre, R.N., Kernat, B. and Briskey, E.J. 1964. Processing characteristics of porcine muscle related to pH and temperature during rigor mortis development and to gross morphology 24 hr. postmortem. J. Food Sci. 29: 175.

Topel, D.J., Miller, J.A., Berger, P.J., Rust, R.E., Parrish, F.C. Jr. and Ono, K. 1976. Palatability and visual acceptance of dark, normal and pale colored porcine M. longissimus. J. Food Sci. 41: 628.

Ms received 9/3/77; revised 1/5/78; accepted 1/10/78.

Muscle Biology Paper No. 118.

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and in part by State appropriation for cooperative research. The authors express their appreciation to Dick's Supermarket, Platteville, WI and Oscar Mayer and Co., Inc., Madison, WI for their cooperation.

CHARACTERISTICS OF SKINNED PORK CARCASSES . . . From page 1146

Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D. and Merkel, R.A. 1975. "Principles of Meat Science." W.H. Freeman and Co., San Francisco.

Fredeen, H.T., Martin, A.H., Harbison, D.S. and McAndrews, J.G. 1975. Criteria of belly bacon desirability. 1. Fat-lean ratio of the pork belly in relation to backfat, carcass weight and specific gravity. Can. J. An. Sci. 55: 641.

Hansen, N.H. 1962. A simplified method for the measurement of bacterial surface contamination in food plants and its use in the evaluation of pressure cleaners. J. Appl. Bact. 25: 46.

Jabaay, R.W. 1977. Bacon. "Proc. Meat Ind. Res. Conf.," p. 69. American Meat Institute Foundation, Washington, D.C.

Jensen, L.B. and Hess, W.R. 1941. A study of ham souring. Food Res. 6: 273.

Kolb, Q., Mowafy, M. and Cassens, R.G. 1976. Hogs: Should they be scalded or skinned? Wis. Ag. Ext. Bull. #A2835.

Locker, R.H., Davey, C.L., Nottingham, P.M., Haughey, D.P. and Law, N.H. 1975. New concepts in meat processing. Adv. Fd. Res. 22: 157.

Montgomery, R.E., Kemp, J.D. and Fox, J.D. 1976. Shrinkage, palatability and chemical characteristics of dry cured country ham as affected by skinning procedure. J. Food Sci. 41: 1110.

Mowafy, M. and Cassens, R.G. 1975. Comparative study of different scalding methods and their effect on the quality of the pig skin. J. An. Sci. 41: 1291.

Neter, J. and Wasserman, W. 1974. "Applied Linear Statistical Models." Richard D. Irwin, Inc.

Romans, J.R. and Ziegler, P.T. 1974. "The Meat We Eat." The Interstate Printers and Publishers, Inc. Danville, IL.

Snidjers, J.M.A. 1973. Comparative bacteriological studies on the effect of circular saws and band saws and chipping on the carcasses of slaughtered pigs. Tijdschr, Diergeneesk 11: 551.

Wang, H., DeBeukelaer, J.L. and Maynard, N. 1954. Histological study of the hog skin dehairing process. J. Amer. Leather Chem. Assoc. 50: 728.

Ms received 10/27/77; revised 1/23/78; accepted 1/30/78.

Journal paper no. 6931, Agricultural Experiment Station, Purdue University, West Lafayette, IN 47907.

POTENTIAL FOR WATER REUSE IN AN EGYPTIAN POULTRY PROCESSING PLANT

AHMED HAMZA, SAMIA SAAD and JACK WITHEROW

ABSTRACT

The water usage and waste loads were determined for ten unit operations in a modern poultry processing plant in Alexandria, Egypt. Concentrations of 10 wastewater parameters and 9 heavy metals, identification of 12 bacteria and enumeration of both total aerobic and coliform bacteria in the wastewater from these unit operations were determined to evaluate the potential for water reuse. In the scalder, washer and chillers, bacterial counts in the water and on the carcasses were directly related and increased with the length of water usage. Based on the study, countercurrent water use through the chillers and washer was planned after applying renovation measures for grease and bacterial reductions. Compressor cooling water quality was satisfactory for reuse in the scalder.

INTRODUCTION

THE PAST DECADE has been marked by unprecedented global activity directed toward man's concern for environmental quality. In many nations, this concern has been translated into initiation of intensive research programs and adoption of strict measures to protect and enhance the quality of the environment.

In Egypt, population and industrial growth have made increasing demands on the scarce water resources, and at the same time people are demanding upgrading of water quality for public health, aesthetic and recreational purposes. The emergence of these paradoxical demands has presented a problem in providing water to meet the rising demands and obtaining the desired water quality. In addition to this problem, the poultry industry faces a unique situation, since new production farms and processisng plants will be located in remote areas as a part of an integral plan to revive the Egyptian deserts. The stringency of water supply for the new plants coupled with the compelling need for prodigious quantities of water have led to the recognition of used water as a vital part of the water cycle in the poultry processing.

Water recycling in poultry processing is restricted as it confronts public health regulations and established traditions. Recognizing that applied research is needed in this vital area, Alexandria University was awarded a U.S. Environmental Protection Agency grant to study application of water recycling in poultry processing. Field studies were initiated at Alexandria Poultry Processing Plant (APPP), during April 1976.

Research efforts in the first phase of the study were directed toward evaluation of water and poultry characteristics during processing, and documentation of necessary information for selection of potential sources of water for reuse. The second phase will focus on application of a multiple water reuse system, assessment of its technical and economic aspects and most important, its impact on sanitation of the process and product.

MATERIALS & METHODS

CHEMICAL ANALYSES were performed in accordance with EPA methods (U.S. EPA, 1974). Samples for trace metal analyses were concentrated according to a method described by Price (1967), and measurements by atomic absorption techniques were in accordance with standard methods of the American Public Health Association (APHA 1971). Composite samples were collected by automatic samplers, and grab samples were collected by individuals. Samples were taken at the locations shown on Figure 1.

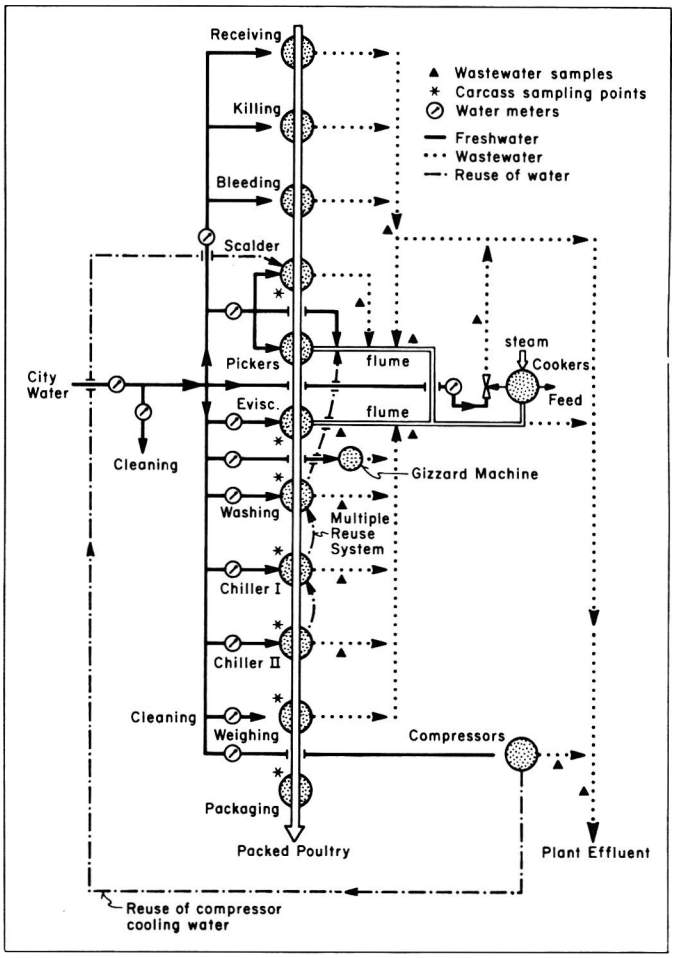


Fig. 1—Flowsheet for Alexandria poultry processing plant.

Water samples for bacteriological analyses were collected in 100 ml sterile glass containers, while carcass samples were kept in specially prepared sterile polyethylene bags. To each bag, 0.2L distilled water was poured followed by shaking for 5 min. The washing was poured on a sterile funnel and filtered through sterile no. 1 filter paper. Total bacterial and coliform counts were done by plating on blood-agar and Mac-Conkey agar, respectively, according to methods by Cruickshank (1973). Procedures of bacteria identification are described elsewhere by Hallman and Burkhardt (1974). Anti-microbial residues were tested according to the method suggested by Huber et al. (1969).

RESULTS

Unit operations at APPP

A brief description of water using operations at APPP is given for understanding of the available water sources for reuse. The operations are almost identical to those in the United States. A flow diagram of APPP and water usage by

Authors Hamza and Saad are with Alexandria University, Higher Institute of Public Health, 165 El Horreya, Alexandria, Egypt. Author Witherow is with the U.S. Environmental Protection Agency, Food & Wood Products Branch, 200 S.W. 35th St., Corvallis, OR 97330.

Table 1—Average water usage for operations at APPP

Sample station	Mean[a] (m^3/day)	Std dev[a] (m^3/day)	Process water (%)	Total water (%)
Killing & bleeding	9.7	6.6	3.9	2.4
Scalding & defeathering	116.7	19.7	47.0	28.3
Evisceration	31.7	9.1	12.8	7.7
Giblet processing	10.6	4.3	4.3	2.5
Washing tank	27.7	10.5	11.2	6.7
Chiller I	16.5	7.2	6.6	4.0
Chiller II	9.5	2.9	3.8	2.3
Cleaning packing area	11.5	3.2	4.6	2.8
Cleaning evisceration area	14.6	4.3	5.8	3.5
Condenser effluent	95.5	28.5		23.2
Compressor cooling	50.7	10.6		12.3
Process water	248.2	27.7	100.0	60.2
Process & feed & cool	394.4	48.3		95.7
Domestic uses	17.9	12.4		4.3
Total water	412.3	54.9		100.0
Carcasses processed	13083	1907		
Process water (L/bird)	19.0	4.7		
Total water (L/bird)	31.5	3.5		

[a] n = 69

Table 2—Average waste load[a] for operations at APPP

Sampling station	COD kg/d	COD kg/kkg LWK	BOD kg/d	BOD kg/kkg LWK	TR kg/d	TR kg/kkg LWK
Bleeding effluent	30.8	2.1	22.8	1.6	41.7	2.9
Feather flume	217.5	15.1	165.7	11.5	225.6	15.7
Evisc. & giblet process.	60.9	4.2	39.4	2.7	70.9	4.9
Washing effluent	39.7	2.8	33.4	2.3	48.3	3.4
Chiller I effluent	30.9	2.1	23.6	1.6	35.0	2.5
Chiller II effluent	12.9	0.9	9.7	0.7	14.8	1.0
Condenser effluent	132.5	9.2	108.9	7.6	163.5	11.4
Final effluent	530.0	36.8	426.8	29.6	609.6	42.3

[a] kg/d = kilogram/day; kg/kkg LWK = kg/1000 kg Live Weight Killed [Processed birds averaged 14.4 kkg LWK/day]

various operations is shown in Figure 1 and Table 1, respectively. The variation in water usage is large for individual unit operations on either a daily or per bird basis, but small for the combined operations.

Killing and bleeding. The live birds are stunned by low voltage current, and slaughtered by cutting the jugular vein and allowing the blood to drain in a restricted area. A sizable waste load is associated with this area due to the placement of blood into the sewer system. Saving the blood for rendering is common in the U.S.

Scalding and defeathering. Birds are subscalded at 54–60°C, while adjusting the daily overflow to 5L per bird, (L/bird). The scalder effluent is highly contaminated with bacteria, blood and other residues. The scalded birds are defeathered in three consecutive picking machines. The feathers are flumed to the rendering area with 9.2L/bird using the effluents from the scalder and pickers in addition to freshwater. This is the largest water usage in the plant.

Evisceration. The carcass cavity is opened for removal of entrails. Giblets are cleaned and wrapped for bulk selling. After opening and washing the gizzards, they are transferred manually to the wrapping area without fluming to save water

Table 3—Analyses of wastewater grab samples from effluents of APPP (mg/L)

Sample[a]	Temp°C	pH	D.O.	NH_3-N	$SO_4^=$	COD	BOD_5	TR	TVR	Grease
Final $\bar{X}$	31.0	6.8	0.2	29	50	1581	1306	1974	1368	102
Effl. S.D.	1.5	0.4	0.5	14	7	365	323	331	327	76
r							0.7	0.5	0.1	
Bleeding $\bar{X}$	26.2	6.7	0.0	45	63	3165	2341	4288	3163	88
Effl. S.D.	1.6	0.3	0.0	19	14	1571	858	1882	1640	51
r							0.8	0.7	0.8	
Scalder $\bar{X}$	58.0	7.0	0.0	28	49	1330	978	1556	1121	68
Effl. S.D.	1.7	0.2	0.0	22	8	597	573	558	462	42
r							0.9	0.9	0.9	
Feather $\bar{X}$	40.5	7.0	0.5	28	52	1449	937	1687	1069	111
Flume S.D.	4.8	0.3	1.3	16	9	707	472	611	420	63
r							0.8	0.8	0.6	
Eviscer. $\bar{X}$	26.2	6.4	0.4	29	53	2195	1678	2369	1806	316
Effl. S.D.	1.5	0.4	0.6	12	14	748	509	854	800	202
Offal $\bar{X}$	25.5	6.9	2.0	28	48	1582	1156	1784	1218	208
Flume S.D.	1.1	0.4	1.7	17	6	802	525	820	828	101
r							0.9	0.9	0.9	
Washing $\bar{X}$	26.5	7.1	4.4	28	51	1303	1061	1485	981	229
Effl. S.D.	2.6	0.3	2.3	21	7	358	422	385	373	124
r							0.9	0.9	0.9	
Chiller I $\bar{X}$	14.8	7.1	2.9	28	47	1193	956	1411	985	270
Effl. S.D.	3.1	0.3	1.7	20	9	471	475	509	497	219
r							0.9	0.9	0.9	
Chiller II $\bar{X}$	8.6	7.4	4.3	29	45	884	758	1093	644	239
Effl. S.D.	1.0	0.6	1.5	20	8	477	744	454	346	82
r							0.3	0.9	0.7	
Condenser $\bar{X}$	55.9	7.4	2.0	24	49	1123	942	1297	960	182
Effl. S.D.	1.2	0.3	2.4	17	11	197	236	291	307	131
							0.9	0.7	0.8	
Compresser $\bar{X}$	28.7	7.7	6.4	36	39	30	9	378	123	< 5
Effl. S.D.	0.6	0.5	1.0	48	6	13	6	273	77	< 5

[a] $\bar{X}$ = Mean; S.D. = Standard deviation; r = Correlation between COD and BOD, TR and TVR; and n = 12

and to eliminate the problem of treating the fluming effluent. The inedible viscera are dropped into a trough which is flushed by water from the hand washing faucets located along the evisceration line.

Washing and chilling. The eviscerated carcasses are washed with freshwater in a horizontal immersion tank. The daily water usage averages 2 L/bird. Chilling is accomplished in two consecutive tanks at a combined daily overflow rate of 2 L/bird which is equal to USDA requirements. Effluents from both operations are discharged to the offal flume.

Other water uses. The screened feathers and offal are cooked by direct contact steam to produce a poultry feed ingredient. Waste steam is withdrawn from the cooker by jet ejector and condensed before final discharge to the sewer system. The steam condenser has once through water usage which averages about $95m^3$ of fresh water per day. In addition, about $50m^3$ of freshwater are used daily to cool the ammonia compressors which operate continuously to refrigerate the storage coolers and produce ice used in the chilling operation.

Characteristics of poultry and process

The waste loads of all operations presented in Table 2 were calculated from average flow, concentration and production data. From each wastwater sample point (Fig. 1), six sets of composite samples were collected during July and 12 sets of grab samples were collected between April and August. The average values for the 12 grab samples were generally higher than the average values for the six composite samples. A summary of the data from the grab samples is presented in Table 3 to characterize the wastewater effluents. The results indicate that the compressor cooling water is virtually clean, except for trace contamination by ammonia leaked from the compression cylinders. This water is suitable for reuse in the scalding or defeathering operations. The chemical oxygen demand (COD) for most effluents highly correlated to 5-day biochemical oxygen demand (BOD_5), total residue (TR), and total volatile residue (TVR), which implies the possible use of COD as an indicator of pollution in poultry processing. The dissolved oxygen (DO) values are depressed by oxygen demanding pollutants and increased temperature.

Discharge of the washing and chilling effluents to the offal flume results in noticeable dilution of the concentrated effluent from the evisceration process. The mean concentrations of COD and TR are less in chiller II than in chiller I or in the washer. This offers an opportunity for reuse of water from chiller II in chiller I and the washer.

Trace metal concentrations in various effluents are shown in Table 4. The relatively high iron concentration in the killing, scalding and eviscerating effluents is attributed to the blood contamination and the possible corrosion of fittings and pipelines at elevated scalding temperatures. Trace metal concentrations in the compressor cooling water are nearly identical to those in the freshwater. In general, concentratons of trace metals in various effluents are similar to those of freshwater, which indicates the near absence of pollution by trace metals during poultry processing. Magnesium and iron concen-

Table 4—Trace metals in grab samples of wastewater from APPP (μg/L)

Sample[a]	Co	Cr^{+6}	Zn	Ni	Pb	Mn	Cu	Fe	Cd
Final Effluent	R	2–16	2–17	34–117	1–6	1–12	19–180	23–60	130–800	2–3
	$\bar{X}$	5.8	5.6	61.8	3.0	3.6	72.3[b]	36.1	485[b]	2.1
	S.D.	4.7	5.1	39.0	1.9	3.7	51.7	11.3	220	0.4
Bleeding Effluent	R	1–18	1–13	25–200	1–4	1–12	21–210	20–40	180–630	1–4
	$\bar{X}$	7.0	4.9	68.9	2.6	2.8	61.4[b]	27.1	470[b]	3.0
	S.D.	5.7	4.0	60.5	1.1	4.1	66.2	7.2	165	1.1
Scalding Effluent	R	1–18	1–12	21–170	1–5	1–14	26–210	21–40	201–810	1–6
	$\bar{X}$	7.1	6.0	57.4	2.6	3.5	62.5[b]	29.4	524[b]	2.7
	S.D.	5.3	4.2	48.1	1.4	4.6	62.9	7.8	222	1.6
Feather Flume	R	2–15	0–12	22–190	2–5	1–12	13–180	15–40	215–640	1–4
	$\bar{X}$	7.4	5.4	64.3	3.4	3.0	53.3[b]	28.3	427[b]	2.9
	S.D.	4.2	4.9	54.3	1.2	3.7	55.0	7.6	132	0.1
Evisceration Effluent	R	1–17	1–8	31–150	1–4	1–14	14–130	20–40	210–730	1–5
	$\bar{X}$	7.8	4.4	64.5	3.1	2.9	53.5[b]	30.6	466[b]	2.7
	S.D.	5.9	3.1	37.9	1.3	4.5	36.1	6.8	165	1.4
Offal Flume	R	1–17	1–14	28–130	1–5	1–22	13–140	20–40	310–530	1–4
	$\bar{X}$	7.8	5.3	67.5	3.0	4.1	49.5	28.0	419[b]	2.3
	S.D.	5.8	4.9	34.4	1.5	7.3	40.8	8.1	68	1.0
Washing Effluent	R	1–17	1–12	28–90	1–8	1–12	12–72	20–40	190–400	1–2
	$\bar{X}$	6.3	5.8	62.0	3.5	4.0	26.7	29.7	311[b]	1.8
	S.D.	6.3	4.0	25.8	2.6	4.6	22.5	6.4	95	0.5
Chiller I Effluent	R	2–19	1–8	21–110	1–9	1–10	10–160	21–180	90–500	1–3
	$\bar{X}$	8.3	3.8	66.4	3.6	3.3	44.0	51.9	279	2.4
	S.D.	5.2	2.8	27.4	2.7	3.9	52.2	53.7	142	0.8
Chiller II Effluent	R	3–18	1–6	30–120	1–5	1–9	10–220	18–32	210–650	1–3
	$\bar{X}$	9.1	3.4	68.4	3.1	3.5	61.4[b]	27.1	374[b]	2.0
	S.D	4.7	1.9	34.1	1.6	3.3	86.3	6.0	147	0.8
Condenser Effluent	R	2–14	1–9	26–140	1–9	1–8	9–140	13–100	240–890	1–5
	$\bar{X}$	8.3	3.5	61.5	3.7	2.3	50.3[b]	38.7	454[b]	2.6
	S.D.	3.8	3.1	44.7	2.9	2.8	58.7	35.2	255	1.8
Compressor Cooling	R	2–18	1–15	32–190	1–12	1–14	10–190	9–40	120–600	1–3
	$\bar{X}$	7.9	4.9	70.1	3.1	2.8	63.5[b]	25.4	362[b]	2.0
	S.D.	4.7	4.4	53.1	3.7	4.6	66.3	12.8	155	0.2
U.S. drinking water standards		–	50	–	–	50	50	1000	300	10

[a] n = 8; R = Range; and X = Mean.
[b] Values exceed drinking water standards

Table 5—Bacteria identification and counts of wastewater samples

Sampling station	No. of observations	(Percent of Positive Results) Escherichia coli	Proteus	Pseudomonas	Salmonella typhi	Salmonella para typhi A	Salmonella para typhi B	Para colon	Shigella	Staphylococci	Streptococci	Micrococci	Total aerobic[a]	Coliform[a]
Final effluent	19	76	10	57	5	5	5	23	5	19	5	5	9.9×10^8	4.3×10^6
Scalding effluent	18	66	16	11	5	32	22	32	66	77		11	3.3×10^7	2.9×10^5
Feather flume	21	100	23	5	5	5	5	9.5	9.5	19			3.5×10^7	4.0×10^5
Eviscerating trough	21	95	33	33				5	9.5				6.4×10^{11}	7.4×10^{10}
Offal flume	21	95	39	19	9.5	5	9.5	19	5	14	5	19	1.9×10^8	2.7×10^6
Wash tank	20	85	33	28	5	19		28	14	9.5	14	28	1.0×10^5	2.0×10^3
Chiller I	21	85	33	57	9.5	9.5	5	9.5	9.5	5	14	14	4.9×10^4	2.1×10^3
Chiller II	21	76	14	62	23	9.5		19	5	14	5	14	6.9×10^4	1.3×10^3
Condenser water	14	64	28		14	14	7	7			7	7	1.2×10^4	1.5×10^3
Compressor water	14	28		14	7								1.1×10^2	—

[a] Average counts per ml

trations exceed the desired levels for drinking water in the United States.

Antimicrobial residues in the effluents from the scalder, evisceration trough, washing and chilling tanks were either free or contained antimicrobial residues less than the sensitivity limit of 25 μg/L of chloramphenicol. In addition, four sets of wastewater samples were found free of parasites, except for the bleeding and killing effluent in which *Ascaridia* was detected twice.

Bacterial identification and counts of the various effluents are shown in Table 5. The highest counts were found in the eviscerating effluent. Less contamination was observed in the scalding and feather flume effluents. This may be due to a reduction of viable cells from scalding or by dilution with freshwater.

The decrease in bacterial counts in the offal flume in comparison with the eviscerating effluent is due to the dilution effect of the washing and chilling effluents. The most prevalent bacteria in the effluents were *E. coli, Pseudomonas* and *Proteus. Salmonella, Para colon, Shigella* and *Staphylococci* were identified in several samples. Cooling water from the compressors was generally free from bacterial contamination.

The results presented in Table 6 show the identified bacteria and counts associated with the carcass samples. Heavy contamination was encountered after both scalding and evisceration. Washing decreased the bacterial counts on the carcasses by 3–4 orders of magnitude and chilling decreased the bacterial counts on the carcasses by two additional orders of magnitude. The bacterial counts increased again during the final weighing and packaging operations. This striking recontamination indicates the need to modify the process by eliminating or minimizing the human contact during the weighing and packaging operations.

The identified bacteria are significant to the sanitary quality, shelf life and safety in consumption of the product. The data were collected for comparison with similar data after water reuse was initiated. In cases where the bacteria were below detectable limits the space was left blank in Tables 5 and 6. In the scalding, eviscerating, washing and chilling operations the percent of positive results associated with the carcasses were usually similar to those found in the wastewater effluents. Some of the identification measurements were found to have limited meaning and have been replaced by salmonella count measurements.

A study of build-up of contaminants in the scalding, washing and chilling tanks was undertaken to provide information concerning the quality of water which comes in direct contact with the carcasses during processing.

The usual operation involves initial fillings of the tanks every morning and discharging the used water and refilling with freshwater once or twice during the processing period. Refilling frequencies depend on the operator's judgment for the need to renew the process water.

During this study no refilling was done. However, overflow from the tanks was kept constant according to the rates previously mentioned. Water samples were taken from the tanks after one hour of operation, and every hour after that for a total period of six hours. Samples were not collected during the lunch hour. This sampling scheme was repeated four times in May.

Table 6—Bacteria identification and counts of processed carcasses

Sampling station	No. of observations	(Percent of positive results) Escherichia coli	Proteus	Pseudomonas	Salmonella typhi	Salmonella para typhi A	Salmonella para typhi B	Shigella	Streptococci	Micrococci	Total aerobic[a]	Coliform[a]
After scalding	18	72	16.6	11.1						5.6	1.8×10^9	1.7×10^7
During evisceration	18	88.9	38.9	50.0	16.0	5.7	5.7	11.1		11.1	3.0×10^{12}	4.6×10^{10}
After evisceration	12	83.3	25	58.3	8.3						3.9×10^{12}	4.6×10^{10}
After washing	12	100	8.3	16.7	16.7					16.7	2.2×10^8	9.6×10^6
After chiller I	12	100	33.3	41.7	25.0	8.3	8.3	16.7	25.0		1.6×10^6	9.8×10^4
After chiller II	18	88.9	44.4	38.9	27.8	11.1	33.3				1.9×10^6	5.6×10^4
During weighing	12	100	33.3	75.0		8.3			25.0	8.3	1.6×10^7	4.2×10^5
After packaging	18	77.8	27.8	66.7	22.2	11.1	27.8	16.6	55.6		4.6×10^7	3.1×10^6

[a] Average counts per carcass

Accumulation of total aerobic and coliform counts in the process water is illustrated in Figure 2. Bacterial contamination of the scalding water was greater than in the washing and chilling waters. During the first processing period (8–11 a.m.) bacterial counts in all tanks increased, and continued to increase during the second processing period (12–3 p.m.). The results of this study support the value of continual removal of water contaminants in these "immersion processes."

To evaluate the effect of process water on the bacterial quality of the carcasses, the accumulation study was repeated seven times during Nov./Dec. Figure 3 shows parellel increases in bacterial counts on the carcasses and in the water in the scalder, the washer and the chillers. The logarithmic increase in the water could be due to increasing counts on the carcasses which is the case for the carcasses entering the chillers and washer (Fig. 3). However, it is unrealistic to assume that the bacterial counts on birds entering the scalder increase during the day, thus, the cause and effect relationship is not certain. What is certain is that the levels of bacteria in the water and on the carcasses are directly related, and that the means of improving the product quality are through continual improvement of the quality of the water in these processes.

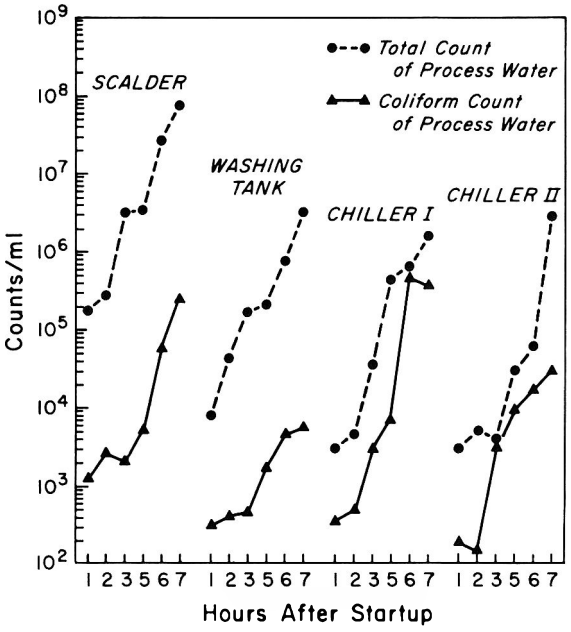


Fig. 2—Accumulation of bacteria in process water.

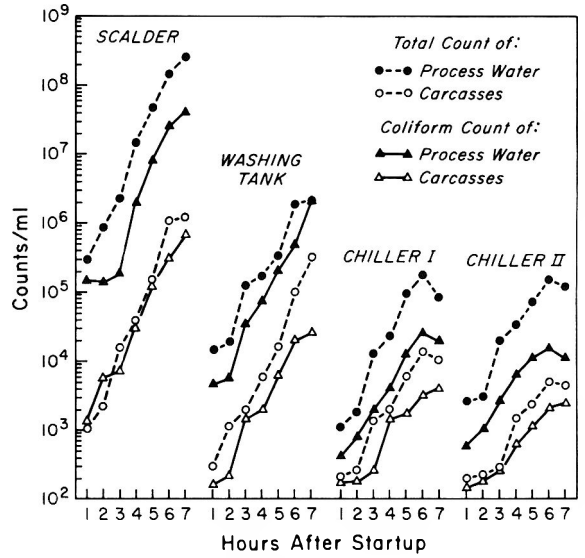


Fig. 3—Accumulation of bacteria in process water and carcasses.

DISCUSSION

EGYPTIAN HEALTH REGULATIONS agree in principle with those of the USDA which requires the use of potable water in all operations involving direct contact between water and the carcass or giblets. However, in the common water immersion and water transport operations used in poultry processing the potable quality is lost when the first carcass or giblet enters the tank or flume. The addition of freshwater does not regain potable quality but does cause considerable energy losses in maintaining water tempertures in the scalder and chillers.

The available option to save process water and conserve energy is water renovation and reuse. Although used water may not be potable, it can be treated to a better quality than the process water which is presently used in contact with the carcasses. Contrary to popular belief, Rogers (1976) showed recycling of renovated water can result in improving the quality of water in the chiller and this study indicates a similar improvement in the quality of product can be expected.

Present U.S. regulations specify quality and quantities of freshwater in poultry processing to maintain processing methods which experience has shown satisfactory to obtain a product safe for consumption. The purpose of this research project is to demonstrate environmentally desirable modifications of water use in several unit operations which will maintain or improve the water quality in these operations and produce a product equally safe for consumption. Most of the data gathered in phase one has been to establish a baseline of water and product quality in each unit operation. These data are to be compared with similar data collected in phase two to document expected improvements in water and product quality.

Multiple use system

The results of the first phase of the U.S. EPA demonstration project add support to the premise of water reuse in poultry processing. The multiple use system shown in Figure 1, has been initiated with the second phase of this project which began in March 1977.

The system involves use of freshwater in the final chiller and recycling its overflow to the chiller I. The overflow of chiller I will be reused in the washing tank. Effluent of the washing will be reused in the scalder to replace the freshwater used in this operation. Water used for cooling the composers will be reused as a continuous feed to the scalding tank, which will reduce both the freshwater and energy requirements of the scalding operation. This multiple system is flexible, since the ratio of freshwater/used water can be adjusted to insure the quality of the water used in each unit operation.

Multiple water use together with modifying the evisceration operation will save 40% of the current process water usage.

In the final phase of the study, the once-through condensing water will be renovated and recycled which will save an additional 25% of the water usage.

SUMMARY

THE RESULTS obtained from this study have provided the following:

Manual handling during weighing and packaging results in noticeable recontamination of the processed carcasses. Elimination of human contact in this operation is required to upgrade the quality of the product.

Water used for cooling the compressors can be reused in the scalder without treatment as it is virtually free from contamination.

—Continued on page 1161

BIOCONVERSION OF SHELLFISH CHITIN WASTES: PROCESS CONCEPTION AND SELECTION OF MICROORGANISMS

PAUL A. CARROAD and RAYMOND A. TOM

ABSTRACT

A process was conceptualized for bioconversion of shellfish chitin wastes to single-cell protein of value in animal or aquaculture feed formulations or to other products. An extracellular chitinase enzyme system obtained by a submerged culture of microorganisms is contacted with the chitin waste, hydrolyzing it to smaller sugar units. The hydrolysate is converted to a marketable product. Experimental results indicate that *Serratia marcescens* QMB1466 is suitable for use in the proposed process. Hydrolysis of various chitinous waste preparations shows the culture filtrate to be effective in decomposing the substrate. For crude preparations, hydrolysis slows after approximately 40 hr. Colloidal chitin is almost completely dissolved after 60 hr.

INTRODUCTION

ENVIRONMENTAL and energy restrictions have forced the nation's seafood processors to consider alternatives to the inland dumping or the hauling out to sea of waste materials. Affected are the processors of shellfish, such as shrimp and crab, who must cope with the disposal of chitin. Recent years' shrimp and crab landings in the United States have been approximately 0.340 billion kilograms (Anon., 1977). Approximately 75% of the total weight of these shellfish is considered as waste, and from 20–58% of the dry weight of the waste is chitin depending upon the processing method (Kreag and Smith, 1973; Mauldin and Szabo, 1974; Ashford et al., 1977). The harvesting and processing of shrimp and crab in the United States thus represents both an important problem and also an opportunity in waste resource utilization (Kohn, 1976).

Chitin is a naturally occurring biopolymer, chemically described as β-1,4-N-acetyl-2-amino-2-deoxy-D-glucose. In structure chitin is similar to cellulose, a polymer of glucose units, particularly since the intermonomer bondings of both are of the β-1,4-type. The difference between the two polymers is that the hydroxyl group in the second position in cellulose is replaced in chitin by an acetamido group ($-NHCOCH_3$), making chitin a polymer of N-acetylglucosamine units. The structure of purified chitin has been the subject of much investigation, especially with respect to determination of polymer chain lengths which have been shown to range in the thousands of monomer units (Chan, 1970). The chitin from many different sources has been shown to be structurally similar, although its association with protein and calcium carbonate may differ among shellfish (Averbach, 1975; Berger, 1957).

The chitin waste is available mostly along the Western United States and Alaskan coasts and the Gulf of Mexico. Of the approximately 70 shrimp canners in the United States, 25 are located on the Gulf Coast. Canning plants range in size from those processing 10–20 tons of raw shrimp per day to those processing up to 60 tons per day (Mauldin and Szabo, 1974). The nature of chitin waste, then, is that it tends to be chemically similar as far as the chitin protion is concerned and to be available in given areas rather than dispersed widely.

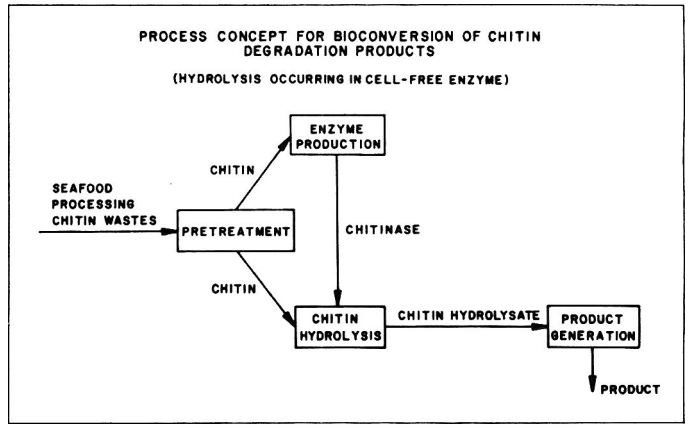


Fig. 1—Four-step process concept for chitin bioconversion

A process was conceptualized for the bioconversion of chitin wastes into single-cell protein suitable for animal feed or aquaculture feed. Design concepts were drawn from the process development work concerning the bioconversion of cellulose to ethanol and single-cell protein (Cysewski and Wilke, 1976; Wilke et al., 1976). The chitin bioconversion process under investigation is a specific waste treatment application for a problem in which local economic factors will dictate success and is without the national energy impact of the cellulose process. Supplies of chitin are much less abundant on land than are those of cellulose. The similarity in chemical nature between chitin and cellulose, however, makes much of the work on the latter substrate applicable to the former.

For maximum flexibility in substrate utilization a four-step process is envisioned as illustrated in Figure 1. In the first step the chitin waste receives some pretreatment which would at a minimum involve drying and size reduction. Chemical purification measures may also be required as the chitin waste contains considerable amounts of protein and calcium carbonate. A small fraction, perhaps one-tenth, of the waste is diverted to an enzyme production step. Chitin waste is used to induce a selected microorganism to secrete into solution an extracellular chitinase system in submerged culture. The enzyme is harvested by filtration and, in a third step, is combined with the bulk of the chitin waste for chitin hydrolysis. The chitin is enzymatically hydrolyzed, principally to the monomer N-acetylglucosamine and its dimer. The hydrolysate is filtered free of undigested solids and in a fourth step is fed to a product generation stage where, for example, the hydrolysis product is fermented in submerged culture to single-cell protein by an appropriate microorganism. Product purification may follow as necessary.

Alternatively, if the enzyme production, hydrolysis, and product generation steps can be combined into a single vessel, then the process could be reduced to two steps as shown in Figure 2. Such would be the case if the organism producing the chitinase had value as a protein source, if the desired product was the chitinase enzyme system, or if the product

Authors Carroad and Tom are with the Dept. of Food Science & Technology, University of California at Davis, Davis, CA 95616.

0022-1147/78/0004-1158$02.25/0
© 1978 Institute of Food Technologists

generating organism could be co-cultured in the same fermentor. Following pretreatment, concurrent reactions in a single vessel or stage would yield the desired product.

The objective of this study was to select an appropriate microorganism for extracellular chitinase production. Sources include in-house isolates as well as those organisms requested from the University of California, Davis, culture collection and from other researchers. The organism(s) selected will be used in further studies concerning optimization of enzyme production and chitin hydrolysis. A final process feasibility study is to be the result.

EXPERIMENTAL

Chitin

Shrimp shell chitin (24.4 wt % chitin) is the waste product obtained from a California shrimp processing plant and milled through a 0.635 cm (¼ inch) screen. Commercial chitin (85.8 wt % chitin) is the preparation available from the chemical market (Calbiochem, San Diego, CA). Milled chitin is commercial chitin which has been ball milled for 24 hr. Colloidal chitin (98.7 wt % chitin) can be prepared from milled chitin in two ways. In the first, chitin is stirred into 10 volumes of hydrochloric acid at room temperature for 48–72 hr, washed, heated in 10 volumes of 1N sodium hydroxide at 100°C for 1 hr, washed, soaked in 2% potassium manganate at room temperature for 8 hr, suspended in 1% oxalic acid, washed successively with 2N hydrochloric acid, water, ethanol and ether, dried, moistened in acetone, added to 10 volumes of concentrated hydrochloric acid, filtered, and precipitated in 50% ethanol. The precipitate was resuspended in water and dialyzed (Berger, 1957). In the second method, 10g milled chitin is stirred into 100 ml of 85% phosphoric acid in the cold for 48 hr, reprecipitated in excess water, and centrifuged (Monreal and Reese, 1969). The first method is herein cited as the Berger method, the second as the Reese method.

Organisms

Serratia marcescens QMB1466 was obtained from Dr. Elwyn T. Reese, Food Science Laboratory, U.S. Army Natick Research & Development Command, as was *Serratia marcescens* W200. *Enterobacter liquefaciens* 3354-ICPB, *Aeromonas liquefaciens* 2327-ICPB, and *Serratia marcescens* 2875-ICPB were obtained from Dr. Mortimer Starr, Dept. of Bacteriology, University of California at Davis. The organism identified as *Bacillus cereus* 21.5 is an in-house isolate.

Media formulations

The standard liquid culture medium to which chitin and yeast extract were added was (in grams per liter): $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.3; KH_2PO_4, 1.36; pH initially adjusted to 8.5 to yield pH 7.7 after sterilization (Monreal and Reese, 1969). The agar plates used for observation of chitinolytic zones around microorganism colonies were double layer, the bottom layer consisting of 0.5% yeast extract and 2% agar and the top layer also containing 1% colloidal chitin.

Methods of analysis

Gravimetric measurements. Determination of chitin decomposition (weight loss) by pure cultures was determined by heating chitin for 1 hr at 100°C with 10% potassium hydroxide to digest cell matter, filtering onto glass filters, and drying for 24 hr at 100°C. Decomposition by cell-free enzyme extracts was similarly determined, omitting the digestion step.

N-acetylglucosamine. The N-acetylglucosamine concentration was determined with p-dimethylaminobenzaldehyde reagent which turns a reddish-purple color upon reaction in acetic acid at room temperature for 45 min (Morgan and Elson, 1934).

Reducing sugar. The total reducing sugar concentration was determined with 3,5-dinitrosalicylic acid reagent which turns a reddish color upon reaction in boiling water for 5 min. A standard curve based upon N-acetylglucosamine was used (Sumner and Somers, 1944).

Chitin composition. Percentage of chitin on a weight basis in shellfish samples was determined by a gravimetric method following protein and calcium carbonate solubilization (EPA, 1971).

Enzyme activity

For chitinase activity in solution, 1 ml of enzyme (culture filtrate or concentrate) was incubated with 1 ml of 1% swollen chitin (Reese method), in 0.05M citrate-phosphate buffer (pH 6.6) for 1 hr at 50°C. Reducing sugar was measured on 1 ml of supernatant using the N-acetylglucosamine standard curve. One unit of enzyme activity will produce 0.5 micromoles of N-acetylglucosamine under these conditions (Monreal and Reese, 1969).

Activity ratio is defined as the ratio of the diameter of the chitinolytic zone to the diameter of the bacterial colony incubated on a double layer agar plate at 30°C for 7 days (Chan, 1970).

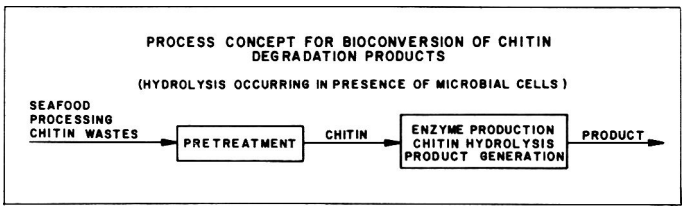


Fig. 2—Two step process concept for chitin bioconversion

Table 1—Activity ratios of chitinolytic organisms

Organism	Activity ratio[a]
Serratia marcescens QMB1466	2.8
Bacillus cereus isolate 21.5	2.4
Serratia marcescens W200	2.0
Enterobacter liquefaciens 3354-ICPB	2.0
Aeromonas liquefaciens 2327-ICPB	1.0
Serratia marcescens 2875-ICPB	0.8

[a] Ratio denotes diameter of chitinolytic zone to diameter of bacterial colony in solid culture on agar with 60.0 g/L colloidal chitin (Berger method), 0.5 g/L yeast extract, 1.0 g/L Bacto-peptone (Difco), 20.0 g/L agar, pH adjusted to 7.6. Incubation at 30°C, 7 days.

Hydrolysis procedures

For hydrolysis with intact cells related to selection of organisms, the microorganisms were cultured with 1% chitinous solids (colloidal by Berger method and shrimp shells) and 0.05% yeast extract, at 30°C for 7 days. Precipitates remaining after incubation were treated with 10% potassium hydroxide for 1 hr to lyse intact cells, and were washed, filtered, and dried to determine weight loss of chitin.

For hydrolysis of various substrates by *S. marcescens* QMB1466 culture filtrate, enzyme was produced by culturing the organism in shake flasks on the standard medium with either milled or commercial chitin at 1.5%, 2.5%, and 3.5% solids at 30°C for 9 days. Solutions were combined to give a final enzyme activity after addition of substrate for hydrolysis of 83 units in each hydrolysis vessel. Chitinous solids were added to the 5% level and the hydrolysis conducted at 50°C in a rotary shaker at 250 RPM. Initial pH was 6.6, established by a 0.5M phosphate buffer.

RESULTS & DISCUSSION

Selection of microorganism

Three hundred microorganisms were isolated from a variety of sources of chitin including soil, insects and marine residues. Based on activity ratios, 17 were judged to have sufficiently active extracellular chitinase systems to be considered further. Gravimetric measurements of chitin decomposition by pure cultures confirmed the chitinolytic ability of the isolates. The most active isolate (Isolate 21.5), tentatively identified as *Bacillus cereus*, achieved a 29.12% weight loss of commercial chitin from a medium constituted at the 1% chitin level, in 7 days.

The isolate 21.5 was tested with seven microorganisms received from the University of California, Davis culture collection and from other researchers for activity ratios. In Table 1 are listed the ratios for the six most active chitinolytic organisms. The mutant *Serratia marcescens* QMB1466 is seen to secrete the most active chitinase by this measure.

The organisms were incubated with chitin for hydrolysis in the presence of cells. The percentage weight losses of the chitin substrates in the culture flasks are listed in Table 2. As expected, weight loss of colloidal chitin is substantially greater than that of shrimp shell chitin since the former has con-

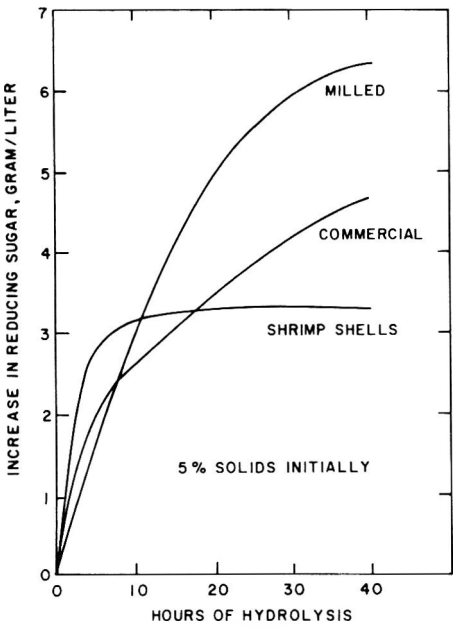


Fig. 3—Hydrolysis curves of chitin samples by Serratia marcescens QMB1466 culture filtrate. Hydrolysis, expressed as increase of reducing sugar concentration in solution, was conducted at 50°C, initial pH 6.6. Enzyme activity was 83 units.

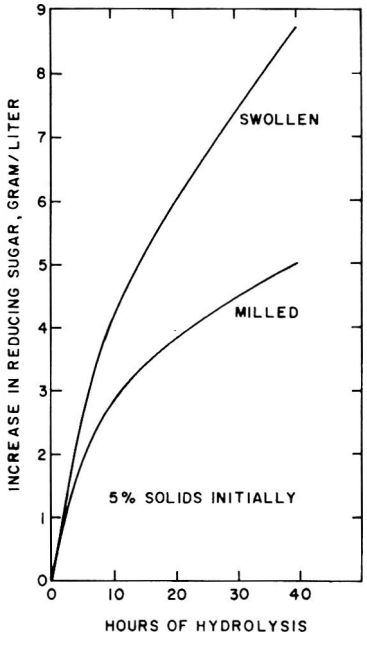


Fig. 4—Hydrolysis curves of chitin samples by Serratia marcescens QMB1466 culture filtrate. Hydrolysis, expressed as increase in reducing sugar concentration in solution, was conducted at 50°C, initial pH 6.6. Enzyme concentration in solution was 53% of that used in Fig. 3 hydrolysis.

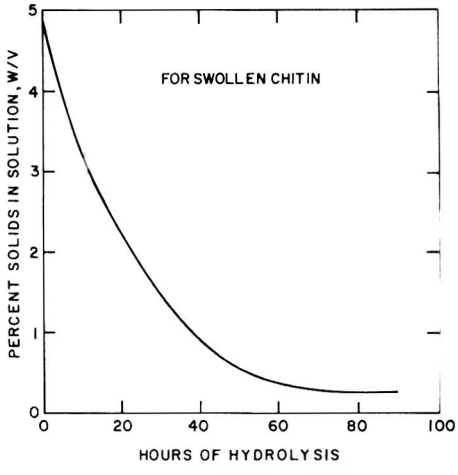


Fig. 5—Weight loss of colloidal chitin resulting from hydrolysis by Serratia marcescens QMB1466 culture filtrate. Curve corresponds to hydrolysis curve of colloidal chitin in Fig. 4.

Table 2—Percentage weight loss of colloidal and shrimp shell chitins from incubation flasks

Organism	Percentage weight loss[a]	
	Colloidal chitin (Berger method)	Shrimp shell chitin
Serratia marcescens QMB1466	92.6	57.6
Bacillus cereus isolate 21.5	93.3	62.3
Serratia marcescens W200	92.6	50.2
Enterobacter liquefaciens 3354-ICPB	89.6	44.5
Aeromonas liquefaciens 2327-ICPB	90.6	44.3
Serratia marcescens 2875-ICPB	96.3	46.2

[a] Cultures were grown on standard medium, 1% chitinous solids, 0.5% yeast extract, at 30°C, initial pH 7.6, for 7 days.

siderably more exposed surface area for reaction, and may be less crystalline. On colloidal chitin, *Serratia marcescens* 2875 effected the greatest weight loss while *S. marcescens* QMB1466 and *B. cereus* 21.5 yielded best results on shrimp shell chitin.

The culture filtrates of the three organisms most effective in achieving weight loss on shrimp shells as a growth substrate were tested on colloidal chitin (Berger method), commercial chitin, and shrimp shell chitin. The organisms were grown in shake flasks on standard liquid medium with 2% of the different chitins added. Cell-free culture filtrates were incubated with 0.5% levels of the same chitin preparations as used in each respective culture vessel. For both colloidal and shrimp shell chitin, the total concentration of N-acetylglucosamine measured in solution following a 5-hr hydrolysis was 3.6 times greater for *S. marcescens* QMB1466 than for *S. marcescens* W200 and 2.6 times greater for *S. marcescens* QMB1466 than for *B. cereus* Isolate 21.5. On commercial chitin the ratios were 2.0 and 1.8, respectively.

Since the substrate to be used in a practical shellfish treatment process will be raw shrimp chitin having undergone minimum pretreatment, emphasis is placed on organisms effecting greatest conversion of this substrate. In this regard, overall chitin conversion and culture filtrate enzyme activity are significant parameters. From Table 2 it is seen that the *B. cereus* isolate 21.5 effected the greatest weight loss of chitin as a growth substrate. The results in Tables 1 and those discussed above for total N-acetylglucosamine generation on different substrates, however, show that the most active cell free culture filtrate is that of *S. marcescens* QMB1466. An interaction between the microbial cell and the solid substrate may be responsible for the very slight advantage of *B. cereus* Isolate 21.5 exhibited in Table 3. Since the process as conceptualized is concerned primarily with use of a cell-free enzyme extract (four step process) in order to gain maximum flexibility in conversion to final products and because of the greater possibility of mutating *S. marcescens* to improve enzyme production, use of *Serratia marcescens* QMB1466 is indicated.

Hydrolysis of chitin

The hydrolyses of various chitin preparations by cell free culture filtrates of *S. marcescens* QMB1466 were followed as functions of time by measuring the reducing sugar increase in solution. The results for milled, shrimp shell, and commercial chitins are shown in Figure 3. It is seen that after approximately 40 hr the increase in reducing sugar had slowed. Little increase was evident after 100 hr. Final pH values were shrimp shell 8.3, commercial 7.2, and milled 7.1.

Swollen chitin (Reese method) was similarly hydrolyzed. Since swollen chitin contains considerable water and drying might have altered its structure, hydrolysis was conducted at a lower enzyme activity than for the results shown in Figure 3. For swollen chitin hydrolysis, enzyme activity was 53% of that previously used, due to dilution of the protein by the moisture contained in the chitin preparation. A comparison hydrolysis of milled chitin was conducted at this enzyme strength. Results are shown in Figure 4. Final pH values were 6.6 for swollen chitin and 7.0 for milled chitin. Hydrolysis of swollen chitin is essentially complete in 60 hr as seen from the weight loss curve in Figure 5.

These hydrolysis results demonstrate that enzymatic chitin hydrolysis is possible and may have value as part of a treatment configuration for shellfish processing waste.

REFERENCES

Anon., 1977. Fisheries '76. The Fish Boat. 22(10): 22.
Ashford, N.A., Hattis, D. and Murray, A.E. 1977. Industrial prospects for chitin and protein from shellfish wastes. M.I.T. Sea Grant Program report number MITSG-77-3, index number 77-703-Zle. Massachusetts Institute of Technology, Cambridge, MA.
Averbach, B.L. 1975. The structure of chitin and chitosan. M.I.T. Sea Grant Program report number MITSG-75-17, index number 75-317-IlW. Massachusetts Institute of Technology, Cambridge, MA.
Berger, L.R. 1957. Digestin of chitin by a Streptomyces species. Ph.D. thesis, University of California.
Chan, J.G. 1970. The occurrence, taxonomy, and activity of chitinoclastic bacteria from sediment, water, and fauna of Puget Sound. Ph.D. thesis, University of Washington.
Cysewski, G.R. and Wilke, C.R. 1976. Utilization of cellulosic materials through enzymatic hydrolysis. 1. Fermentation of hydrolysate to ethanol and single-cell protein. Biotech. Bioeng. 18: 1297.
EPA. 1971. Pollution abatement and by-product recovery in shellfish and fisheries processing. Project number 12130FJQ. Environmental Protection Agency, Washington, D.C.
Kohn, P.M. 1976. Shellfish wastes vie for CPI role. Chem. Eng. 83(19): 107.
Kreag, R. and Smith, F. 1973. Seafood solid waste in Oregon: Disposal or recovery? Special report 395. Oregon State University Extension, Marine Advisory Program. Corvallis. OR.
Mauldin, A.F. and Szabo, A.J. 1974. Shrimp canning waste treatment study. Environmental Protection Agency study EPA-660/2 74-061. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.
Monreal, J. and Reese, E.T. 1969. The chitinase of Serratia marcescens. Canad. J. Microbiol. 15: 689.
Morgan, W.T. and Elson, L.A. 1934. A colourimetric method for the determination of N-acetylglucosamine and N-acetylchondrosamine. Biochem. J. 28: 988.
Sumner, J.R. and Somers, F. 1944. "Laboratory Experiments in Biological Chemistry." Academic Press, Inc. New York.
Wilke, C.R., Cysewski, G.R., Yang, R.D. and von Stockar, U. 1976. Utilization of cellulosic materials through enzymatic hydrolysis. 2. Preliminary assessment of an integrated processing scheme. Biotech. Bioeng. 18: 1315.
Ms received 6/21/77; revised 11/21/77; accepted 12/1/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

This work was supported in part by the NOAA office of Sea Grant, Dept. of Commerce, under Grant No. 04-7-158-44121 (R/F-34) and was aided by a Grant-in-Aid of Research from Sigma XI, the Scientific Research Society of North America. The U.S. Government is authorized to reproduce and distribute reprints for governmental purpose notwithstanding any copyright notations that may appear hereon.

WATER REUSE IN POULTRY PROCESSING . . . From page 1157

A study of accumulation of contaminants in the immersion processes (scalding, washing and chilling) revealed a direct relationship between the bacterial counts in the water and on the carcasses. Continual renovation of water to retard the rate of build-up of contaminants is needed. Use of renovated water can enhance the quality of the process water in these operations, besides saving part of the energy required for cooling and heating processes.

Counter current water use through the chillers, washer and scalder has been initiated with a comprehensive evaluation of the water and carcass quality.

REFERENCES

APHA. 1971. "Standard Methods of Examination of Water and Wastewater." American Public Health Association, 1740 Broadway, New York, NY.
Cruickshank, R. 1973. "Medical Microbiology," 12th ed., Vol. 1. Longman, Inc., New York, NY.
Hallman, L. and Burkhardt, F. 1974. "Klinische Microbiologie," George Thieme, Verlage. Thieme Stuttgart, D-7000 Stuttgart 1, Herdweg 63.
Huber, W.G., Carlson, M.B. and Lepper, M.H. 1969. Penicillin and anitmicrobial residues in domestic animals. J. Amer. Vet. Med. Assoc. 154: 1590.
Price, W.J. 1967. Atomic adsorption analysis of waters and effluents. Effl. and Water Treatment J. 7(4): 218.
Rogers, C.J. 1976. Recycling of water in poultry processing plants. Tech. Report on Project S-800930, EPA, IERL, Cincinnati, OH.
USDA. 1968. "Regulations Governing the Inspection of Poultry and Poultry Products." U.S. Department of Agriculture, Consumer Marketing Service, Poultry Division, Washington, DC.
USEPA. 1974. "Manual of Methods for Chemical Analysis of Water and Wastewater." Tech. Transfer, EPA 625/6-74-003, U.S. Environmental Protection Agency, Washington, DC.
Ms received 8/20/77; revised 12/8/78; accepted 12/14/77.

This research is supported by Grant No. PL3.54202 form the EPA. Grateful acknowledgment is extended to Dr. Ali Mourad, Faculty of Medicine, Alexandria University, for his help in the bacterial analyses.

FATTY ACID COMPOSITIONS OF 15 SPECIES OF FISH FROM TROPICAL WATERS

P. G. VISWANATHAN NAIR and K. GOPAKUMAR

ABSTRACT

Fatty acid composition of five species of freshwater fish, three marine fish and seven brackish water fish were determined by GLC. Of the saturated fatty acids $C_{16:0}$ and $C_{18:0}$ are found to be the dominant ones with $C_{16:0}$ accounting for 50–55% of the total saturated acids. Among the monounsaturated fatty acids $C_{16:1}$ and $C_{18:1}$ and polyunsaturated fatty acids $C_{18:2}$, $C_{18:3}$, $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$ are found to be the important ones. Stinging cat fish contained the highest amount of $C_{16:1}$ (16.52%). The level of arachidonic acid was fairly high in most of the fish lipids analyzed. The ratio $C_{18:1}/C_{18:2}$ is higher for marine fish than for the freshwater fish and is fairly constant for each group. The fatty acid composition of brackish water fish do not show any definite pattern.

INTRODUCTION

FISH LIPIDS are known for the complexity of their fatty acid composition. The lipids of marine and freshwater fish have certain characteristic differences in the fatty acid composition (Lovern, 1951; Hilditch and Williams, 1964). These distinctions are based mainly on the chain lengths rather than any other fundamental properties (Ackman, 1967). The most characteristic features in freshwater fish are the higher levels of C_{16} and C_{18} acids and the lower levels of C_{20} and C_{22} acids compared to marine fish (Ackman, 1967; Yamada and Hayashi, 1975). It has been observed that these differences are mainly due to the dietary fat rather than the habitat (Ackman, 1967). While these two form somewhat distinct groups, the lipids from the fish of estuarine or brackish water origin do not conform to any of the two groups (Ackman, 1967; Pathak and Reddy, 1964). The lipids of fish of marine and freshwater origin of this region have been studied in detail for their fatty acid make up (Gopakumar and Rajendranathan Nair, 1966, 1967, 1972; Gopakumar, 1975), while the lipids of the brackish water fish have not so far received much attention. This paper describes the fatty acid compositions of the lipids of some marine, freshwater and brackish water fishes obtained from the waters in and around Cochin and compares their differences.

MATERIALS & METHODS

THREE SPECIES of marine, five species of freshwater and seven species of brackish water fish were studied. The temperature of the water from which these fish were caught varied from 25–28°C. All the samples were collected during the period from May to June 1976 from adjoining and interconnecting water bodies. The samples selected for analysis were of normally adult size.

The numbers of fish from which representative samples were taken for analyses are given in Table 1. The muscle was removed from each fish and the whole muscle was blended together in a Waring Blendor. Five hundred grams of blended muscle was taken for lipid extraction. Total lipids were extracted from the muscle of the fish by the method of Bligh and Dyer (1959). The iodine value (Wijs, 30 min) of the lipid samples were determined by the method of AOAC (1975). For the preparation of methyl esters, the lipid samples were saponified, unsaponifiable matter was removed and the fatty acids were collected. The free acids were esterified with methanolic BF_3 (Morrison and Smith, 1964). All these operations were carried out in an atmosphere of nitrogen. The methyl esters were analysed on a gas chromatograph (Toshniwal Ltd., Bombay) using flame ionization detector and strip chart recorder. The column was of stainless steel, 6 ft × ¼ in. (o.d.), with 15% diethyleneglycol succinate on chromosorb W (60–80 mesh). Operating conditions were as follows: column temperature, 192°C; injection port temperature 250°C; detector temperature, 250°C; carrier gas, nitrogen at 40 ml/min. The following standards were used: Saturated acids–the methyl esters of C_{12}, C_{13}, C_{14}, C_{16}, C_{17}, C_{18} acids (A.G. Fluka, Switzerland) and C_{20} acid (E. Merck). Unsaturated acids–the methyl esters of oleic, linoleic, linolenic, arachidonic, erucic and decoshexaenoic (Sigma, 99%), 11-methyl eicosaenoic (90%) and 5, 8, 11, 14, 17-eicosapentaenoic acids (94.6%) (N.I.H. standards). The instrument was calibrated with fatty acid methyl ester mixtures as described by Horning et al. (1964). Identification and quantitation of peaks were done as described by Gopakumar and Rajendranathan Nair (1972). Results are expressed as area percentages.

Both authors are with the Central Institute of Fisheries Technology, Indian Council of Agriuclture Research, Willingdon Island, Cochin-682029, India.

0022-1147/78/0004-1162$02.25/0
© 1978 Institute of Food Technologists

RESULTS & DISCUSSION

THE DETAILS OF FISH specimens analyzed, their lipid contents and the iodine values of the lipids are given in Table 1. All the fish were of low lipid content. The maximum was for grey mullet (5.06%), whereas frog fish had only 0.45% of lipids. Iodine values were found to vary widely, 138 for pearl spot and 170 for Indian halibut.

The fatty acid compositions are given in Table 2. In general, 16:0 and 18:0 are the most predominant saturated acids, as is the case with all fish lipids. Ackman and Eaton (1966) have pointed out that 16:0 is a key metabolite in fish and its level is not influenced by the diet. They have also pointed out that in Atlantic herring oil $C_{16:0}$ accounted for almost 60% of the total saturated acids. In the present study the level of this acid is slightly lower and also shows wider variations. Out of the 15 lipid samples analysed 11 have values between 40 and 55 (Table 3). On the other hand, for the depot fat of milk fish this value is 66.8% (unpublished data) which is similar to the values reported for the depot fats of squid and cod (Ackman and Sipos, 1965). This variation is, therefore, due to the contribution by phospholipids to the total fatty acid make up. $C_{17:0}$ is found to be present in fairly good amounts (up to 7.67% in silver jew fish) in all the fish studied.

Among the monoethylenic fatty acids, $C_{18:1}$ and $C_{16:1}$ are found to be the major constituents. Only in stinging cat fish did the $C_{16:1}$ record a level (16.48%) higher than that of $C_{18:1}$ (15.28%). The principal acids in the polyunsaturated group are 18:2, 18:3, 20:4, 20:5 and 22:6. The level of arachidonic acid ($C_{20:4}$) is fairly high in all the species examined.

The fatty acid composition of the five fresh water fish are compared with those of the marine fish analyzed in this study. These marine lipids had fatty acid compositions similar to that reported earlier (Gopakumar and Rajendranathan Nair, 1967, 1972). The C_{16} and C_{18} acids were generally lower (Table 3) in the marine lipids compared to the freshwater lipids. Marine lipids are found to be richer in C_{22} acids. Unlike earlier reports, there was no great difference in the proportions of C_{20} acids between the two groups. Considering the individual

Table 1—Fish species analyzed, lipid content and iodine values

Sl. no.	Common name	Scientific name	No. analyzed	Habitat	Lipid content (% of fresh muscle)	Iodine value (Wijs 30 min.)
1.	Indian halibut	*Psettodes erumei* (Bloch)	10	Marine	0.92	170
2.	Short-necked sea pike	*Sphyraena langsar* Bleeker	50	Marine	1.51	156
3.	Silver jew fish	*Johnius argentatus* (Houtuyn)	8	Marine	1.63	152
4.	Waigue Snapper	*Lutianus waigiensis* (Quay and Gaimard)	10	Marine	3.13	128
5.	Spiny eel	*Mastacembelus armatus* (Lacepede)	75	Freshwater	0.56	147
6.	Green Snake head	*Ophiocephalus punctatus* Bloch	75	Freshwater	0.77	156
7.	Filamented barb	*Puntius filamentosus* (Valenciennes)	80	Freshwater	1.14	160
8.	Orange chromide	*Etroplus maculatus* (Bloch)	150	Freshwater	1.16	154
9.	Stinging catfish	*Heteropneustes fossilis* (Bloch)	50	Freshwater	0.86	141
10.	Pearl spot	*Etroplus suratensis* (Bloch)	12	Freshwater	2.30	138
11.	One spotted golden snapper	*Lutianus fulviflamma* (Forskal)	70	Brackish water	0.84	143
12.	Frog fish	*Batrachus grunniens* (Linnaeus)	10	Brackish water	0.46	135
13.	Marine Catfish	*Arius dussumieri* Day	7	Brackish water	4.86	150
14.	Giant catfish	*Netuma thalassinus* (Ruppel)	20	Brackish water	2.03	142
15.	Grey mullet	*Mugil cephalus* (Linnaeus)	10	Brackish water	5.06	128

acids, $C_{18:0}$, $C_{18:1}$ and $C_{20:4}$ acids are comparatively lower and $C_{20:5}$ and $C_{22:6}$ are higher in marine species. Higher levels of 16:1 has been described as a characteristic of fresh water fish oils (Ackman, 1967), but no such conclusion can be drawn from this study. The percentage of $C_{16:1}$ varied from 5.8–16.5, the majority falling in the range of 6–8. The distribution of 16:0 also does not show any pattern characteristic of any group. The ratio 16:0/16:1 + 18:1 showed a character-

Table 2—Fatty acid composition of the lipids studied

	Species of fish														
	Indian halibut	Short-necked sea pike	Silver jew fish	Waigue snapper	Spiny eel	Green snake head	Filamented barb	Orange chromide	Stinging catfish	Pearl spot	One-spotted golden snapper	Frog fish	Marine catfish	Giant catfish	Grey mullet
Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Saturated															
12:0	–	–	0.38	–	2.10	–	0.33	–	1.18	–	–	0.38	–	–	0.09
13:0	0.25	4.50	1.62	0.23	0.70	0.26	2.00	0.16	1.37	0.97	0.78	0.89	0.15	0.45	0.17
14:0	5.21	1.25	5.01	2.44	3.59	0.68	3.43	3.35	3.28	3.82	2.65	2.13	4.41	6.92	3.89
15:0	3.93	–	1.38	0.97	2.99	2.06	1.54	1.12	2.23	2.82	3.64	2.90	2.20	3.86	5.84
16:0	15.31	19.75	18.59	17.56	13.66	23.96	13.43	24.71	17.62	19.92	18.34	26.18	16.35	22.01	20.34
17:0	7.67	3.46	5.02	2.64	3.80	3.02	2.25	2.76	2.51	0.35	3.95	4.89	5.04	4.28	5.87
18:0	11.33	11.73	11.89	10.81	12.59	13.86	16.82	12.00	9.36	15.66	13.32	11.76	18.38	13.26	5.16
19:0	1.82	2.45	1.23	1.59	1.80	1.34	0.94	0.88	1.57	–	–	1.67	1.58	1.08	1.72
Total saturated	45.52	43.14	45.12	36.24	41.23	45.18	40.74	44.98	39.12	43.54	42.68	50.80	48.11	51.86	43.08
Monoenoic															
13:1	–	–	0.11	–	–	–	–	–	1.24	0.11	0.96	–	–	–	–
14:1	–	–	–	0.58	3.04	–	–	0.19	2.34	–	0.92	–	–	–	–
15:1	–	–	–	–	–	1.79	–	–	–	–	2.14	–	–	–	–
16:1	7.74	7.74	10.38	7.45	7.46	5.83	7.93	6.28	16.48	11.09	6.82	6.66	7.62	7.90	10.63
17:1	–	1.46	–	–	1.13	2.02	1.55	1.23	–	0.43	5.90	3.24	–	1.90	5.69
18:1	12.09	17.27	16.15	31.98	20.43	13.95	18.60	13.68	15.28	20.32	15.27	16.66	17.97	12.33	14.44
20:1	0.80	0.78	0.73	0.82	1.00	0.68	–	0.92	–	1.60	0.93	1.01	0.48	0.77	5.12
22:1	–	0.53	–	0.93	0.51	0.26	1.14	0.67	0.86	0.97	–	–	–	0.84	1.12
24:1	–	–	–	–	–	–	–	–	–	–	–	0.43	–	–	–
Total monoenoic	20.63	27.78	27.37	41.76	33.57	24.53	29.22	22.97	36.20	34.52	32.94	28.00	26.07	23.74	37.00
Polyenoic															
18:2	3.64	2.85	2.94	4.92	8.17	3.81	10.35	2.10	4.11	7.55	5.43	2.68	0.65	3.31	–
18:3	2.21	2.23	1.57	3.62	1.90	0.54	3.97	0.56	0.85	5.46	–	1.71	5.82	–	3.99
18:4	0.68	1.57	2.52	1.56	1.59	1.81	1.20	3.59	1.73	2.37	2.84	1.14	0.48	3.51	0.82
20:2	–	0.93	–	1.10	–	0.18	0.60	1.01	1.01	–	0.44	–	–	1.68	–
20:3	–	–	–	0.57	1.51	0.20	1.24	0.70	2.08	–	0.49	0.16	–	1.64	–
20:4	6.32	3.21	4.31	3.22	7.06	6.05	6.69	2.98	6.30	3.46	8.14	4.54	3.33	3.60	2.92
20:5	5.87	3.88	7.08	0.89	1.16	5.98	1.37	2.21	3.78	0.50	1.23	2.44	5.45	4.36	3.25
22:2	0.63	–	0.65	–	–	–	–	–	–	–	0.12	0.33	0.61	–	–
22:3	0.96	0.93	0.52	–	–	–	0.27	–	1.30	0.52	0.79	1.78	1.45	0.60	0.70
22:4	1.77	1.89	1.49	0.91	0.85	1.43	0.80	2.83	0.94	–	1.21	0.79	–	2.05	2.75
22:4	–	–	–	0.49	0.46	0.96	–	3.02	–	–	–	–	–	–	0.35
22:5	–	1.14	1.53	0.73	0.34	2.24	0.20	5.06	0.49	0.99	0.72	1.65	3.20	2.07	1.80
22:6	10.41	10.33	4.95	0.99	2.16	6.72	2.57	7.99	0.28	1.02	2.98	4.00	4.83	1.59	2.84
Total polyenoic	32.49	28.96	27.56	19.00	25.20	29.92	29.26	32.05	22.87	21.87	24.39	21.22	25.82	24.41	19.42
Unidentified															
1	0.46	0.14		0.05		0.22	0.78		1.81	0.85					0.50
2	0.90			2.96		0.15									

Table 3—Distribution in terms of major chain lengths and some characteristic ratios of fatty acids

Property compared	Indian halibut	Short-necked sea pike	Silver jew fish	Waigue snapper	Spiny eel	Green snake head	Filamented barb	Orange chromide	Stinging catfish	Pearl spot	One-spotted golden snapper	Frog fish	Marine catfish	Giant catfish	Grey mullet
$C_{16:0}$ as % of total saturated acids	33.88	45.78	41.20	48.45	33.86	53.03	32.97	54.94	45.04	45.75	42.97	51.52	34.00	42.64	47.21
Total C_{16} acids	23.01	27.49	28.94	25.01	21.12	29.79	21.36	30.99	29.91	31.01	25.16	32.84	23.97	34.10	30.97
16:0/16:1 + 18:1	0.7714	0.7898	0.7008	0.4453	0.5006	1.212	0.5063	1.238	0.558	0.6343	0.8322	1.122	0.6392	1.087	0.8114
Total C_{18}	29.95	35.65	35.07	52.89	44.68	38.97	50.94	31.93	31.33	51.36	36.86	33.95	43.30	32.41	29.14
Poly unsaturated C_{18}	6.53	6.65	7.03	10.10	11.66	6.16	15.52	6.25	6.69	15.38	8.32	5.53	6.95	6.62	4.81
Total C_{20}	12.99	8.80	12.12	6.60	10.73	13.09	9.90	7.82	13.17	5.56	11.23	8.15	9.26	12.05	11.29
Total C_{22}	13.77	14.82	9.14	4.05	4.32	11.61	4.98	19.57	3.87	4.35	5.82	8.55	10.09	7.15	9.56
18:1/18:2	3.32	6.06	5.49	6.50	2.50	3.66	1.80	6.82	3.72	2.69	2.81	6.22	27.65	3.73	–

istic pattern. Marine fish have higher values. This can be attributed to the higher levels of 18:1 acid in the lipids of freshwater fish. The distribution of 18:2 follows the pattern of lower levels in marine and higher levels in fresh water lipids. No such distinction can be observed in the distribution of the 18:3 acid. When the total C_{18} polyunsaturated acids are considered there is a notable difference between the two groups, marine species generally having lower proportions of these acids compared to fresh water species. Another important difference noted in this study is the relative proportions of 18:1 and 18:2 acids. The ratio 18:1/18:2 was much higher for marine lipids compared to the fresh water lipids and fairly constant for each group. The proportions of total monounsaturated acids is lower in marine lipids and higher in fresh water lipids. Values for total saturated and total polyunsaturated fatty acids show much scattering. There appears to be no sharp distinction in these aspects.

The fatty acid compositions of the lipids of estuarine or brackish water fish do not follow any definite pattern. In general it may be said that their composition is an average of the other two groups. In many instances some of them resemble marine lipids and some, fresh water lipids (in the distribution of total C_{18} and total monoenoic acids). Since most of the fish samples were obtained from inter-connecting water bodies there is the possibility of overlapping of the above factors.

REFERENCES

Ackman, R.G. 1967. Characteristics of the fatty acid composition and biochemistry of some freshwater fish oils and lipids in comparison with marine oils and lipids. Comp. Biochem. Physiol. 22: 907.

Ackman, R.G. and Eaton, C.A. 1966. Some commercial Atlantic herring oils: Fatty acid composition. J. Fish. Res. Bd. Canada. 23: 991.

Ackman, R.G. and Sipos, J.C. 1965. Isolation of the saturated fatty acids of some marine lipids with particular reference to normal odd-numbered fatty acids and branched chain fatty acids. Comp. Biochem. Physiol. 15: 445.

AOAC. 1975. 28.020. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.

Gopakumar, K. 1975. Fatty acid composition of three species of freshwater fish. Fish. Technol. 12: 21.

Gopakumar, K. and Rajendranathan Nair, M. 1966. Studies on fish lipids. 2. Fatty acid composition of lipids of oil sardine. Fish. Technol. 3: 21.

Gopakumar, K. and Rajendranathan Nair, M. 1967. Studies on fish lipids 1. Fatty acid composition of lipids in marine fish as determined by gas liquid chromatography. Ind. J. Biochem. 4: 229.

Gopakumar, K. and Rajendranathan Nair, M. 1972. Fatty acid composition of eight species of Indian marine fish. J. Sci. Fd. Agric. 23: 493.

Hilditch, T.P. and Williams, P.N. 1964. "The Chemical Constitution of Natural Fats, Fourth ed, p. 62. Chapman & Hall, London.

Horning, E.C., Ahrens, Jr. E.H., Lipsky, S.R., Mattson, F.H., Mead, J.F., Turner, D.A. and Goldwater, W.H. 1964. Quantitative analysis of fatty acids by GLC. J. Lipid Res. 5: 20.

Lovern, J.A. 1951. "Biochemistry of Fish," p. 49. Biochemical Symposia No. 6. Cambridge University Press.

Morrison, W.R. and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride-methanol. J. Lipid. Res. 5: 600.

Pathak, S.P. and Reddy, B.R. 1964. The component acids of Indian 'boundary' fish fats. J. Sci. Fd. Agric. 15: 529.

Yamada, M. and Hayashi, K. 1975. Fatty acid composition of lipids from 22 species of fish and mollusk. Bull. Jap. Soc. Sci. Fish. 41: 1143.

Ms received 7/18/77; revised 12/4/77; accepted 12/8/77.

The authors are grateful to Shri G.K. Kuriyan, Director, C.I.F.T. for permission to publish this paper, to Shri M.R. Nair, for helpful suggestions, and to Dr. Shahul Hameed, for identifying the fish species.

DEFINITION OF A PREDICTION MODEL FOR DETERMINATION OF THE EFFECT OF PROCESSING AND COMPOSITIONAL PARAMETERS ON THE TEXTURAL CHARACTERISTICS OF FABRICATED SHRIMP

HONG-MING SOO, EUGENE H. SANDER and DOUGLAS W. KESS

ABSTRACT

The designs of Scheffe [J. Royal Stat. Soc., Series B (1963) 25: 235], and of McLean and Anderson [Technometrics (1966) 8(3): 447] were used to study the effect of processing (mixing times and temperatures) and compositional (shrimp, isolated soy protein, NaCl, and sodium tripolyphosphate) parameters on the textural changes of fabricated shrimp. Prediction equations were developed to investigate the effect of those parameters on textural changes of the cooked shrimp patties. There is not a significant interaction effect between the processing and compositional parameters. Mixing temperature has a greater effect on texture of cooked shrimp patties than does mixing time. The directional derivatives of the response surface and the contour plots were used to examine the compositional effects. Sodium tripolyphosphate (STP) causes the greatest change in response among components studied. The optimum level of NaCl for springiness is 2%, whereas the optimum level of STP is dependent upon the isolated soy protein (ISP) level. Springiness of cooked shrimp patties increases with increasing ISP and decreasing shrimp levels in the mixture. The calculated optimum processing and compositional parameters for fabricated shrimp texture were obtained by using a non-linear programming routine, and agree with the measured values. The designs and data analysis procedures can be used to develop accurate prediction models for the effect of processing and compositional parameters on textural characteristics of fabricated shrimp.

INTRODUCTION

FABRICATION of shrimp shapes involves starting with comminuted raw shrimp, combining this with a binding matrix agent, followed by mechanically shaping (Sander et al., 1976). Textural characteristics of fabricated shrimp are influenced by compositional and processing parameters. Geographic source of shrimp and amount, binding matrix agent amount and composition, and comminuting and mixing method used, are important parameters which contribute to the texture of the final product (Soo and Sander, 1977b). The addition of sodium chloride (NaCl) and phosphate to cod, shrimp, comminuted fish muscle and ground meat increase the water holding capacity of muscle proteins and result in a product of desirable texture (Garnatz et al., 1949; Love, 1968; Ahmed et al., 1973; Webb et al., 1975; Lee and Toledo, 1976; Shults et al., 1972; Shults and Wierbicki, 1972).

In order to develop optimum textural characteristics of fabricated shrimp, based on a knowledge of compositional and processing parameters, it is essential to determine the relative effect of each process variable and each formula, and if there is a significant interaction effect between variables. In studies on the effect of product ingredient (composition effect), the use of a mixture design is advantageous because it permits determination of the relative effect of each ingredient and any interaction among them. This type of experimental design is advantageous as opposed to factorial experimentation where it is impossible to vary one ingredient or component while holding all others constant in mixture. In factorial experimentation, the variables studied are independent. The level of each variable can change without changing the level of any of the other variables. But in mixture experimentation, the variables are not independent. Changing the level of one variable will change the level of at least one other variable in the experiment. The applications and advantages of using mixture designs in food research were discussed in an excellent and comprehensive review by Hare (1974). However, no information is available in which the relative factors of compositional and processing parameters have been studied.

Ingredients such as sodium tripolyphosphate (STP) which represent a relatively small proportion of the total mixture as compared to main ingredients (i.e., shrimp or binding matrix agent) can be treated as processing variables. In examining the effect of processing parameters and ingredients constituting a relatively small proportion of the total mixture, response surface statistical design (Box and Wilson, 1951; Meyer, 1971) and factorial experimentation can be applied. However, it is impossible to determine the interaction effect among all ingredients in the mixture and the processing parameters.

Scheffé (1963) introduced the simplex-centroid design for experiments with mixtures. Statistical designs permit the introduction of processing variables in addition to mixture variables from experiments. The effect of processing and mixture variables can be obtained from the fitted regression equation. In designs, the proportion of each component is from 0 to 1.0. However, frequently in mixture experimentation it is impossible to vary the proportion of each component from 0 to 1.0. Each component is constrained within certain limits. For example, the percentages of NaCl and STP are limited in the fabricated shrimp mixture, because use levels are based on flavor and USDA regulations, respectively. The solution for this type of experimental design is to use the extreme vertices design suggested by McLean and Anderson (1966).

The purpose of this study was to apply the experimental designs of Scheffé (1963) and McLean and Anderson (1966), to study the effect of processing and compositional parameters on the texture of fabricated shrimp. The contribution to final fabricated shrimp texture of compositional parameters (shrimp, binding matrix agent, NaCl, STP) and of processing parameters (comminuting temperature and time used) was investigated. A regression equation which can be used to predict textural changes, based on a knowledge of the above compositional and processing parameters, was developed.

EXPERIMENTAL

Sources of shrimp and binding-matrix agent

Alaskan shrimp (from Alaskan water) in frozen blocks of whole and broken pieces (400–600 count/kg) from a single production lot, were obtained from the New England Fish Company (Seattle, WA). Shrimp from water around India was purchased from a seafood processor who routinely uses this source in fabricating extruded shrimp.

Two binding-matrix agents described by Soo and Sander (1977a) were used: (1) Hercules 30, a commercial product containing starch and gums, and (2) isolated soy protein (ISP), (Supro 620 T, Ralston Purina Company).

–Text continued on page 1166

Author Sander is with the Dept. of Food Science & Nutrition, and Author Kess with the Dept. of Applied Statistics, University of Minnesota, St. Paul, MN 55108. Author Soo, formerly with the Univ. of Minnesota, is now affiliated with Ralston Purina Co., Checkerboard Square, St. Louis, MO 63188.

0022-1147/78/0004-1165$02.25/0
© 1978 Institute of Food Technologists

Effects of geographic sources, binding matrix compositions and their mixtures on shrimp texture

A 2 × 3 complete factorial experiment with two replications was utilized to investigate the effects of geographic sources (Alaskan and Indian shrimp) and their mixtures (at 3 levels) on textural changes in cooked shrimp patties. The design of this study is summarized in Table 1. The order of the 12 treatments (2 × 3 × 2) was randomized to minimize the experimental bias. A 2 × 3 × 2 experimental design was also used to determine the contributions of binding matrix composition and their mixtures to textural properties of cooked shrimp patties. Two types of binding matrix agent (ISP and Hercules 30) with 3 different shrimp binding matrix mixtures were investigated. The experimental design of this investigation is presented in Table 2. In order to evaluate the interaction effect between geographic source of shrimp or binding matrix composition, the levels of salt and phosphate were held constant in two experiments.

A frozen block of shrimp (7.3 kg) tempered to 4°C, was cut for 1 min using a Hobart Silent Cutter (Hobart Manufacturing Company, Troy, OH). ISP, NaCl and STP were added and mixing continued for an additional 2 min. The cooked shrimp patties were prepared, and textural properties were measured by Instron Texture Profile Analysis (TPA) using the method of Soo and Sander (1977a).

Table 1—Compositions for experimental design used to evaluate the contribution of geographic source of shrimp and final mixtures to the texture of cooked shrimp patties

Treatment[a] no.	Shrimp %	ISP[b] %	NaCl	STP[c]
1	Alaskan 97.7	0	2	0.3
2	Alaskan 92.7	5	2	0.3
3	Alaskan 87.7	10	2	0.3
4	Indian 97.7	0	2	0.3
5	Indian 92.7	5	2	0.3
6	Indian 87.7	10	2	0.3

[a] Replicate blends for each treatment were prepared.
[b] Isolate soy protein
[c] Sodium tripolyphosphate

Table 2—Experimental design used to study the contribution of specific binding matrix agents and final mixtures to the texture of cooked shrimp patties

Treatment[a] no.	Binding matrix agent %	Alaskan shrimp	NaCl %	STP[c] %
1	ISP 4[b]	95.55	0.3	0.15
2	ISP 8	91.55	0.3	0.15
3	IPS 12	87.55	0.3	0.15
4	Hercules 30 4	95.55	0.3	0.15
5	Hercules 30 8	91.55	0.3	0.15
6	Hercules 30 12	87.55	0.3	0.15

[a] Replicate blends for each treatment were prepared.
[b] Isolate soy protein
[c] Sodium tripolyphosphate

Table 3—Compositions of extreme vertices design used to study the effects of compositional parameters on the texture of cooked shrimp patties

	Treatment[a] no.	Shrimp %	ISP[b] %	NaCl %	STP[c] %
Vertex points	1	86.5	10.0	3.0	0.5
	2	86.9	10.0	3.0	0.1
	3	88.5	10.0	1.0	0.5
	4	88.9	10.0	1.0	0.1
	5	91.5	5.0	3.0	0.5
	6	91.9	5.0	3.0	0.1
	7	93.5	5.0	1.0	0.5
	8	93.9	5.0	1.0	0.1
Face points	9	87.7	10.0	2.0	0.3
	10	89.2	7.5	3.0	0.3
	11	90.0	7.5	2.0	0.5
	12	90.4	7.5	2.0	0.1
	13	91.2	7.5	1.0	0.3
	14	92.7	5.0	2.0	0.3
Center point	15	90.2	7.5	2.0	0.3

[a] Replicate blends (from treatment No. 1, 3, 6, 8 and 15) were also made. A total of 20 treatments were determined.
[b] Isolate soy protein
[c] Sodium tripolyphosphate

Effect of compositional and processing parameters on texture of cooked shrimp patties

The simplex-centroid design with mixtures, as described by Scheffé (1963), and the extreme vertices experimental design of McLean and Anderson (1966), were applied to study the effect of processing compositional parameters on the textural changes of cooked shrimp patties. Two process variables (mixing temperature and time) at 2 × 2 levels were included along with the mixture variables (Alaskan shrimp, ISP, NaCl, and STP). Four extreme vertices experiments were subsequently carried out with each pair of process variables (mixing temperature and time).

The extreme vertices experimental design is a procedure for conducting experiments with compositional constraints on mixtures. The following limited ranges were designated for fractions;

$$85\% \leq \text{Alaskan shrimp} \leq 100\%$$
$$5\% \leq \text{ISP} \leq 10\%$$
$$1\% \leq \text{NaCl} \leq 3\%$$
$$0.1\% \leq \text{STP} \leq 0.5\%$$

The levels of shrimp and ISP were limited, based on the optimum extruded performance and textural properties (Soo and Sander, 1977b). NaCl was limited, based on flavor. STP was limited, based on USDA regulations on meat (USDA, 1975). A total of 15 experimental compositions (Table 3) from four components (eight vertex points, six face points, and one center point) were determined for each variety. Five replicate blends for vertex points (treatment No. 1, 3, 6, and 8 in Table 3) and one center point were also made. A total of 20 experimental treatments for each extreme vertices experiment were thus carried out.

Two mixing temperatures (−2°C and 4°C) were used to study the contributions of frozen and unfrozen shrimp to (1) chopped shrimp size, (2) rehydration of binding matrix and mixing uniformity of the complete formulated mixture, and (3) the textural changes in the cooked shrimp patties. Two mixing times, 2 and 6 min were also studied. A 2 × 2 complete factorial experiment, crossing over the extreme vertices experiment (20 treatments) was carried out to study the effects of processing of cooked shrimp patties. The order of the 80 experimental treatments (2 × 2 × 20) was randomized to minimize the experimental bias.

Frozen block shrimp tempered to the desired temperature (−2°C or 4°C) was cut for 1 min using a Hobart Silent Cutter. ISP, NaCl and STP were added and mixing was continued for an additional 1 or 5 min. All mixing procedures were carried out in a temperature-controlled (4°C) room to eliminate temperature changes in the shrimp mixture during mixing. The cooked shrimp patties were prepared and textural properties were measured using the methods described by Soo and Sander (1977a).

Computation of directional derivatives

The general form of the quadratic polynomial in q components for the extreme vertices mixture designs (McLean and Anderson, 1966) is

$$Y = \sum_{i=1}^{q} \beta_i X_i + \Sigma \sum_{i<j}^{q} \beta_{ij} X_i X_j$$

where Y denotes the expected response, the β's are coefficients to be estimated and X's are the level of the variables.

The derivative or slope of response surface with respect to the ith component can be obtained as following

$$\frac{dy}{dx_i} \quad \beta_i + \sum_{i \neq j}^{q} \beta_{ij} X_j$$

To be more specific, if the mixture contains four components as used in this study (shrimp, ISP, STP, and NaCl), the directional derivative (D_a) in the direction which decreases the first component and holds the other three components in the same relative proportions is

$$D_a = \frac{\frac{dy}{dx_1}(X_1 - 100) + \frac{dy}{dx_2}X_2 + \frac{dy}{dx_3}X_3 + \frac{dy}{dx_4}X_4}{\sqrt{(X_1 - 100)^2 + X_2^2 + X_3^2 + X_4^2}}$$

where $X_1 + X_2 + X_3 + X_4 = 100\%$.

RESULTS & DISCUSSION

Contribution of geographic source of shrimp and binding matrix compositions to the textural properties of cooked patties

The contributions of geographic source of shrimp and binding matrix compositions were discussed earlier by Soo and Sander (1977b). It was concluded that frozen blocks of shrimp from different geographic regions of the world, binding matrix compositions (protein and/or carbohydrate), and shrimp-binding matrix mixture compositions all affect the final Instron-measured texture of cooked shrimp patties. Our objective was to develop the functional relationships of these variables (source of shrimp, binding matrix composition, and shrimp-binding matrix mixtures) and their contribution to the final texture of cooked shrimp patties. In other words, an attempt was made to see if there was a significant interaction effect between either geographic source of shrimp, or binding matrix composition, and their mixtures. Consider two situations: if an increase in the amount of binding matrix used causes an increase in Instron TPA values of cooked shrimp patties using binding matrix (A), and the same increase in amount of binding matrix used causes a decrease in Instron TPA values of cooked patties using binding matrix (B), then the one-variable-at-a-time design (i.e., designs described in Tables 1 and 2) would fail to detect this interaction effect.

Analysis of variance (ANOVA) from factorial design for contributions of geographic sources of shrimp and shrimp-binding matrix agent mixtures to the texture of cooked shrimp patties is presented in Table 4. The F values for mixture (M) and their interaction effect (M × S) for three Instron textural properties (hardness, springiness and cohesiveness) were highly significant (<1%), with the exception of interaction effect for cohesiveness (significant at 5% level). The large M.S. for sources of shrimp should not be taken to infer differences between sources since only one sample or block per source was used. In another study (formulations described in Table 2), the F values for interaction effect between binding matrix compositions (ISP and Hercules 30) and shrimp-binding matrix mixtures for Instron springiness and hardness (140.7, 22.95, respectively) were also highly significant (<1%); however, the Instron cohesiveness was not significant (>5% level). This finding suggests that in order to determine the effect of geographic source of shrimp and binding matrix composition on textural changes (Instron springiness and cohesiveness) in cooked shrimp patties, a plan that studies geographic source of shrimp at a fixed binding matrix agent, and then studies binding matrix composition when the source of shrimp is fixed, can reveal very little about the real interaction effects of these variables. In simple terms, to apply the one-variable-at-a-time design for these evaluation studies, within the range of variables studied, may lead to incorrect conclusions about the effect of the variables on changes in cooked shrimp patties texture.

Table 4—Analysis of variance for contribution of geographic source of shrimp, and shrimp-binding matrix agent mixtures to the texture of cooked shrimp patties as measured by Instron textural profile analysis (TPA)

Source	Degree of freedom	F Value[a] Hardness	Springiness	Cohesiveness
Geographic source (S)	1			
Mixture (M)	2	366.0	198.9	298.8
Interaction (M X S)	2	22.58	54.28	6.51
Error	6			

[a] F values are significant ($P \leq 0.001$) with the exception of interaction effect for cohesiveness (significant at $P \leq 0.05$).

Contribution of compositional and processing parameters to texture of cooked shrimp patties

The choice of the experimental design used to evaluate the effect of compositional and processing parameters on texture changes in cooked shrimp patties can directly influence the conclusions about the effect of these parameters. This can be the case if there is a significant interaction effect between any two parameters. As can be seen from previous experiments, a significant effect between either geographic source of shrimp or binding matrix composition and their mixtures was occurring. Therefore, it is not appropriate to include several sources of shrimp and binding matrix agents in mixture design, since the effect of ingredients in a mixture will be different for each source of shrimp and binding matrix agent.

In developing regression equations relating textural changes in cooked shrimp patties to compositional and processing parameters, it is essential to include all the parameters in the same experiment. Geographic source of shrimp and binding matrix composition can be treated as a separate processing variable, which can be included in the extreme vertices design. However, the number of geographic source of shrimp and binding matrix composition is very high. Therefore, the number of experimental treatments could be very large. For example, with two types of each variable (geographic source of shrimp and binding matrix agent), the design would require as many as 320 treatments (2 × 2 × 80), based on the experimental designs described in the experimental section (mixture design described in Table 3, with two levels of mixing time and temperature 2 × 2 × 20). The regression equation resulting from these 320 treatments would be useful only for those two particular variables, geographic source of shrimp and binding matrix composition. It is also difficult to determine the representative numerical value (which is an essential requirement to fit a regression equation) for each geographic source of shrimp and binding matrix agent.

In view of the complexity of these interactions, Alaskan shrimp and ISP were used to investigate the effect of shrimp-binding matrix mixtures and processing parameters on texture changes in cooked shrimp patties.

Among three Instron textural characteristics (hardness, springiness and cohesiveness), springiness is the best objective predictor of sensory response to shrimp textural differences (Soo and Sander, 1977a). Instron springiness values for cooked shrimp patties resulting from compositional and processing parameters are given in Table 5.

Attempts were made to fit the data to a quadratic equation. These parameters were evaluated by minimizing the squared differences between experimental data points and the equation. The following quadratic equation (refereed to in this study as equation A) is obtained:

$$\begin{aligned} Y = {} & 0.0916 X_1 + 6.5065 X_2 - 28.0657 X_3 \\ & + 119.2494 X_4 - 0.07176 X_1 X_2 + 0.2934 X_1 X_3 \\ & - 1.2232 X_1 X_4 + 0.2149 X_2 X_3 - 0.9932 X_2 X_4 \\ & - 0.8502 X_3 X_4 + 0.06927 X_5 + 0.0092 X_6 \\ & - 0.0122 X_5 X_6 \end{aligned}$$

Where Y = predicted springiness value; X_1 = percent of

Table 5—Contribution of compositional and processing parameters to the texture of cooked shrimp patties measured by Instron texture profile analysis (TPA)

Treatment no.	Percent composition				Instron springiness values[a] (mm)			
	Shrimp	ISP[b]	NaCl	STP[c]	−2°C 2 min	4°C 2 min	−2°C 6 min	4°C 6 min
1	86.5	10.0	3.0	0.5	9.88	10.23	10.08	9.98
2	86.9	10.0	3.0	0.1	9.35	9.65	9.45	9.45
3	88.5	10.0	1.0	0.5	9.85	10.20	9.98	9.98
4	88.9	10.0	1.0	0.1	9.65	9.90	9.66	9.75
5	91.5	5.0	3.0	0.5	9.35	7.55	7.53	7.50
6	91.9	5.0	3.0	0.1	7.90	8.13	8.05	8.03
7	93.5	5.0	1.0	0.5	7.65	7.73	7.75	7.70
8	93.9	5.0	1.0	0.1	7.85	8.05	8.02	7.90
9	87.7	10.0	2.0	0.3	9.75	9.78	9.85	9.86
10	89.2	7.5	3.0	0.3	8.03	8.20	8.05	8.06
11	90.0	7.5	2.0	0.5	8.03	8.83	8.73	8.80
12	90.4	7.5	2.0	0.1	8.60	8.93	8.85	8.38
13	91.2	7.5	1.0	0.3	8.05	8.30	8.18	8.09
14	92.7	5.0	2.0	0.3	7.65	7.98	7.74	7.80
15	90.2	7.5	2.0	0.3	8.18	8.55	8.25	8.60
16	86.5	10.0	3.0	0.5	9.60	9.88	9.75	9.78
17	88.5	10.0	1.0	0.5	9.55	9.90	9.58	9.77
18	91.9	5.0	3.0	0.1	7.72	8.00	7.86	7.95
19	93.9	5.0	1.0	0.1	7.63	8.05	7.80	7.93
20	90.2	7.5	2.0	0.3	8.48	8.78	8.75	8.15

[a] Mean based on 3 measurements at each treatment. Frozen block of shrimp tempered to indicated temperature was cut and mixed for indicated time.
[b] Isolated soy protein
[c] Sodium tripolyphosphate

Alaskan shrimp; X_2 = percent of ISP; X_3 = percent of NaCl; X_4 = percent of STP; X_5 = mixing temperature (°C); X_6 = mixing time (min); and $X_1 + X_2 + X_3 + X_4 = 100\%$.

The adjusted multiple correlation coefficient (Snee, 1971), $R_A = 0.985$, indicated that the model developed was highly significant ($P < 0.001$) with $F = 168.61$. Thus, the adjusted R^2 ($R^2_A = 0.97$) statistic suggests that the model accounts for 97% of the total variation. The coefficient of variability (CV) is 1.895%. The residual difference between observation and prediction based on regression plot showed normal distribution. The F test for lack of fit ($F = 0.054$, in Table 6) was not significant, which indicated that the model is appropriate.

ANOVA for the above quadratic equation is given in Table 6. The sum of squares (SS) of the interaction effect between compositional variables and processing variables [M × (T, t, Tt) in Table 6] was computed by subtracting the sum of the individual error SS when fitting the four levels of the process variables separately (SS of error in Table 8) from the error obtained when all variables were fitted. Thus, it represents the failure of mixture variables to produce a simple additive response across the process variables. The F test for the interaction effect between compositional parameters and processing parameters was statistically not significant ($P > 0.5$). This finding along with other features of the data suggest that the type II error associated with assuming the interaction to be zero is small, or that the interaction itself is small and the consequences of assuming it absent are negligible. This suggests that effects of compositional parameters on Instron springiness can be obtained from one of four extreme vertices mixture design results.

Table 6—Analysis of variance for effects of compositional and processing parameters on Instron springiness values of cooked shrimp patties

Source	Degree of freedom	Mean of square	F Test[a]
Mean	1	6043.7314	
Mixture (M)			
Linear	3	4.6042	121.26
Quadratic	6	4.5561	199.99
Temperature (X_5)	1	0.2991	7.88
Time (X_6)	1	0.0036	0.095 NS
$X_5 X_6$	1	0.4224	11.12
M × (X_5, X_6, $X_5 X_6$)	27	0.02397	0.63 NS
Lack of fit	20	0.002056	0.054 NS
Pure error	20	0.03797	

[a] NS = nonsignificant ($P > 0.25$)

The springiness values of cooked shrimp patties from each of four extreme vertices designs were subjected to least square computation. The four quadratic equations for prediction of Instron springiness values for cooked shrimp patties, resulting from effects of compositional parameters, are presented in Table 7. Four regression equations accounted for almost 98% of the variance ($R^2_A \geq 0.973$, Table 7) and were highly significant ($P < 0.001$). The validity of each regression was based on a low coefficient of variability (≤2.27%, Table 7) and a normal residual distribution. F tests for coefficient of regression equation terms (Table 7) are also significant ($P \leq 0.075$) for four equations. However, F values for lack of fit for four equations (Table 8) were not significant ($P \geq 0.5$). All of the above findings suggest that a regression equation has high accuracy with respect to overall prediction.

Since the response of Instron springiness values is essentially a function of mixture variables, the relative effect of any single mixture variable on the Instron springiness of cooked shrimp patties is dependent upon the other variables present in the mixture. Therefore, the importance of any single variable is a local issue, because the effect of changing that variable is dependent upon the amount present and the relative proportions of the other variables in the composition.

One way to measure the relative importance of a mixture variable at a fixed level in the composition is to examine the change in response for a small decrease in that variable, when the other variables of the mixture are kept in the same relative

Table 7—Regression equations for effects of compositional parameters of Instron springiness of cook shrimp patties

Terms of regression equation	−2°C, 2 min[a]		4°C, 2 min		−2°C, 6 min		4°C, 6 min	
	Regression coefficient	F Test	Regression coefficient	F Test	Regression coefficient	F Test	Regression coefficient	F Test
Percent shrimp (X_1)[b]	0.08318	110.67	0.0848	140.11	0.0842	76.65	0.0952	128.67
Percent ISP (X_2)[c]	5.0378	13.69	4.4589	13.06	4.6367	7.83	6.6680	21.29
Percent NaCl (X_3)	−30.6235	10.29	−31.7074	13.43	−35.1895	9.18	−28.1723	7.73
Percent STP (X_4)[d]	632.5365	6.55	740.2351	10.93	763.7447	6.45	545.0071	4.32
$X_1 X_2$	−0.0544	11.71	−0.0478	11.01	−0.0512	6.71	−0.0736	19.03
$X_1 X_3$	0.3197	10.34	0.3310	13.50	0.3667	9.19	0.2948	7.81
$X_1 X_4$	−6.3815	6.59	−7.4750	11.01	−7.7065	6.49	−5.5054	4.36
$X_2 X_3$	0.2638	6.18	0.2792	10.02	0.3216	7.38	0.2126	4.24
$X_2 X_4$	−6.1897	7.35	−7.1718	10.11	−7.4634	6.07	−5.2639	3.97
$X_3 X_4$	−6.0805	6.08	−7.1065	10.11	−7.2898	5.90	−5.1919	3.93
Regression F value	579.43		167.57		106.55		137.16	
R_A^2[e]	0.982		0.986		0.973		0.981	
CV[f] (%)	1.898		1.668		2.275		1.990	

[a] Frozen block of shrimp tempered to indicated temperature was cut mixed with ISP, NaCl and STP for indicated time.
[b] $X_1 + X_2 + X_3 + X_4 = 100\%$.
[c] Isolate soy protein
[d] Sodium tripolyphosphate
[e] Adjusted R-squared (Snee, 1971)
[f] Coefficient of variability

proportion. This is a similar concept to using the gradient surface to aid in the interpretation of response surfaces (Cornell and Ott, 1975; Snee, 1975). Mathematically, the directional derivative (D_a) in the direction which corresponds to the decrease in one component when the others are held in the same proportions can be used to interpret the response. Doing this for each variable at each point tells us which variables are important at what levels in each composition. Further, the D_a correspond to the direction of change in response surface when the percentage of one of the variables in the mixture is changed. Therefore, the D_a can provide information with a four-components mixture that would be difficult to obtain from marginal contour plots.

The D_a for some particular mixture components were computed and are summarized in Table 9. The magnitude of D_a, which is a measure of how fast the surface is changing, showed that D_a for STP are the highest values among the four components studied. This indicated that STP is the most sensitive component which causes the change in response (springiness). Specifically, if STP is fixed at a high level (0.5%), a subsequent decrease in STP causes a large decrease in response (shown by high negative D_a for STP at treatment No. 1, 3, 5, 7, and 11 in Table 9). Whereas at a low STP level (0.1%), a subsequent decrease in STP tends to cause an increase in response (high positive D_a at treatment No. 2, 4, 6, 8, and 12 in Table 9. At moderate level (0.3%), STP is generally not the most sensitive component (small D_a at treatment No. 9, 10, 13, 14, and 15 in Table 9).

The positive D_a are associated with high levels of NaCl (3%) indicating that, if NaCl is fixed at 3%, a subsequent decrease in NaCl causes an increase in response surface. Conversely, negative D_a are associated with low levels (1%) of NaCl indicating that a subsequent increase in NaCl causes a further increase in response. These findings therefore suggest that the optimum level of NaCl is at 2% which is within the range of NaCl investigated.

The hydration (Lee and Toledo, 1976) and swelling (Love, 1968; Ahmed et al., 1973) effect of NaCl and STP can change the water-holding capacity of fish and shrimp. The binding mechanism which actually determines the textural characteristics of comminuted products is a complex system. Schnell et al. (1970) reported that the mechanism of binding turkey meat is a summation of water-holding capacity, protein solubility and cell disruption. This suggestion supports our observation of the effect of the different optimum levels of STP at high and low levels of ISP (Fig. 2A and 2C). Even though STP is the most sensitive component among the four components at the same percentage change, the proportion of STP in the total mixture is relatively small (≤0.5%). Therefore, a 5% of

Table 8—Sum of squares of lack of fit and pure error for regression equations used to predict the compositional parameters effect on Instron springiness values for cooked shrimp patties

Source	Degree of freedom	Sum of squares			
		−2°C[a] 2 min	4°C 2 min	−2°C 6 min	4°C 6 min
Lack of fit	5	0.0948	0.0759	0.0897	0.1508
Pure error	5	0.1696	0.1412	0.3017	0.1470

[a] Frozen block of shrimp tempered to −2°C was cut for 1 min, then ISP, NaCl and sodium tripolyphosphate were added and mixing continued for an additional minute.

Table 9—The response (springiness) directional derivatives for regression equation obtained from mixtures prepared using mixing conditions at 4°C for 2 min

Treatment no.	Mixture composition (%)				Response directional derivatives			
	Shrimp	ISP[a]	NaCl	STP[b]	Shrimp	ISP	NaCl	STP
1	86.5	10.0	3.0	0.5	0.40	−0.52	0.60	−2.78
2	86.9	10.0	3.0	0.1	0.20	−0.43	0.58	1.71
3	88.5	10.0	1.0	0.5	0.63	−0.50	−0.40	−2.68
4	88.9	10.0	1.0	0.1	0.41	−0.41	−0.41	1.77
5	91.5	5.0	3.0	0.5	0.01	−0.18	0.51	−1.47
6	91.9	5.0	3.0	0.1	−0.20	−0.09	0.50	2.92
7	93.5	5.0	1.0	0.5	0.34	−0.16	−0.45	−1.38
8	93.9	5.0	1.0	0.1	0.09	−0.07	−0.46	2.97
9	87.7	10.0	2.0	0.3	0.41	−0.46	0.08	−0.49
10	89.2	7.5	3.0	0.3	0.07	−0.31	0.53	0.10
11	90.0	7.5	2.0	0.5	0.39	−0.33	0.06	−2.07
12	90.4	7.5	2.0	0.1	0.17	−0.24	0.05	2.35
13	91.2	7.5	1.0	0.3	0.32	−0.29	−0.44	0.17
14	92.7	5.0	2.0	0.3	0.05	−0.12	0.02	0.71
15	90.2	7.5	2.0	0.3	0.24	−0.29	0.05	0.14

[a] Isolated soy protein
[b] Sodium tripolyphosphate

ISP increase resulted in the different optimum STP levels because of the relatively smaller change (0.4%) of STP levels.

With one exception, the D_a (Table 9) in the direction of subsequently decreasing level of shrimp are positive, and the D_a in the direction of subsequently decreasing level of ISP are negative. This indicates that it is generally better to increase ISP and decrease the amount of shrimp. Isolated soy protein is hydrophilic and would therefore be expected to absorb and retain water during cooking (Wolf, 1970). The increase in springiness of cooked shrimp patties, which corresponds to an increase in ISP level, could result from the increase of water-holding capacity of ISP and the interaction strength between ISP and extractable myofibrillar protein from comminuted shrimp (Soo and Sander, 1977b). The water-holding capacity of ISP and physical protein-protein interactions could therefore generate the scanning electron microscopy spongy microstructure of shrimp-ISP mixture, and result in the higher springiness of cooked shrimp patties (Soo et al., 1978). However, the shrimp-ISP mixture consistency (softness), which actually determines the extruder performance, decreases as the ISP levels are increased (Soo and Sander, 1977b). To make a target extruded shrimp weight of 7g, previous investigations (Soo and Sander, 1977b) showed that mechanical formation of a shape is difficult above ISP levels of 12% because of low consistency values (harder) of shrimp-ISP mixture as measured by penetrometer.

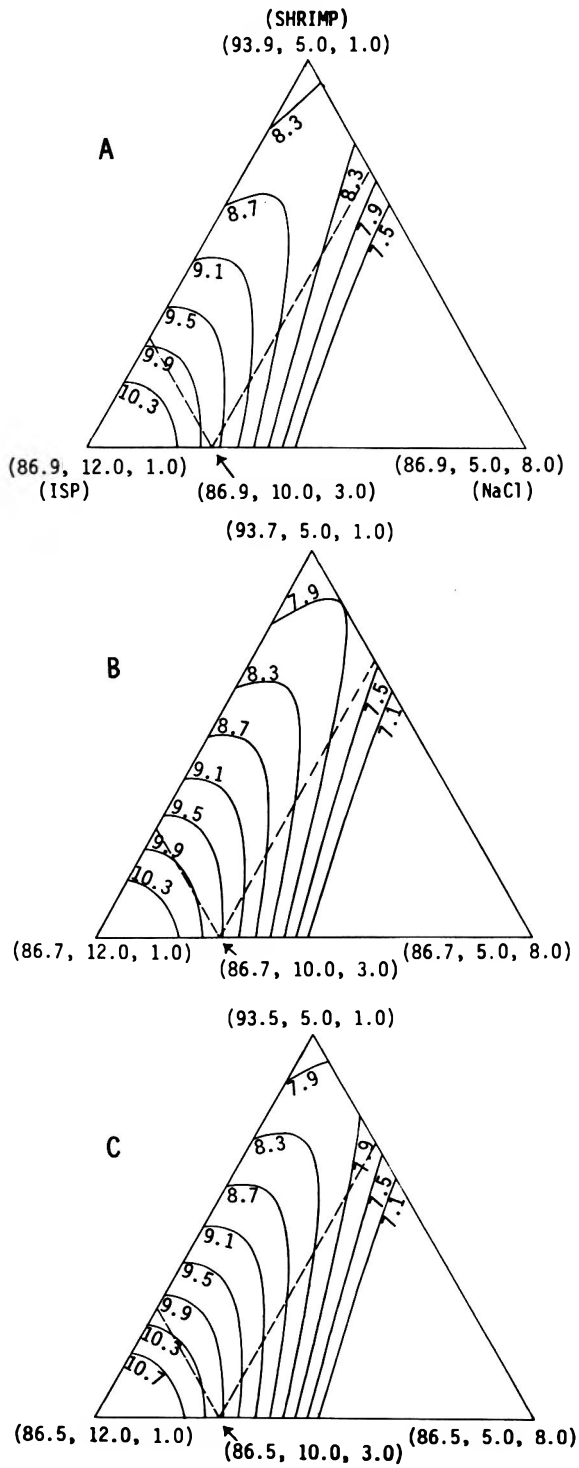


Fig. 1—Contour plots of predicted Instron springiness of cooked shrimp patties prepared using mixing conditions at 4°C for 2 min: (A) Sodium tripolyphosphate (STP) fixed at 0.1%; (B) STP fixed at 0.3%; (C) STP fixed at 0.5%.

Among three comminuting and mixing methods studied (silent cutter, grinder/mixer, and upright paddle mixer methods), Soo and Sander (1977b) reported that the use of the silent cutter method results in the highest values for textural characteristics (hardness, springiness and cohesiveness) in cooked shrimp patties.

Using the silent cutter, the mixing times and temperatures are the two major processing contributions to the textural changes in cooked shrimp patties. Data summarized in Table 5 showed the effect of mixing time and temperature on the textural changes of cooked shrimp patties. The effect of these two processing variables can be obtained from F tests (Table 6) of regression equation A. F test, which is a measure of how significant the variable is in the regression equation, indicated that the value for mixing temperature (7.88) was much higher than the value for mixing time (0.095). Mixing temperature thus has a greater effect on textural characteristics than does mixing time. In these studies, all comminuting mixing trials were carried out in a temperature controlled (4°C) room. The increase of temperature in shrimp mixtures by extensive mixing time (6 min) is too small (a 1°C increase) to cause a textural change. The nonsignificant F test for mixing time (Table 6) supports this finding. However, the statistically significant ($P < 0.025$) F test for mixing temperature (Table 6) indicated that the contributions of using frozen (−2°C) and unfrozen (4°C) shrimp for mixing significantly affected the textural changes of cooked shrimp patties. Using unfrozen shrimp for mixing resulted in better rehydration of ISP and mixing uniformity of the complete shrimp-ISP mixture; consequently, a higher Instron springiness was obtained for cooked shrimp patties.

In view of the interaction effect of compositional and processing parameters, the optimum processing and compositional variables required for the texture of cooked shrimp patties can be obtained from the regression equation A. In order to consider all the necessary constraints and the concave function (i.e., equation A) which defines response surface, a nonlinear programming routine (Cohen and Stein, 1976) was used which has been suggested by McLean and Anderson (1966) to determine the optimum treatment combination.

The solution to the equation A indicates that the optimum treatment combination is to use a mixture of 87.53% of Alaskan shrimp, 10.0% of ISP, 1.97% of NaCl, and 0.5% of STP, and then mix at 4°C for 2 min. The predicted Instron springiness for this optimum combination is 10.29 mm. This value agrees with the previous discussion (Fig. 1 and 2).

In conclusion, we have developed an experimental design using the designs of Scheffé (1963) and McLean and Anderson (1966) to evaluate the textural changes of fabricated shrimp resulting from the processing and compositional parameters with the constraints on mixture components. Regression equations, based on a knowledge of the processing (mixing times and temperatures) and compositional (Alaskan shrimp, ISP,

NaCl, and STP) parameters, were developed. Predictions of the textural changes of fabricated shrimp resulting from the processing and compositional parameters can be accurately obtained using these equations.

There is not a significant interaction effect between the compositional and processing parameters. In the processing parameters studied, mixing temperature has a greater effect on textural characteristics than does mixing time.

The D_a of the response surface which was generated from the equation and the contour plots of this estimated surface were used to examine the effect of the compositional parameters on response. Among the four components studied, STP causes the greatest change in response. The optimum level of NaCl for springiness of cooked shrimp patties is 2%, whereas the optimum STP level is dependent upon the ISP level used. Springiness of cooked shrimp patties increases with increasing ISP and decreasing shrimp levels in the mixture.

A nonlinear programming routine was used to determine the effect of optimum processing and compositional parameters on the textural changes of cooked shrimp patties. The calculated optimum processing and compositional parameters for the fabricated shrimp agree with the measured values.

The developed experimental designs and the procedures used for data analysis can be applied to study the effect of processing and compositional parameters on texture. More specifically, the developed design could be used to study the contribution of compositional parameters including the use of the different geographic sources of shrimp, binding matrix agents, and processing parameters (time, temperature) to changes in fabricated shrimp texture.

REFERENCES

Ahmed, E.M., Mendenhall, U.T. and Koburger, J.A. 1973. Modification of cocktail shrimp texture. J. Food Sci. 38: 356.

Box, G.E.P. and Wilson, K.B. 1951. On the experimental attainment of optimum conditions. J. Roy. Stat. Soc. 13: 1.

Cohen, C. and Stein, J. 1976. LEMKE program. In "Mult. Purpose Optimization System, Version 3." Northwestern U., Evanston, IL.

Cornell, J.A. and Ott, L. 1975. The use of gradients to aid in the interpretation of mixture response surfaces. Technometrics 17(4): 409.

Garnatz, G., Volle, N.H. and Deatherage, F.E. 1949. Processing of shrimp. U.S. Patent 2,488,184.

Hare, L.B. 1974. Mixture designs applied to food formulation. Food Technol. 28(3): 50.

Lee, C.M. and Toledo, R.T. 1976. Factors affecting textural characteristics of cooked comminuted fish muscle. J. Food Sci. 41: 391.

Love, R.M. 1968. Histological observations on the texture of fish muscle. In "Rheology and Texture of Foodstuffs," p. 120. S.C.I. Monograph No. 27, London.

Meyer, R. 1971. "Response Surface Methodology." Allyn and Bacon, Inc., Boston.

McLean, R.A. and Anderson, V.L. 1966. Extreme vertices design of mixture experiments. Technometrics 8(3): 447.

Sander, E.H., Soo, H.-M. and Mindham, D.R. 1976. The shape of things to come. Food Engr. June: EF-24.

Scheffé, H. 1963. The simplex-centroid design for experiments with mixtures. J. Roy. Stat. Soc., Series B 25: 235.

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

Shults, G.W. and Wierbicki, E. 1972. Process for stabilizing structure of ground meat. U.S. Patent 3,642,500.

Shults, G.W., Russell, D.R. and Wierbicki, E. 1972. Effect of condensed phosphates on pH, swelling and water-holding capacity of beef. J. Food Sci. 37: 860.

Snee, R.D. 1971. Design and analysis of mixture experiments. J. Quality Technol. 3(4): 159.

Snee, R.D. 1975. Discussion: The use of gradients to aid in the interpretation of mixture response surfaces. Technometrics 17(4): 425.

Soo, H.-M. and Sander, E.H. 1977a. Prediction of sensory response to textural parameters of breaded shrimp shapes using Instron texture profile analysis. J. Food Sci. 42: 163.

Soo, H.-M. and Sander, E.H. 1977b. Textural and mechanical shaping characteristics of comminuted shrimp-binding matrix agent compositions. J. Food Sci. 42: 1522.

Soo, H.-M., Davis, E.A. and Sander, E.H. 1978. Application of scanning electron microscopy to texture definition in natural and fabricated shrimp. J. Food Sci. 43: 202.

USDA. 1975. Meat and Poultry Regulations. Animal and Plant Health Inspection Service, U.S. Dept. of Agriculture, Washington, D.C. Sec. 318, 7(4).

Webb, N.B., Howell, A.J., Barbour, B.C., Monroe, R.J. and Hamann, D.D. 1975. Effect of additives, processing techniques and frozen storage on the texture of shrimp. J. Food Sci. 40: 322.

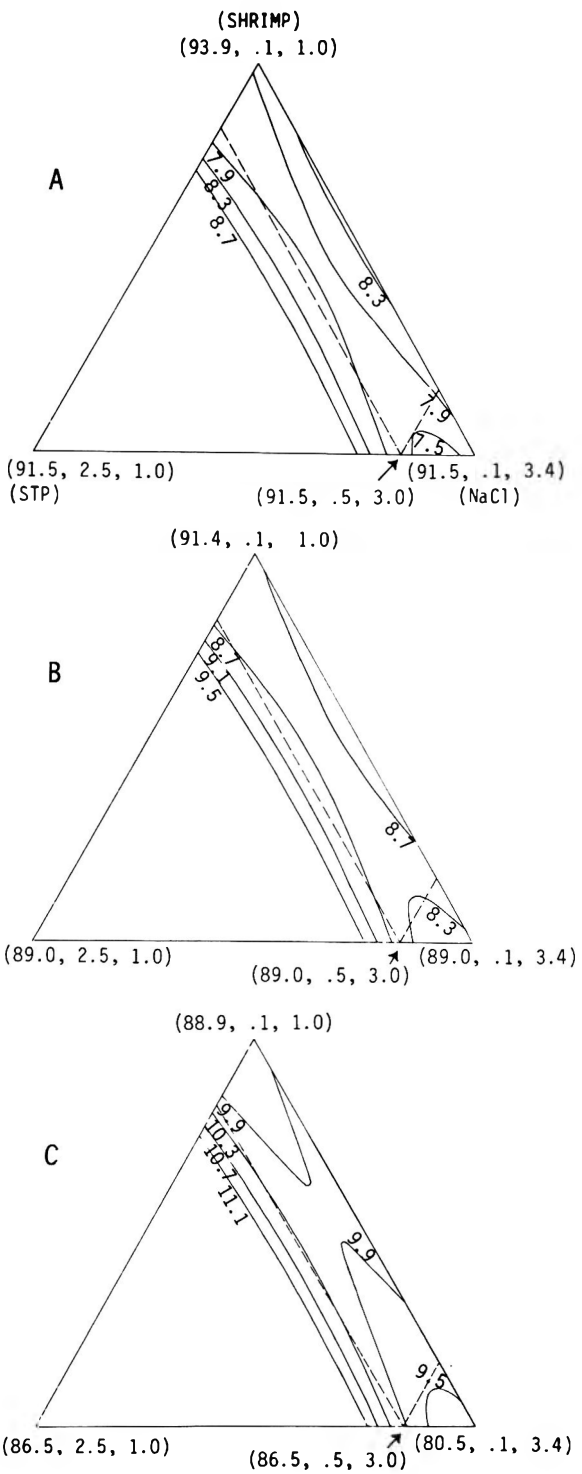


Fig. 2—Contour plots of predicted Instron springiness of cooked shrimp patties prepared using mixing conditions at 4°C for 2 min: (A) Isolated soy protein (ISP) fixed at 5%; (B) ISP fixed at 7.5%; (C) ISP fixed at 10.0%.

Wolf, W. 1970. Soybean protein: Their functional, chemical and physical properties. J. Agr. Food Chem. 18: 969.

Ms received 9/16/77; revised 2/6/78; accepted 2/15/78.

Scientific Journal Series Paper No. 10,024, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

The authors thank Professor David V. Hinkley, Dept. of Applied Statistics, for his review of this manuscript, and Dr. David R. Thompson for his helpful suggestions in conducting experimental designs. The authors also with to thank New England Fish Company for their contribution of shrimp, and the Computer Center, Univ. of Minnesota, for providing computer time used in this study.

EFFECT OF PREPROCESS HOLDING ON THE QUALITY OF CANNED MAINE SARDINES

BOHDAN M. SLABYJ and RUTH H. TRUE

ABSTRACT

The effect of preprocess holding conditions (3 and 12% brine at 7.2 and 0.6°C) on the quality of canned sardines (*Clupea harengus*) was examined. The sardines appeared to have a relatively short shelf life and had to be canned within 36 hr when lightly salted (3% brine) and held at 7.2°C. At a lower temperature (0.6°C) and a higher salt concentration (12% brine) the sardines could be held up to 3 days. Low viable bacterial counts detected during storage experiments indicated spoilage not to be primarily due to bacterial activity. Sensory evaluation of the raw sardines could not be used with any accuracy to predict quality of the canned product, because raw sardines, which were still considered acceptable before canning, resulted in an unacceptable product. Of the objective quality tests performed on raw sardines (TMA, TBA, tyrosine and pH) only TMA-N content appeared to possess high accuracy ($P \leq 0.001$) in predicting quality of the canned products.

INTRODUCTION

ABOUT 1 MILLION CASES of sardines (*Clupea harengus*) are packed annually by the Maine industry. Despite improved quality control procedures developed by the Maine Sardine Laboratory, the industry annually experiences substantial losses due to decreased grade of marketable product as well as to substandard and unmarketable product.

Very little information is available in the literature describing the effect of preprocess holding conditions on the quality of canned sardines. Farber (1955) published a graph showing the relationship between storage temperature and fitness of sardines (*Sardinops caerula*) for canning. This investigator indicated that fish held up to 1 wk in brine at 1.7–4.4°C resulted in an acceptable product. However, no information was given on brine concentration or size of the fish used. Sigurdsson (1947) examined various tests as to their suitability for determining the quality of raw sardines (*Clupea harengus*), but the chemical analyses were not related to the quality of the canned product. Quality loss during brine storage of Maine sardines has been investigated by Clark (1959), but this study was restricted to the loss of nitrogenous material. The difficulty encountered in predicting the quality of canned sardines from sensory evaluation of the raw material is apparent from a study reported by MacCullum et al. (1956). These investigators observed that sardines considered organoleptically acceptable prior to canning resulted in a canned product which was not acceptable.

The present investigation was conducted to determine the effect of preprocess holding conditions (time, temperature and brine concentration) on the quality of the canned sardines (*Clupea harengus*) and to evaluate the relation of objective quality tests (TMA, TBA, tyrosine and pH) performed on the raw material to sensory evaluation of the raw and canned fish.

Authors Slabyj and True are with the Dept. of Food Science, Life Science and Agriculture Experiment Station, University of Maine, Orono, ME 04473.

0022-1147/78/0004-1172$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

MAINE SARDINES, caught in early March in Sheepscott Bay by purse seining, were used in the present study. These were salted lightly (0.5 kg salt/27 kg fish) when pumped into the boat's hold which was subsequently flooded with seawater at the dock. The fish were then transferred to an insulated tank truck, arriving at a processing plant 13 hr after catch. At the plant the sardines were flumed with bay water into metal holding tanks from which 300 kg of sardines were obtained. The sardines were placed in four plastic 95 liter barrels and transported to the laboratory arriving 2 hr later with an internal temperature of 5°C.

Upon arrival, samples were removed for canning and chemical analyses. The remainder was transferred to four 95 liter plastic barrels. Two of these contained 20 liters of 3% and two contained 12% NaCl solution (11 and 42° brine, respectively). One container of each brine concentration was stored at 7.2°C and the other at 0.6°C. At daily intervals samples were removed for canning as well as for chemical, microbiological and sensory tests.

Canning was performed by packing, on the average, 7 sardines with heads and tails removed (without evisceration) in 405 × 301 × 014.5 in. rectangular, aluminum cans (3¾ oz net wt). These were steamed for 30 min, and then drained and cooled for 30 min. Fifteen ml of soybean oil were added, the cans closed without vacuum and retorted at 116°C for 50 min.

Sensory evaluations of raw sardines were performed at daily intervals for 3 days by 8 judges, the majority of whom had previous experience. The samples, representing 4 different treatments, were coded and presented in a randomized block design, with three replications. There were at least four fish per treatment; two of these were opened, exposing abdominal cavity and muscle along the backbone for examination. Evaluation included external appearance (eyes, gills, skin and belly flaps), flesh (color of muscle near backbone, belly flaps, abdominal cavity and color of kidney blood), texture (back muscle, belly flaps, and tail) and odor (whole fish, gills, abdomen and flesh). Sardines frozen in a blast freezer at −34°C on day zero and stored at −40°C until the day prior to their use were thawed overnight in a refrigerator at 1.5°C and used as external reference samples. The panelists were informed of the history of the reference samples. Scoring was based on a 6-point descriptive scale with a score of 5 representing the best and 0 the poorest quality fish. A score of ≤2.5 was considered unacceptable.

Sensory evaluation of canned sardines was performed by 9 judges, the majority of whom had previous experience. The cans were stored at 22°C for 6 wk before examination. The samples representing 13 treatments (one can per treatment) were coded and presented in a randomized block design in three separate sessions. Two external reference samples (an excellent and a poor quality product) were used. The panelists were instructed to evaluate appearance, flesh cracks, ventral breaks, texture and odor on a 6-point scale with a score of 5 representing the best and 0 the poorest quality fish. A score of ≤2.5 was considered unacceptable.

Microbiological analyses were performed on duplicate composite samples of cleaned raw fish (head, tail and viscera removed) prepared by homogenizing 50g of tissue in 150 ml of phosphate buffer (Butterfield, 1932). Appropriate serial decimal dilutions were prepared with the above buffer and used for inoculating duplicate pour plates of Plate Count Agar (Difco.) containing 3% NaCl (PCA-3) and for surface inoculating duplicate PCA-3 and PCA plates. All inoculated plates were incubated at room temperature (22°C) for up to 5 days at which time the colonies were counted.

For chemical analyses sardines were cleaned (head, tail and viscera removed) briefly washed with tap water to remove blood, and four 200g composite samples weighed. Two 200g samples were used the same day for pH determination and assay of thiobarbituric acid number (TBA) and the other two were frozen in Whirl Pack bags at −34°C and stored at −40°C. The frozen samples were analyzed within 2 wk for trimethylamine (TMA) and tyrosine.

All chemical analyses were performed on two subsamples drawn from each of the two composite (200g) samples after these were passed three times through a meat grinder. The frozen samples were thawed overnight in a refrigerator at 1.5°C before being ground as above. Trichloroacetic acid (TCA) extract was obtained by adding 30 ml H_2O to 30g of ground tissue and homogenizing with 60 ml of 10% TCA at room temperature (Bradley and Bailey, 1940). The homogenate was filtered through a double layer of Whatman #1 filter paper using a Buchner funnel and suction. The clear TCA extract was used to determine TMA-N (Dyer, 1945), TBA value (Vyncke, 1970) and tyrosine (Folin and Ciocalteau, 1927). The TBA value (malonaldehyde equivalent) was calculated by dividing 1,1,3,3-tetraethoxypropane equivalent by 3.06. The pH was determined after thoroughly mixing 30g of ground muscle tissue with 30 ml of distilled water (Love and Muslemuddin, 1972) using a Corning Model 12 pH meter with an expanded scale.

Analysis of variance was performed for sensory scores and chemical data to determine the effect of preprocess storage conditions (treatment effect) and Duncan's Multiple Range Test was used (Duncan, 1955) to detect significant differences among the treatment means. Treatment × judge mean square was used as the error term to test for significant differences among the treatment means in the sensory analyses (Bliss, 1960).

Pooled sensory scores (quality) for raw and canned material were obtained by adding individual scores for each attribute and dividing the total by the number of attributes involved. Correlation coefficients between objective and subjective tests as well as analysis of covariance and stepwise multiple linear regression tests were performed wherever appropriate. In all cases significance of statistical analysis was reported at the 1% level ($P \leq 0.01$) except where indicated.

RESULTS & DISCUSSION

ANALYSIS OF VARIANCE of the sensory scores for appearance, flesh, texture and odor of raw sardines revealed significant differences among sensory mean scores due to preprocess holding conditions (each sampling was handled as a separate treatment). Duncan's Multiple Range Test revealed considerable ($P \leq 0.01$) overlap among sensory score means for all attributes examined of sardines stored under different conditions (Table 1). However, the mean scores reveal a definite trend of quality loss with storage conditions.

As far as appearance is concerned, only sardines held 3 days at 7.2°C in 3% brine received a score of <2.50, which was significantly different from remaining scores, indicating their unacceptability (Table 1). Samples held at 7.2°C were considered spoiled on the third day of storage (regardless of brine concentration used) when evaluating quality of flesh. However, these samples were not significantly different from other samples receiving a mean score of >2.50, as revealed by the Duncan Multiple Range Test. Texture scores indicated softening of the flesh with increased storage, although no samples were rejected based on this characteristic within the storage period tested. Only sardines held 3 days at 7.2°C in 3% brine developed strong off odors and were considered unacceptable for canning. MacCallum et al. (1956) also observed discrepancy between different sensory attributes of raw sardines during storage.

Table 1—Mean sensory score and results of Duncan's Multiple Range Test for raw sardines (Clupea harengus) stored in 3 and 12% brine at 7.2 and 0.6° C[a]

Days stored	7.2°C		0.6°C	
	3% brine	12% brine	3% brine	12% brine
		Appearance		
1	4.42 a	3.67 abcd	4.33 ab	3.83 abc
2	3.96 abc	3.46 abcd	4.08 abc	3.33 d
3	2.46 e	2.83 d	3.38 cd	2.83 d
		Flesh		
1	3.63 ab	3.79 ab	3.71 ab	3.88 ab
2	3.83 ab	3.63 ab	4.13 a	3.38 abc
3	2.50 c	2.46 c	3.33 abc	2.88 bc
		Texture		
1	4.33 a	3.92 ab	4.17 a	4.21 a
2	3.88 abc	3.79 abc	4.00 ab	3.75 bc
3	2.75 d	2.92 cd	3.04 bcd	3.13 bcd
		Odor		
1	4.42 a	4.25 a	4.21 a	4.20 a
2	3.71 ab	3.63 ab	3.69 ab	3.67 ab
3	0.88 d	2.79 c	3.33 bc	3.17 c

[a] Average scores not showing the same letters are significantly different at the 1% level ($P \leq 0.01$). Values below the solid lines are ≤ 2.50 and considered unacceptable.

The observed overlap ($P \leq 0.01$) between sensory mean scores (Table 1) is in part due to the fact that fish held in brine does not undergo changes which are known to occur with fish held in ice or under refrigeration (Shewan et al., 1953). In the present study the eyes were not observed to become opaque at any time, although they tended to sink, especially when the fish was held in 12% solution, while gills became bleached after 24 hr of storage regardless of storage conditions. More noticeable changes were observed when evaluating condition of flesh. In this case reddening of the dorsal protion of the abdominal cavity was observed and the kidney blood darkened with time. With prolonged storage, belly flaps also appeared to become sensitive to physical damage.

Analysis of variance of the sensory scores for appearance, flesh cracks, ventral breaks, texture and odor of the canned sardines revealed significant differences among treatment mean scores. Examination of the mean sensory scores for appearance, flesh cracks and ventral breaks (Table 2) along with Duncan's Multiple Range Test revealed distinct quality loss with preprocess holding conditions. There was a clear cut difference in sensory score means between acceptable (>2.50) and unacceptable (≤2.50) canned sardine samples. Fish held at 7.2°C for 2 days or longer, regardless of the brine concentration used, resulted in a canned product that was not acceptable (appearance, flesh cracks and ventral breaks). Similarly, sardines held 3 days at 0.6°C in 3% salt solution did not result in an acceptable product as judged by these criteria.

It is interesting to note that all canned samples in the present investigation were considered acceptable as far as texture (except sardines held in 3% brine at 7.2°C for 2 days only) and odor were concerned, including sardines which had strong off odors prior to canning (compare Tables 1 and 2). This observation is not too surprising, in view of the fact that sardines were steamed and drained prior to sealing and retorting. Although, normally, in our laboratory it has been observed that fish which had strong odors prior to canning, tended to retain some of the odor in the canned product. McLay (1968) also observed that taste panelists could not distinguish rancidity among cans prepared using fresh fish and fish held up to 10 days at 4°C. No odor evaluation of the fish prior to canning was reported in that study.

Pooling sensory scores of various sensory attributes examined is desirable as an index of the overall quality of fish and its applicability has been proven by the sardine industry (Clark, 1959). The data presented in Table 3 show the effect of preprocess holding conditions on the overall quality (pooled sensory scores) of the raw and canned sardines. It is apparent that the pooled sensory scores of ≤2.50 agree quite closely with unsatisfactory score for appearance, flesh and odor of raw sardines (Table 1) and for appearance, flesh cracks and ventral breaks of canned sardines (Table 2). However, raw sardines held 3 days at 7.2°C in 12% brine had a pooled sensory score of 2.75, yet were considered unacceptable as far as sensory evaluation of flesh was concerned, receiving a score of 2.46. Similarly, sardines held 3 days at 0.6°C in 3% brine had a pooled sensory score of 2.53 for the canned product, but were considered unacceptable based on appearance, flesh cracks and ventral breaks, receiving a score <2.50. This discrepancy is the result of the above samples receiving high

Table 2—Mean sensory score and results of Duncan's Multiple Range Test for canned sardines (Clupea harengus) stored in 3 and 12% brine at 7.2 and 0.6°C prior to canning[a]

Days stored	7.2°C		0.6°C	
	3% brine	12% brine	3% brine	12% brine
		Appearance		
0	4.59 a	–	–	–
1	3.74 bcd	3.56 cd	4.19 abc	4.41 abc
2	2.04 ef	1.96 ef	3.41 d	4.19 abc
3	1.70 f	1.52 f	2.48 e	3.26 d
		Flesh cracks		
0	4.67 a	–	–	–
1	3.59 bc	3.59 bc	3.96 ab	4.00 ab
2	1.74 de	1.74 de	3.04 c	4.04 ab
3	1.30 e	1.19 e	2.11 d	2.93 c
		Ventral breaks		
0	4.44 a	–	–	–
1	3.81 ab	3.63 bc	3.74 ab	4.26 ab
2	1.44 f	1.22 f	3.00 cd	4.11 ab
3	1.15 f	0.85 f	2.15 e	2.67 de
		Texture		
0	3.19 bcdef	–	–	–
1	3.11 cdef	3.30 bcde	3.70 abcd	4.33 a
2	2.26 f	3.22 bcde	2.96 def	4.00 abc
3	3.07 def	2.85 def	2.74 ef	4.07 ab
		Odor		
0	4.63 a	–	–	–
1	4.07 abc	3.89 abcd	4.30 ab	4.30 ab
2	3.37 cde	3.04 e	4.11 abc	3.78 bcde
3	3.26 de	3.04 e	3.19 de	3.78 bcde

[a] Average scores not showing the same letters are significantly different at the 1% level ($P \leq 0.01$). Values below the solid lines are ≤2.50 and considered unacceptable.

scores for other attributes examined. In order to overcome this problem, weighted averages should perhaps be employed. In practice, however, the "limiting rule" is applied which specifies that if a product is graded substandard in any one of the attributes examined, the overall quality automatically becomes substandard regardless of the overall score.

It is important to note, that the quality of sardines before and after canning does not coincide. Thus raw sardines held 2 days at 7.2°C (regardless of brine concentration) as well as those held 3 days at 0.6°C in 3% brine were considered acceptable as far as each sensory attribute was concerned (Table 1). However, this fish resulted in a canned product which had excessive flesh cracks and ventral breaks and was not considered acceptable (Table 2). This observation indicates the difficulty that can be encountered in attempting to predict the quality of canned sardines based on sensory evaluation of the raw material. Similar discrepancy in quality between raw and canned sardines was observed by MacCallum et al. (1956).

Results of objective quality tests (TMA, TBA, tyrosine and pH) for raw sardines stored in brine are given in Table 4. Analysis of variance for these data revealed significant differences among samples, reflecting preprocess holding conditions. Duncan's Multiple Range Tests were performed on all the data, except in case of TMA content, where samples with TMA-N of > 1.0 mg/100g of tissue were excluded in order to detect small significant differences among treatment means.

TMA content of freshly caught sardines observed in the present investigation is relatively lower (threefold) than the concentration reported by Sigurdsson (1947) studying the same species. Sigurdsson determined TMA concentration using a microdiffusion procedure developed by Beatty and Gibbons (1937), which admittedly could include other volatile bases. However, Dyer (1945) showed rather close agreement in TMA content using colorimetric, microdiffusion and distillation procedures for cod and haddock. It would appear, therefore, that the observed difference in TMA content between these two studies may be due to variation in season or fishing grounds (Shewan and Jones, 1957; Takada and Nishimoto, 1958; Castell et al., 1961b).

In the present study TMA at first increased very gradually in raw sardines but at the stage of incipient spoilage increased logarithmically. It should be pointed out that there was no corresponding increase in viable bacterial counts (Table 5). Thus it appears that, in the present study, TMA production is not primarily due to bacterial activity. In fact, Castell (1970) indicated that heavy metals and various haem pigments can contribute to the reduction of TMA oxide.

Beatty and Gibbons (1937), working with cod, noticed that off odors began to appear at 0.95–1.45 mg TMA-N/100g and they were quite definite at 2.37 mg/100g of tissue. Sigurdsson (1947) recommended these values to be increased to 1.18–1.66 and 2.84 mg TMA-N, respectively, for raw sardines. In the present study the mean odor score of sardines with 1.68

Table 3—Pooled sensory score means (quality) of raw and canned sardines (Clupea harengus)[a]

Days stored	7.2°C		0.6°C	
	3% brine	12% brine	3% brine	12% brine
		Raw sardines		
1	4.20	3.91	4.11	4.03
2	3.84	3.63	4.04	3.53
3	2.15	2.75	3.27	3.00
		Canned sardines		
0	4.30	–	–	–
1	3.66	3.59	3.98	4.26
2	2.17	2.24	3.30	4.02
3	2.10	1.89	2.53	3.34

[a] Values below the solids lines are ≤2.50 and considered unacceptable

Table 4—Mean values and results of Duncan's Multiple Range Test of trimethylamine (TMA), thiobarbituric acid (TBA), tyrosine and pH for sardines (Clupea harengus) held in 3 and 12% brine at 7.2 and 0.6°C[a]

Days stored	7.2°C		0.6°C	
	3% brine	12% brine	3% brine	12% brine
		TMA-N (mg/100g muscle)		
0	0.296 a	–	–	–
1	0.394 bc	0.396 bc	0.352 ab	0.371 ab
2	1.681 –	0.592 e	0.476 cd	0.345 ab
3	23.418 –	0.715 f	0.825 g	0.508 d
		TBA value (mg malonaldehyde/kg muscle)		
0	0.224 a	–	–	–
1	0.598 a	0.726 a	0.502 a	1.175 abc
2	1.933 bcd	2.232 d	1.047 ab	2.115 cd
3	1.740 bcd	3.833 e	2.531 d	5.000 f
		Tyrosine equivalent (mg/100g muscle)		
0	31.2 ab	–	–	–
1	29.7 a	33.9 abc	30.3 a	41.6 abc
2	36.0 abc	38.9 abc	39.9 abc	37.1 abc
3	41.9 abc	49.0 c	38.9 abc	43.2 abc
		pH		
0	5.87 a	–	–	–
1	6.58 bc	6.48 bc	6.57 bc	6.48 bc
2	6.61 c	6.51 bc	6.65 c	6.54 bc
3	6.61 c	6.39 b	6.58 bc	6.45 bc

[a] Average values not showing the same letters are significantly different at the 1% level ($P \leq 0.01$). TMA-N values >0.550 mg/100g of tissue were separated with a solid line to set off unacceptable samples as judged by appearance, flesh cracks and ventral breaks.

Table 5—Mean aerobic plate count for sardines (Clupea harengus) held in 3 and 12% brine at 7.2 and 0.6° C

Days stored	Total viable count/g			
	7.2°C		0.6°C	
	3% NaCl	12% NaCl	3% NaCl	12% NaCl
	PCA pour plate			
0	2.1×10^3	–	–	–
1	8.8×10^2	6.2×10^2	1.8×10^3	6.6×10^2
2	8.0×10^3	6.2×10^3	2.7×10^3	3.3×10^2
3	1.5×10^4	7.6×10^3	5.9×10^3	4.5×10^3
	Surface inoculated PCA-3 plate			
0	1.5×10^3	–	–	–
1	1.8×10^3	1.0×10^3	1.2×10^3	1.2×10^3
2	7.3×10^3	6.8×10^3	3.2×10^3	4.7×10^2
3	3.0×10^4	9.9×10^3	5.5×10^3	4.9×10^3
	Surface inoculated PCA plate			
0	1.2×10^3	–	–	–
1	1.2×10^3	5.8×10^2	1.2×10^3	6.0×10^2
2	6.8×10^3	5.4×10^3	2.8×10^3	3.6×10^2
3	1.5×10^4	7.6×10^3	6.0×10^3	3.5×10^3

Table 6—Linear correlation coefficients (r) between objective tests (TMA, TBA and tyrosine) of sardines (Clupea harengus) prior to canning and sensory scores of raw and canned sardines

	TMA 10 means	TBA 12 means	Tyrosine 12 means
Raw sardines			
Appearance	–0.550	–0.720**	–0.764**
Flesh	–0.533	–0.669*	–0.615*
Texture	–0.807**	–0.713**	–0.706*
Odor	–0.750*	–0.417	–0.573
Pooled score	0.075	–0.017	–0.037
	11 means	13 means	13 means
Canned sardines			
Appearance	–0.882**	–0.523	–0.582*
Flesh cracks	–0.905**	–0.549	–0.631*
Ventral breaks	–0.862**	–0.562*	–0.609*
Texture	–0.562	0.072	0.033
Odor	–0.859**	–0.609*	–0.585*
Pooled score	–0.898**	–0.504	–0.556*

* Significant at the 5% level
** Significant at the 1% level

mg TMA-N/100g of fish was not significantly different from the better quality fish, while at 23.41 mg TMA-N content, the odor was distinctly putrid.

Canned sardine samples, which were not acceptable as far as appearance, flesh cracks and ventral breaks are concerned (Table 2), corresponded to all the raw samples which had a TMA-N content of >0.550 mg/100g of tissue. Duncan's Multiple Range Test revealed no significant overlap ($P \leq 0.01$) in TMA values among samples which were acceptable and those considered unacceptable based on appearance, flesh cracks and ventral breaks. Thus, TMA values of the raw sardines appear to be potentially good quality indicators of the canned material as far as flesh cracks and ventral breaks are concerned, although usually TMA is associated with off odors, as discussed above.

The TBA value obtained in the present investigation (Table 4) at the initiation of the experiment was somewhat lower (2.7-fold for light and 3.5-fold for dark muscle) from that reported by Vyncke (1974) for mackerel. It was further observed that the increase in TBA values for frozen mackerel was at a noticeably slower rate than was observed for brined sardines above. This difference may be due to the salting of sardines after catch and to their being held at considerably higher temperatures in brine than was the case for mackerel. In fact, the increase in TBA values with time in raw sardines, in the present investigation, reflects quite clearly ($P \leq 0.01$) the effect of brine concentration used regardless of temperature. Castell et al. (1965) have also observed an increased rate in malonaldehyde production due to the presence of salts. What is interesting, however, is that while mackerel were considered unacceptable due to rancidity at 2.0 mg malonaldehyde/kg of tissue, raw sardines were not noticed to be rancid at 5.0 mg/kg (Tables 1 and 4). This variation in results may be due to different storage conditions, but perhaps equally important is the sensory evaluation procedure (in the present study, no taste testing of raw sardines was involved). The TBA data obtained in the present study for the raw fish appeared to be more consistent than those observed with other marine fishes (Botta and Shaw, 1975; 1976).

Tyrosine values reported in Table 4 are slightly lower than those observed by Sigurdsson (1947) for whole ground sardines and similar to those for sardine fillets. There was an apparent increase in tyrosine value with storage time, indicating proteolytic breakdown of tissue. This resembles data in the literature; however, the observed overlap ($P \leq 0.01$) between mean values obtained preclude the use of this test as an index of incipient spoilage. Perhaps better correlation could have been attained, had special care been taken to remove all kidney blood.

The pH values for sardine flesh observed in the present study are lower than those reported by Sigurdsson (1947). While the latter investigator observed a gradual increase in pH with storage of refrigerated fish, brined sardines, in the present study, exhibited a significant change only within 24 hr with no significant change observed thereafter. Consequently, this parameter appears to have little value in evaluating quality of brined sardines or predicting quality of the canned product.

Total viable aerobic counts for sardines stored in brine are given in Table 5. Surface inoculated PCA-3 plates gave the overall highest viable count. A general reduction of about 13% was observed when pour plates were prepared using PCA-3 medium, while surface inoculation of PCA plates exhibited on the average 24% lower counts. These observations indicate that only a relatively small proportion of the population was heat sensitive, and a slightly larger portion was salt requiring. It is interesting to note that sardines which developed strong off odors during storage (Table 1) exhibited only a 20-fold increase in viable bacterial count during that period. Highlands and Williams (1944) also detected relatively low viable bacterial counts for fish samples obtained from sardine packing plants. Furthermore, these investigators detected a bacterial load of 175 and 620/g on tissue and in viscera, respectively, for sardines held 4 days at room temperature in 8% brine.

Although simple linear correlation coefficients between chemical data (TMA, TBA and tyrosine) and mean sensory scores for raw sardines show significant correlation in various instances (TMA with texture and odor, and TBA and tyrosine with appearance, flesh and texture), this significance is lost when pooling the sensory scores (Table 6). Thus, inability to define quality of raw sardines, using the above chemical tests, is perhaps due to a combination of excessive variability (TBA and tyrosine) in these tests (Table 4) as well as due to relatively large overlap ($P \leq 0.01$) among sensory mean scores for raw sardines (Table 1). Other investigators (Hoogland, 1958; Shewan and Ehrenberg, 1959) also found imprecise relationship between TMA values and sensory scores which was partially due to the lack of better definition of grades as well as due to some variability in TMA values. Castell et al. (1961a), on the other hand, found definite correlation with assigned quality grades, but the degree of correlation was influenced by seasonal variation.

Chemical tests on the raw material (TMA and tyrosine) and sensory scores of individual attributes of the canned product

(appearance, flesh cracks, ventral breaks and odor) exhibited significant correlation and this significance was retained after pooling the sensory scores (Table 6). Stepwise multiple linear regression of the quality of canned fish (pooled sensory scores) with the objective tests (TMA, TBA and tyrosine) and sensory quality of the raw sardines revealed a high significant correlation ($P \leq 0.001$) between TMA and quality of the canned product. Tyrosine values and pooled sensory scores of raw sardines contributed little to the multiple regression, while TBA values had no effect.

The relationship between TMA content of raw sardines and quality (pooled sensory score) of the canned product is shown in Figure 1. This graph was prepared, omitting TMA-N values >0.80 mg/100g of tissue, to indicate accuracy with which raw material can be rejected that could result in an unacceptable product. The above cut-off point was selected, since it is apparent from the data presented (Tables 2 and 4) that a 0.6 mg/100g concentration in raw sardines was associated with a product that was not acceptable. From this figure it can be estimated that for raw material, a TMA-N content of 0.55 mg/100g will be associated with a product which 99% of the time may receive an overall quality score between 2.53 and 3.12. It follows, that at a lower TMA-N content, chances of having sardines not meeting minimum requirements are significantly reduced.

In view of the fact that TMA content may vary in migratory fish as well as exhibit seasonal variation in marine fish, it is essential to evaluate applicability of TMA on a larger scale.

In conclusion, it should be pointed out that, based on these limited tests, sardines have a relatively short shelf life and should be canned within 36 hr, when lightly salted and held at 7.2°C. At a lower temperature (0.6°C) and a higher salt concentration (12% brine) the sardines held up to 3 days resulted in an acceptable product. Although spoilage of fish is known to be primarily due to bacterial activity, microorganisms appeared to contribute little to the quality loss of sardines in these tests. Significant correlation between various attributes of sensory quality and objective chemical tests of raw sardines was detected, but the overall quality of raw sardines cannot accurately be defined because of absence of distinct quality changes in the fish. Sensory evaluation of the raw material appears to hold little promise for predicting quality of the canned product since fish which was considered acceptable before canning resulted in an unacceptable product. TMA content of the raw fish, on the other hand, was highly significant and inversely related to the overall quality of the canned material. Despite the fact that Dyer's (1945) procedure for TMA determination does not distinguish between TMA and dimethylamine content (Castell et al., 1973), the test itself appears to have a high degree of accuracy in predicting quality of canned Maine sardines. In practice perhaps both TMA and tyrosine values should be employed in quality control.

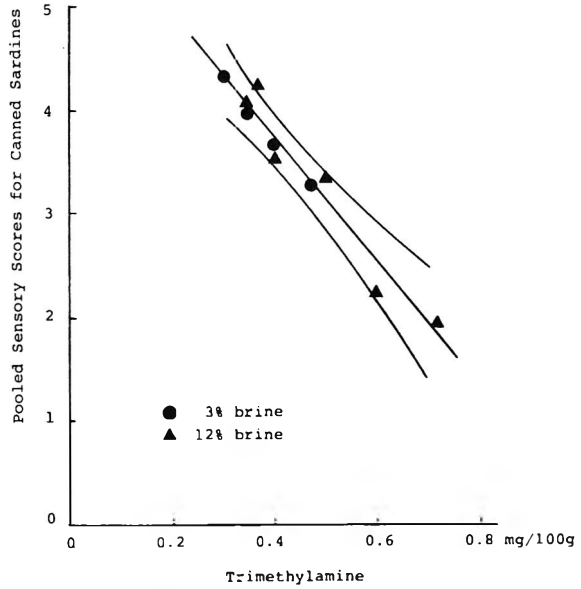


Fig. 1—Relationship between trimethylamine content of raw sardines (Clupea harengus) held in brine and average sensory values of the canned product with confidence intervals at the 99% level.

REFERENCES

Beatty, S.A. and Gibbons, N.E. 1937. The measurement of spoilage in fish. J. Biol. Board Can. 3: 77.

Bliss, C.I. 1960. Some statistical aspects of preference and related tests. Appl. Statistics. 9: 1.

Botta, J.R. and Shaw, D.H. 1975. Chemical and sensory analysis of round head grenadier (Macrourus berglax) stored in ice. J. Food Sci. 40: 1249.

Botta, J.R. and Shaw, D.H. 1976. Chemical and sensory analysis of roundnose grenadier (Coryphaenoides repestris) stored in ice. J. Food Sci. 41: 1285.

Bradley, H.C. and Bailey, B.E. 1940. Estimation of decomposition of fish muscle. Food Res. 5: 487.

Butterfield, C.T. 1932. The selection of a dilution water for bacteriological examinations. J. Bacteriol. 23: 355.

Castell, C.H. 1970. Current status of the TMA test as a measure of spoilage in fish. Fisheries Can. 22(9): 16.

Castell, C.H., Elson, M.F. and Giles, J.G. 1961a. Grading fish for quality. 4. Variation in the relation between trimethylamine values and grades for gutted, trawler-caught Atlantic cod and haddock. J. Fish. Res. Board Can. 18: 303.

Castell, C.H., Greenough, M.F., Rodgers, R.S. and Macfarlane, A.S. 1961b. Spoilage of fish in the vessels at sea. 7. Further studies on seasonal variations in the landed quality of gutted trawler-caught Atlantic cod and haddock. J. Fish. Res. Board Can. 18: 295.

Castell, C.H., MacLean, J. and Moore, B. 1965. Rancidity in lean fish muscle. 4. Effect of sodium chloride and other salts. J. Fish. Res. Board Can. 22: 929.

Castell, C.H., Smith, B. and Dyer, W.J. 1973. Simultaneous measurements of trimethylamine and dimethylamine in fish and their use for estimating quality of frozen-stored gadoid fillets. J. Fish Res. Board Can. 31: 383.

Clark, B.S. 1959. Integration of organized food technology into a system for Maine Sardine production control. Food Technol. 13: 289.

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

Dyer, W.J. 1945. Amines in fish muscle. 1. Colorimetric determination of trimethylamine as the picrate salt. J. Fish Res. Board Can. 6: 351.

Farber, L. 1955. Refrigeration of tuna and sardines by sodium chloride brines. Food Technol. 9: 141.

Folin, O. and Ciocalteau, V. 1927. On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. 73: 627.

Highlands, M.E. and Williams, O.B. 1944. A bacteriological survey of sardine canning in Maine. Food Res. 9: 34.

Hoogland, P.L. 1958. Grading fish for quality. 2. Statistical analysis of the results of experiments regarding grades and trimethylamine values. J. Fish. Res. Board Can. 15: 717.

Love, R.M. and Muslemuddin, M. 1972. Protein denaturation in frozen fish. 12. The pH effect and cell fragility determinations. J. Sci. Food Agric. 23: 1229.

MacCallum, W.A., Dyer, W.J., Curi, S., Simoncic, J.J., Kovacevic, M., Horne, D.C., McNeil, R.J., Krvaric, M. and Lisac, H. 1956. Quality of sardines (Clupea pilchardus, Walb.) held unfrozen and frozen prior to canning. Food Technol. 10: 432.

McLay, R. 1968. The effect of storage prior to processing on the quality of canned herring. J. Food Technol. 3: 361.

Shewan, J.M. and Ehrenberg, A.S.C. 1959. Comparison of certain Scottish and Canadian experiments in respect of grading fish for quality. J. Fish. Res. Board Can. 16: 555.

Shewan, J.M. and Jones, N.R. 1957. Chemical changes occurring in cod muscle during chill storage and their possible use as objective indices of quality. J. Sci. Food Agric. 8: 491.

Shewan, J.M., MacIntosh, R.G., Tucker, C.G. and Ehrenberg, A.S.C. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J. Sci. Food Agric. 4: 283.

Sigurdsson, G.J. 1947. Comparison of chemical tests of the quality of fish. Anal. Chem. 19: 892.

Takada, K. and Nishimoto, J. 1958. Studies on the choline in fish. I. Contents of choline and similar substances in fish. Bull. Jap. Soc. Sci. Fish. 24: 632.

Vyncke, W. 1970. Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. Fette Seifen Anstrichmittel 72: 1084.

Vyncke, W. 1974. Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (Scomber scombrus L). Fette Seifen Anstrichmittel 76: 239.

Ms received 8/25/77; revised 12/8/77; accepted 12/14/77.

One of the authors (BMS) expresses his gratitude to the Maine sardine industry for making samples available for experiments leading to this study. Mr. Wayne Persons' assistance in computer use is gratefully acknowledged.

RELATIONSHIP OF MYOFIBRIL FRAGMENTATION INDEX TO CERTAIN CHEMICAL, PHYSICAL AND SENSORY CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

R. D. CULLER, F. C. PARRISH JR., G. C. SMITH and H. R. CROSS

ABSTRACT

Loin steaks from 78 carcasses of A, B, C and E maturities were categorized into three tenderness groups on the hasis of sensory tenderness scores and Warner-Bratzler (W-B) shear force values. Myofibril fragmentation index (MFI), sarcomere length, total and soluble collagen, moisture, fat and pH values were determined for these steaks. Carcass characteristics were also measured. The physical, chemical and sensory values were statistically analyzed to determine the relationship of these values, especially the relationship and the importance of myofibril fragmentation to tenderness of loin steaks. Results of this study showed that myofibril fragmentation index (MFI) accounted for more than 50% of the variation in loin steak tenderness and that myofibril fragmentation was a more important effector of tenderness in loin steaks than collagen solubility or sarcomere length. The steaks used in this study varied widely in marbling degree and maturity; therefore, MFI should be an excellent predictor of broiled loin steak tenderness. Although a number of significant correlations were observed between MFI and carcass characteristics, none of these was of practical importance because they accounted for little of the variation in tenderness.

INTRODUCTION

MYOFIBRIL FRAGMENTATION accounts for more than 50% of the variation in tenderness of steaks from the longissimus muscle of conventionally handled carcasses (Olson and Parrish, 1977; MacBride and Parrish, 1977; Moller et al., 1973). Olson and Parrish (1977) have successfully adapted the technique of determining myofibril fragmentation of postmortem muscle (Davey and Gilbert; 1969), and have termed this value, myofibril fragmentation index (MFI). MFI provides a potential method for identifying tough and tender beef carcasses. One of the requirements, however, before MFI can be seriously considered for practical use as a means of categorizing carcasses into tough and tender groups is to know how well MFI is related to tenderness of meat varying widely in composition and palatability. Earlier work by Olson and Parrish (1977) showed that MFI and tenderness were related for steaks from veal and A and C maturity carcasses, but carcass characteristics and other chemical and histological parameters were not measured in this earlier study. It would seem to be worthwhile to further explore the relationship between MFI and steak composition and palatability, and to determine if certain carcass characteristics are related to MFI. For example, if some carcass characteristic was highly related to MFI, then that characteristic could be used to qualitatively identify tenderness, and then this information could be used to classify carcasses and cuts into various tenderness groups.

The purpose of this paper is to report the relationship between myofibril fragmentation index and other chemical, physical and sensory characteristics of bovine longissimus muscle.

Author Parrish is with the Muscle Biology Group, Departments of Animal Science & Food Technology, Iowa State University, Ames, IA 50011. Author Culler is with the Research & Development Dept., Geo. A. Hormel and Co., Austin, MN 55912. Author Smith is with the Dept. of Animal Science, Texas A&M University, College Station, TX 77843. Author Cross is with the USDA Meat Science Research Lab., ARS, Beltsville, MD 20705.

0022-1147/78/0004-1177$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Source of longissimus muscle

Loin steaks from 78 carcasses were categorized into three tenderness groups on the basis of sensory tenderness scores and Warner-Bratzler (W-B) shear values. These 78 loin steaks were selected from 333 loin steaks used in a cooperative Texas A&M University, Colorado State University and ARS project. Marbling degree ranged from moderately abundant to practically devoid (9 moderately abundant, 8 slightly abundant, 8 moderate, 11 modest, 8 small, 13 slight, 10 traces and 11 practically devoid). Steaks were from 25A, 11B, 20C and 22E maturity carcasses. Carcass characteristics were evaluated by three evaluators (College and USDA personnel) sometime between a 24 and 120 hr postmortem selection period. Steaks were cut 2.8 cm in thickness from short loins after 10–14 days postmortem storage at 2°C. Then they were trimmed to 0.65 cm of external fat, double wrapped in freezer paper, stored in a −29°C freezer and subsequently used for sensory panel evaluation, W-B shear force and chemical and histological analyses.

Sensory panel selection and training

A ten-member descriptive attribute panel was trained according to procedures outlined by Cross et al. (1978).

Steak preparation and evaluation

Frozen steaks were thawed for 24 hr, 2 hr at room temperature (22°C) and 22 hr at 5°C. Thawed steaks (5° ± 2°C) were cooked on preheated Model 450A Farberware electric broilers (163° ± 17°C at grill surface) to an internal temperature of 70°C as determined by both thermocouple and thermometer. When steaks reached 70°C, they were removed from the broiler and wrapped in aluminum foil. Before serving, steaks were cut in longitudinal halves, one-half was randomly selected for sensory panel evaluation and the other half was for W-B shear determination. The half for panelists was sectioned into 1.27 cm × 1.27 cm × 1.90 cm samples. Two of these sections, selected at random, were presented to each panelist for evaluation of tenderness, juiciness and amount of connective tissue. Tenderness, juiciness and amount of connective tissue were scored according to an 8-point structured scale (8 = extremely tender or juicy and no detectable connective tissue).

Degree of doneness and W-B shear force determinations

Degree of doneness was subjectively determined by using an 8-point scale (8 = very rare) of color photographs of steaks cooked to different degrees of doneness. Six 1.27-cm cores were removed parallel with the muscle fibers for W-B shear determinations after steaks had equilibrated to room temperature. Each core was sheared twice, and the 12 values were averaged in pounds of force per 1.27 cm core and then converted to kg force per square cm.

Myofibril fragmentation index (MFI) determination

MFI was determined by modification of the procedure of Olson et al. (1976). Three 1.27-cm cores from a frozen (−29°C) steak designated for histological and chemical analyses were finely scissor-minced, and any visible amounts of fat and connective tissue were removed. Four grams of minced muscle were homogenized for 30 sec in 10 vol (v/w) of a 2°C isolating medium consisting of 100mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM $MgCl_2$ and 1 mM sodium azide. The homogenate was sedimented at 1000 × G for 15 min and then the supernatant was decanted. The sediment was next resuspended in 10 vol (v/w) of isolating medium by using a stir rod, sedimented again at 1000 × G for 15 min and the supernatant decanted. The sediment was resuspended in 2.5 vol (v/w) of isolating medium and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris. An additional 2.5 volumes (v/w) were used to facilitate passage of myofibrils through the strainer. Protein concentration of the suspension of myo-

Table 1—Tenderness, juiciness and, connective tissue scores, W-B shear values, cooking loss and degree of doneness of bovine steaks for three tenderness groups[a]

Tenderness Group	T[b]	W-B kg/cm[c]	CT[d]	J[b]	CL[d] %	DD[e]
Tender (27)[f]	6.88a ±0.08	1.94a ±0.05	6.26a ±0.16	5.80a ±0.14	27.26a ±0.68	4.70a ±0.15
Intermediate (24)	5.20b ±0.08	2.81b ±0.06	5.44b ±0.22	5.48b ±0.19	26.07a ±0.73	4.96a ±0.18
Tough (27)	2.98c ±0.15	4.64c ±0.16	4.86b ±0.19	5.43a ±0.16	28.02a ±0.55	5.15a ±0.15
Combined (78)	5.02 ±0.19	3.13 ±0.14	5.52 ±0.13	5.57 ±0.09	27.22 ±0.38	4.94 ±0.09

[a] Mean values and standard errors not followed by the same letter are significantly different at the 1% level of probability as determined by Duncan's New Multiple Range Test
[b] Eight point descriptive scale (8 = extremely tender and 1 = extremely tough; 8 = extremely juicy and 1 = extremely dry)
[c] Eight point descriptive scale (8 = no detectable connective tissue and 1 = abundant connective tissue)
[d] Percentage cooking loss
[e] Eight point degree of doneness descriptive chart (8 = very rare and 1 = very well done)
[f] Number of samples

fibrils was determined by the biuret method of Gornall et al. (1949).

An aliquot of the myofibril suspension was diluted with isolating medium to a protein concentration of 0.5 ± 0.05 mg/ml. Protein concentration was again determined by the biuret method of Gornall et al. (1949). The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately at 540 nm with a Bausch and Lomb Spectronic 20 colorimeter. Absorbance was multiplied by 200 to give a MFI for each steak.

Sarcomere length determination

Sarcomere lengths were measured on the diluted myofibril suspensions (0.5 × 0.05 mg/ml) according to the procedure of Parrish et al. (1973).

Total and soluble collagen determinations

Hydroxyproline content was determined on each of the 78 steaks as a measure of total and soluble collagen. The determination was by modification of the procedures of Goll et al. (1963; 1964). For total collagen, 5g of scissor-minced muscle and 50 ml of 6N HCl were placed in a glass centrifuge bottle and autoclaved for 12 hr at 16–19 pounds pressure (122–126°C). The samples were cooled and then decolorized with 2–3g of a 1:2 (w:w) mixture of activated charcoal and dry Dowex 1–X8 ion exchange resin and then filtered. The filtrate was neutralized to a phenolphthalein end point and diluted. Samples were analyzed in duplicate. Absorbance was read at 557 nm and the amount of hydroxyproline was converted to collagen by using a factor of 7.25 (Goll et al., 1963; 1964).

For soluble collagen, 20 ml of a 0.1M sodium phosphate buffer (pH 7.0) was added to 5g of finely scissor-minced muscle in a centrifuge tube. Each sample was then heated in a 72–74°C water bath with occasional stirring until the sample reached 70°C (approx 25–28 min). After cooling in an ice bath for 10 min, the samples were then centrifuged at 10,000 × G for 15 min. Both the supernatant and residue were retained. The soluble collagen in the supernatant was determined by placing a 10 ml aliquot of the supernatant into a large glass test tube with 10 ml of 6N HCl and autoclaving it for 6 hr at 16–19 pounds pressure (122–126°C). The samples were cooled, decolorized with 1g of a 1:2 (w:w) mixture of activated charcoal and dry Dowex 1-X8 ion exchange resin and filtered. The filtrate was neutralized to a phenolphthalein end point and diluted. The residue was analyzed according to the total collagen procedure used in this study. Samples were analyzed in duplicate. A factor of 7.52 (Cross et al., 1973) was used to convert hydroxyproline to collagen.

Moisture, fat and pH determination

Moisture and fat content was determined according to AOAC procedures. pH was determined on 10g of muscle tissue homogenized in 100 ml of deionized water with a Waring Blendor for 10 sec. pH values were measured with a Radiometer pH meter 26.

Statistical analysis

Data were analyzed for means, standard deviations, standard errors, simple correlations, multiple correlations and significant mean differences by analysis of variance tests by the Statistical Analysis System of the Iowa State University Computation Center.

RESULTS & DISCUSSION

TENDERNESS, as shown by sensory panel scores and W-B shear values in Table 1, was significantly different among the three tenderness groups. Connective tissue scores, which reflect the amount of residue after thorough mastication of the sample, were significantly different between the tender and other two groups. The intermediate and tough groups, however, were not significantly different, although the steaks from the tough group had more connective tissue (lower score). Juiciness changed very little among tenderness groups.

Earlier work has demonstrated the importance of the relationship of myofibril fragmentation to tenderization (Parrish et al., 1973; Olson and Parrish, 1977). In this study myofibril fragmentation index (MFI) was determined to see if it would be useful in characterizing three different tenderness groups. Results presented in Table 2 demonstrate that MFI was significantly different among tenderness groups. A loin steak with a MFI of 60 or above should be very tender, and a steak with an MFI of about 50 should be slightly tender. An MFI much below 50, however, would denote lack of the tenderness desired in a loin steak. These results, which include four maturity groups, A, B, C and E, extend earlier work showing that MFI of loin steaks from tender and from tough longissimus of A maturity carcasses was significantly different (MacBride and Parrish, 1977).

There was no significant difference in sarcomere length of myofibrils among tenderness groups (Table 2). This substanti-

Table 2—Certain chemical and histological properties of bovine loin steaks for three tenderness groups[a]

Tenderness group	Myofibril fragmentation index[b]	Sarcomere length (μm)	Total collagen (mg/g)	Soluble collagen (%)	pH	Fat (%)	Moisture (%)
Tender (27)[c]	60.44a ±1.86	1.82a ±0.01	3.28a ±0.12	6.03a ±0.38	5.68a ±0.02	7.73a ±0.56	70.41a ±0.49
Intermediate (24)	53.71b ±1.57	1.76a ±0.02	3.24a ±0.08	4.34b ±0.36	5.82a ±0.06	3.75b ±0.48	73.98b ±0.46
Tough (27)	36.52c ±2.18	1.77a ±0.02	3.38a ±0.11	4.58b ±0.39	5.85a ±0.04	2.67b ±0.33	74.67b ±0.33
Combined (78)	50.09 ±1.60	1.78 ±0.01	3.28 ±0.06	5.01 ±0.23	5.79 ±0.03	4.75 ±0.37	72.98 ±0.33

[a] Mean values and standard errors not followed by the same letter are significantly different at the 1% level of probability as determined by Duncan's New Multiple Range Test
[b] Absorbance per 0.5 mg myofibril protein X 200
[c] Number of samples

ates an earlier observation in which no significant difference in sarcomere length of myofibrils from tough and tender longissimus muscle conventionally aged in A maturity carcasses was found (MacBride and Parrish, 1977). Values reported in this paper for sarcomere lengths of myofibrils from loin steaks include A, B, C and E maturity carcasses. Sarcomere length has been shown to be of consequence in tenderness, but the importance of sarcomere lengths on tenderness seems associated with the excising of pre-rigor muscle and then subjecting it to cold shortening conditions (Marsh and Leet, 1966; Herring et al., 1967b).

Total collagen values among tenderness groups were not significantly different, but soluble collagen was significantly different between the tender group and the intermediate and tough groups. It is well known that the amount of collagen is not age dependent, but that the chemical nature of collagen is age dependent (Goll et al., 1964; Hill, 1966). Hence, steaks from the intermediate and tough groups were from more mature carcasses than were steaks in the tender group and therefore had less soluble collagen. Values for total collagen and soluble collagen are in close agreement with those values reported by Cross et al. (1973) and Field et al. (1970).

Correlation coefficients between various chemical and histological measurements, sensory attributes and W-B shear values are shown in Table 3. MFI, percentage soluble collagen, pH and W-B shear were significantly correlated with all sensory characteristics, with the exception of juiciness, whereas sarcomere length and total collagen were not significantly related to sensory attributes and W-B shear. Tenderness, determined by both sensory panel evaluation and W-B shear force, and MFI were highly correlated. Consequently, MFI accounts for over 50% of the variation in tenderness of loin steaks varying widely in marbling and maturity. This relationship is substantiated by the work of MacBride and Parrish (1977), Moller et al. (1973) and Olson and Parrish (1977). Thus, these results indicate that the fragmentation of the myofibril at the Z-disk, and reported as a MFI, accounts for more variation in tenderness than any other single relationship. The relationship between MFI and juiciness is considerably lower, however, than an earlier reported correlation between MFI and juiciness (Olson and Parrish, 1977). One possible reason for this lower relationship was the method of cookery. In earlier work an oven-broiling method was used (Parrish et al., 1973), and in this study Farberware electric broilers were used. The direct and intense heat of the Farberware broilers may cause loss of juiciness, but this remains to be proven. The two methods of cookery used, however, do not seem to alter the relationship between MFI and tenderness. This relationship seems to be repeatable under different conditions, but further experiments are needed to further elucidate the factors regulating myofibril fragmentation.

Sarcomere length as a measure of the state of postmortem muscle shortening and the relationship of sarcomere length to tenderness has been of interest since the report of Locker and Hagyard (1963) showing the cold shortening effect in beef muscle. Marsh and Leet (1966) and Herring et al. (1967b) have shown that muscle shortening and sarcomere length are contributing factors to lack of tenderness of meat from cold shortened muscle. The results of this study, (Table 3) however, show that sarcomere length of myofibrils from loin steaks of conventionally handled carcasses was not significantly related to sensory tenderness scores (0.21) or W-B shear values (−0.20).

Collagen has also received considerable attention as a muscle tissue component in beef steak tenderness. Total collagen content of muscle and its relationship to meat tenderness has been described as being meaningless, with the quality of collagen being the important aspect (Bailey, 1972). This is not entirely accurate because there have been reports showing some relationship between total collagen and tenderness (Loyd and Hiner, 1959; Parrish et al., 1962). The relationship between collagen and tenderness, however, is low and is not of significance in this paper. On the other hand, soluble collagen was significantly related to sensory attributes, with the exception of juiciness. The highest correlation coefficient was between soluble collagen and sensory connective tissue amount remaining after mastication. Because several maturities are represented in this study, these values probably reflect the changes in collagen solubility with increasing age (Goll et al., 1964; Hill, 1966). Herring et al. (1967a) found a highly significant relationship between collagen solubility and tenderness of longissimus when all maturities were included, but there was not a significant relationship within maturity. Furthermore, Cross et al. (1973) found a significant relationship between collagen solubility and amount of connective tissue detected by a sensory panel, but they did not find significance between initial sensory tenderness and muscle fiber tenderness rating. Hence, the nature of collagen is important to tenderness, but its relationship to tenderness is not nearly as important as the one between MFI and tenderness of loin steaks from the longissimus. That is, myofibril fragmentation accounts for more than 50% of variation in tenderness whereas collagen solubility accounts for only about 10% of the variation in tenderness of loin steaks.

Table 3—Correlation coefficients between certain chemical and histological properties, sensory attributes and W-B shear for the three tenderness groups

	Tenderness[a]	Juiciness[a]	Connective tissue[b]	W-B shear[c]
MFI	0.75**	0.02	0.32*	−0.72**
Sarcomere length (μm)	0.21	−0.56	0.10	−0.20
Total collagen (mg/gm)	−0.11	0.08	−0.12	0.06
Soluble collagen (%)	0.33**	−0.27	0.62**	−0.14
pH	−0.30**	−0.01	−0.38**	−0.44**
W-B shear	−0.90**	−0.23*	−0.39**	1.00

[a] Eight point descriptive scale (8 = extremely tender, juicy or desirable; 1 = extremely tough, dry or undesirable)
[b] Eight point descriptive scale (8 = none; 1 = abundant)
[c] kg of shear force per cm^2
* $p < 0.05$
** $p < 0.01$

If some carcass characteristics were related to MFI, this would provide a possible means of identifying carcasses with high MFI and acceptable tenderness. Correlation coefficients between various chemical and histological properties and physical carcass measurements are shown in Table 4. Although the relationship between MFI and lean texture, marbling degree and carcass maturity was significant, none of these correlation coefficients was exceptionally high or would facilitate the use of some carcass characteristic as a tenderness indicator. On the other hand, correlation coefficients between percentage soluble collagen and lean color, lean texture and carcass maturity were all highly significantly related. These highly significant values are probably because of the wide variation in carcass maturity. These values further substantiate the relationship between collagen solubility and animal age (Goll et al., 1964; Hill, 1966; Herring et al., 1967a). Unfortunately the relationship between other carcass measurements and MFI and other histological and chemical measurements proved to be of little value.

Results of this study show that MFI accounted for more than 50% of the variation in loin steak tenderness and that myofibril fragmentation is a more important effector of tenderness in loin steaks conventionally handled than is collagen solubility or sarcomere length. These results further substantiate that the term for a state of tenderness, myofibril fragmentation tenderness, is an appropriate one to describe tenderness of conventionally aged bovine longissimus muscle

Table 4—Correlation coefficients between certain chemical and histological properties and physical carcass measurements

	Fat color[a]	Carcass weight (kg)	Fat thickness (cm)	Rib eye area $(cm)^2$	Kidney, heart and pelvic fat (%)	Lean color[b]	Lean texture[b]	Lean firmness[c]	Marbling degree[d]	Carcass maturity[e]
MFI	0.41**	0.29**	0.35**	0.29**	0.35**	0.21	0.31**	0.28*	0.39**	0.29**
Sarcomere length (μm)	0.18	0.24	0.28*	0.26*	0.22*	0.07	0.12	0.21	0.20	0.13
Total collagen (mg/g)	−0.15	0.00	−0.12	−0.12	−0.18	−0.10	−0.10	−0.06	−0.17	−0.03
Soluble collagen (%)	0.61**	0.00	0.21	0.12	0.42**	0.58**	0.49**	0.12	0.17	0.66**
pH	−0.34**	0.18	−0.27*	−0.35**	−0.14	−0.39**	−0.30**	−0.06	−0.16	−0.33**

[a] Five point scale (5 = white; 1 = dark yellow)
[b] Eight point scale (8 = light grayish red)
[c] Eight point scale (8 = very firm or fine)
[d] Twenty-seven point scale (27 = +moderately abundant; 1 = devoid)
[e] Fifteen point scale (15 = A−; 1 = E+)
* $p < 0.05$
** $p < 0.01$

(MacBride and Parrish, 1977). The steaks used in this study varied widely in marbling degree and maturity level; therefore, MFI has the potential of being an excellent predictor of broiled loin steak tenderness. Although a number of significant correlations were observed between MFI and carcass characteristics, none of these was of practical importance because they accounted for little of the variation in tenderness. Further investigations are in progress to determine (1) the effect of other factors on MFI, and (2) if MFI can be of practical use in classifying carcasses and cuts into different tenderness groups.

REFERENCES

Bailey, A.J. 1972. The basis of meat texture. J. Sci. Food Agric. 23: 995.

Cross, H.R., Carpenter, Z.L. and Smith, G.C. 1973. Effects of intramuscular collagen and elastin upon bovine muscle tenderness. J. Food Sci. 38: 998.

Cross, H.R., Moen, R. and Stanfield, M.S. 1978. Guidelines for training and testing a meat descriptive panel. J. Food Sci. (In press).

Davey, C.L. and Gilbert, K.V. 1969. Studies in meat tenderness. 7. Changes in the fine structure of meat during aging. J. Food Sci. 34: 69.

Field, R.A., Pearson, A.M. and Schweigert, B.S. 1970. Labile collagen from epimysial and intramuscular connective tissue as related to Warner-Bratzler shear values. J. Agric. Food Chem. 18: 280.

Goll, D.E., Bray, R.W. and Hoekstra, W.G. 1963. Age-associated changes in bovine muscle composition. The isolation and properties of a collagenous residue from bovine muscle. J. Food Sci. 28: 503.

Goll, D.E., Bray, R.W. and Hoekstra, W.G. 1964. Age-associated changes in bovine muscle connective tissue. 3. Rate of solubilization at 100°C. J. Food Sci. 29: 622.

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

Herring, H.K., Cassens, R.G. and Briskey, E.J. 1967a. Factors affecting collagen solubility in bovine muscles. J. Food Sci. 32: 534.

Herring, H.K., Cassens, R.G., Suess, G.G., Brungardt, V.H. and Briskey, E.J. 1967b. Tenderness and associated characteristics of stretched and contracted bovine muscle. J. Food Sci. 32: 317.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31: 161.

Locker, R.H. and Hagyard, C.J. 1963. A cold shortening effect in beef muscles. J. Sci. Food Agric. 14: 787.

Loyd, E.J. and Hiner, R.L. 1959. Relation between hydroxyproline of alkali-insoluble protein and tenderness of bovine muscle. J. Agric. Food Chem. 7: 860.

MacBride, M.A. and Parrish, F.C. Jr. 1977. The 30,000 dalton component of tender bovine longissimus muscle. J. Food Sci. 42: 1627.

Marsh, B.B. and Leet, N.G. 1966. Studies in meat tenderness. 3. The effects of cold shortening on tenderness. J. Food Sci. 31: 450.

Moller, A.J., Vestergaard, T. and Wismer-Pederson, J. 1973. Myofibril fragmentation in bovine longissimus dorsi as an index of tenderness. J. Food Sci. 38: 824.

Olson, D.G. and Parrish, F.C. Jr. 1977. Relationship of myofibril fragmentation index to measures of beef steak tenderness. J. Food Sci. 42: 506.

Olson, D.G., Parrish, F.C. Jr. and Stromer, M.H. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J. Food Sci. 41: 1036.

Parrish, F.C. Jr., Bailey, M.E. and Naumann, H.D. 1962. Hydroxyproline as a measure of beef tenderness. Food Technol. 16: 68.

Parrish, F.C. Jr., Young, R.B., Miner, B.E. and Andersen, L.D. 1973. Effect of postmortem conditions on certain chemical morphological and organoleptic properties of bovine muscle. J. Food Sci. 38: 690.

Ms received 11/12/77; revised 2/4/78; accepted 2/8/78.

Journal Paper No. J-8992 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project 2049. The technical assistance of Diane Kuhlers, Carol Selvig and Ruth Smith and the statistical analysis by Dr. David F. Cox are gratefully acknowledged. This research was supported in part from a grant by the Meat Science Research Laboratory, Agriculture Research Service, United States Department of Agriculture.

EFFECT OF TENSILE STRESS ON THE ULTRASTRUCTURE OF BOVINE MUSCLE

R. J. CARROLL, F. P. RORER, S. B. JONES and J. R. CAVANAUGH

ABSTRACT

The changes in ultrastructure of bovine semitendinosus, both raw and heated (90°C) and subjected to tensile stress, were determined by scanning electron microscopy. Stress parallel to the fiber axis resulted in the initial rupture of the muscle fiber-endomysium sheath. Perpendicular stress caused initial rupture at the endomysium-perimysium junction with the muscle fibers remaining undisturbed. Similar observations were made on the muscle tissue heated to 90°C and subjected to tensile stress. Heating to 90°C does not alter completely the fibrous nature of the perimysium connective tissue. Some native type collagen fibrils were observed, even after the severe heat treatment. The effect of applied stress on components of the muscle tissue is an important consideration in the development of a more reliable test method for evaluation of meat tenderness.

INTRODUCTION

PROBABLY the most important factor influencing meat quality, apart from wholesomeness, is tenderness. The literature contains a large number of papers related to the identification of those factors contributing to meat tenderness. Some of these considerations include: sarcomere length (Bouton et al., 1974; Hegarty and Allen, 1975; Herring et al., 1965); pH (Hamm and Deatherage, 1960); connective tissue (Dutson, 1974; Paul et al., 1973); age (Bouton and Harris, 1972); cooking temperature (Bouton et al., 1974; Hegarty and Allen, 1975; Jones et al., 1977; Schaller and Powrie, 1972; Schmidt and Parrish, 1971); method of hanging (Herring et al., 1965); location of muscle (Herring et al., 1965); and many others. Laakkonen (1973) in an excellent review paper has examined many of these factors.

Subjective evaluation of meat tenderness is best determined by use of a trained taste panel, but it is expensive and time consuming and gives results after the fact. Pearson (1963) reviewed methods for the objective determination of meat tenderness. He noted that the most widely used is the Warner-Bratzler shear test, but that the correlation of this with sensory methods is poor, averaging about 0.75 (range 0.60–0.85). An analysis of this test indicates that tensile, compression, and shear forces are involved with the measurement.

Stanley et al. (1972) attempted to correlate tensile stress with sensory evaluation of meat tenderness, but the coefficient of variation ranged up to 54%. L'Hirondelle and Martin (1975) reported that an attempt to estimate tenderness of raw and cooked beef muscle by comparison of various objective methods with a trained sensory panel proved unsatisfactory. Herring (1976) discussed some of the more important factors related to meat texture and the instrumental methods used to evaluate tenderness. He classified the instruments according to the principal action: shearing, biting, penetrating, compressing and stretching, and breaking.

In our study, we attempted to isolate a single stress factor, tensile, and determine its effect on the muscle fiber and the connective tissue structure. To carry out this work, we developed a reproducible sample preparatory procedure (Jones et al., 1976) and studied the effects of heating at various temperatures on bovine muscle structure (Jones et al., 1977).

With the techniques of light and electron microscopy, raw and heated tissue samples are characterized to determine the effects of applied tensile stress on the meat system. Structural information of this nature hopefully can lead to a better understanding of these effects and eventually to the development of more precise objective methods for predicting meat tenderness.

All authors are with the USDA Eastern Regional Research Center, ARS, 600 E. Mermaid Lane, Philadelphia, PA 19118.

MATERIALS & METHODS

Sample source

The bovine semitendinosus muscle (eye of round) was obtained either commercially (ten samples) or from a carcass obtained at slaughter and aged in a cold room maintained at 0.5°C for 10 days. Results obtained with muscle from the two sources were indistinguishable.

Heating

For the heating experiments, the tissue was immersed in H_2O in a polyethylene bag and heated in a water bath at 90°C. The sample required 15–20 min to reach equilibrium temperature, as monitored by a thermocouple inserted in the sample and was held at that temperature for 45 min.

Stressing

The semitendinosus muscle was cut parallel to the fiber axis into slices about 6 mm thick. Pieces 6 mm × 20 mm were cut from the large slice for the stress experiments. Raw and heated muscle samples were stressed either parallel or perpendicularly to the muscle fiber axis to various extensions up to and including break. Both raw and heated muscle tissue were evaluated at (a) rest length-control, (b) stressed parallel to fiber axis, and (c) stressed perpendicularly to fiber axis.

Initial stress experiments were carried out as described by Stanley et al. (1972) on an Instron Universal testing apparatus. For carrying out dynamic stressing studies of muscle tissue, a minitensile stage (Fig. 1a) was designed and built. For more general application, this stage was designed to operate in the specimen chamber of the scanning electron microscope. All stress experiments were performed on hydrated specimens and observed under a Bausch and Lomb Stereo 7 zoom microscope at magnifications up to 200X. These experiments were recorded at the appropriate magnification with a GBC-ITC 5000 video camera coupled to the stereo microscope and recorded on an Ampex 5200 video tape deck. Video tape observations were compared to specific areas observed in the scanning electron microscope (SEM).

Handling of stressed tissue

We designed and built several special devices in order to fix and observe the tissue in the stressed position. An adjustable clamping device (Fig. 1b) consisting of two hemostats (one fixed and one variable) permitted fixation of the sample under stress. The sample was transferred to a clamping holder (Fig. 1c) designed to hold the tissue in a stressed position and fit in the specimen holder of the scanning electron microscope. Thus, manipulation of the specimen was minimized. Identical areas of the specimen could be observed and compared in both the light and electron microscopes.

Sample preparation

All muscle samples were restrained and fixed overnight in a modified aldehyde fixative (Jones et al., 1976). The fixative contained 2% glutaraldehyde and 2% paraformaldehyde in 0.05M phosphate buffer. The pH of the fixative was adjusted to the pH of the particular muscle sample which, at 10–14 days postmortem, was 5.6–5.9. The samples were thoroughly washed with water, dehydrated through increasing ethanol water series of 70%, 95% (2X), and 100% (2X). At this point the interior surfaces of the tissue, control or stressed, were exposed for

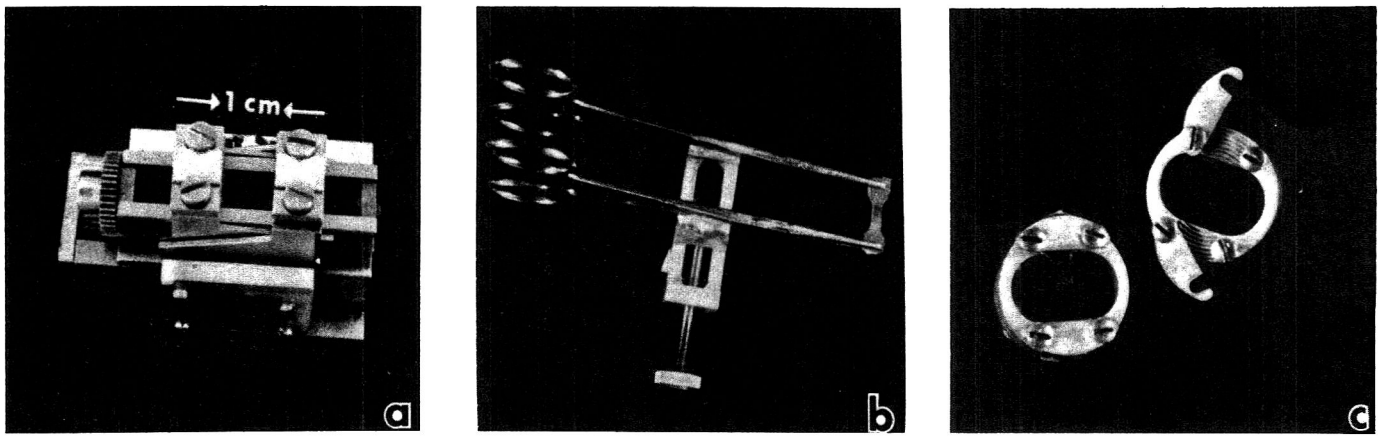


Fig. 1—Mechanical devices used in muscle studies: (a) minitensile stage for stressing tissue; (b) tissue clamping device; (c) tissue clamping holder for SEM.

observation by the cryofracture technique (Humphreys et al., 1974). This procedure involves freezing the sample in ethanol at liquid nitrogen temperature and fracturing the samples while frozen. By this method interior surfaces are obtained which have not been mechanically damaged. The fractured samples were critical point dried with liquid carbon dioxide as a transitional fluid and then mounted on specimen stubs with silver paint. A layer of carbon, followed by gold-palladium, was deposited to minimize charging effects.

Isolation of perimysium

Slices of semitendinosus, approximately 6 mm thick, were cut perpendicularly to the fiber axis. Slices were heated at 90°C as previously described. Samples of perimysial connective tissue from both raw and heated slices were removed by careful dissection under a stereo microscope by use of a scalpel to separate the connective tissue from the muscle fibers. The separated perimysium was fixed in glutaraldehyde, dehydrated, critical point dried, and mounted on stubs for observation on scanning electron microscopy. Portions of the fixed perimysium were dispersed in H_2O in a Waring Blendor and deposited on 200 mesh grids for observation in the transmission electron microscope.

Electron microscopy

A JEOL 50-A scanning electron microscope (SEM) operating at 10–15 kV and an RCA EMU 3-G transmission electron microscope (TEM) operating at 100 kV were used in this investigation.

RESULTS

SCANNING ELECTRON MICROGRAPHS of raw muscle tissue fractured perpendicularly and parallel to the fiber axis are shown in Figure 2. These illustrate the relationships of the various components of the muscle system. As shown in Figure 2a (perpendicular fracture). the perimysium (P) is composed of a random network of fibers which separate muscle fiber

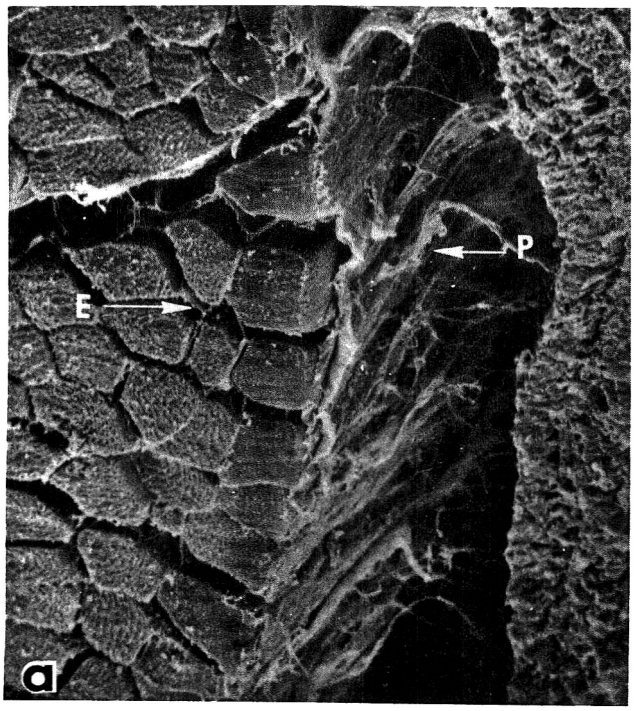


Fig. 2(a)—Raw semitendinosus fractured perpendicularly to fiber axis. The fibers are loosely surrounded by endomysium (E). The perimysium (P) with thicker connective tissue fibers enclose the muscle fiber bundles.

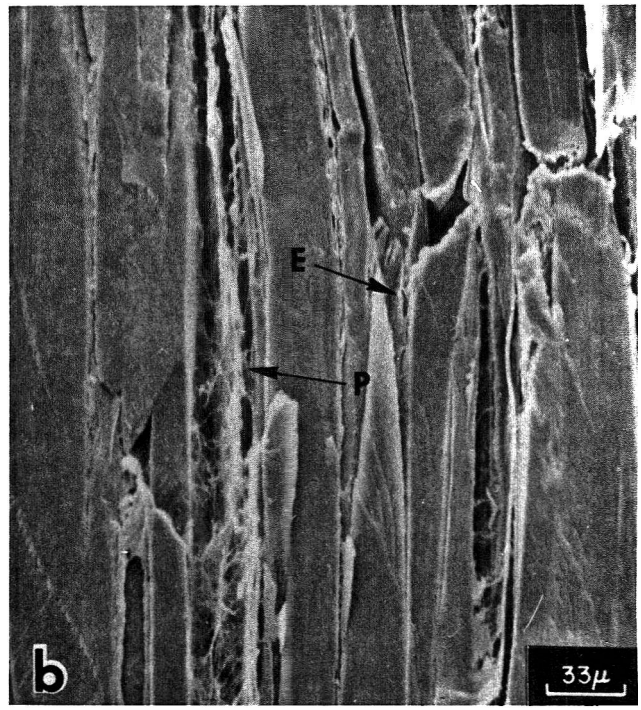


Fig. 2(b)—Raw semitendinosus fractured parallel to fiber axis. Endomysium (E) and perimysium (P) connective tissues are shown.

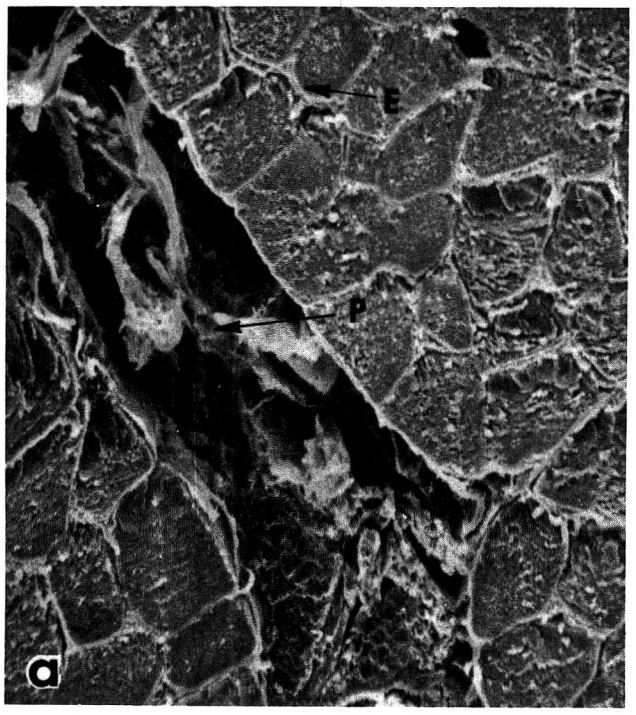


Fig. 3(a)—Heated (90°C) semitendinosus fractured perpendicularly to fiber axis. Endomysium (E) and muscle fibers are in close contact. The perimysium, while still fibrous, contains amorphous material.

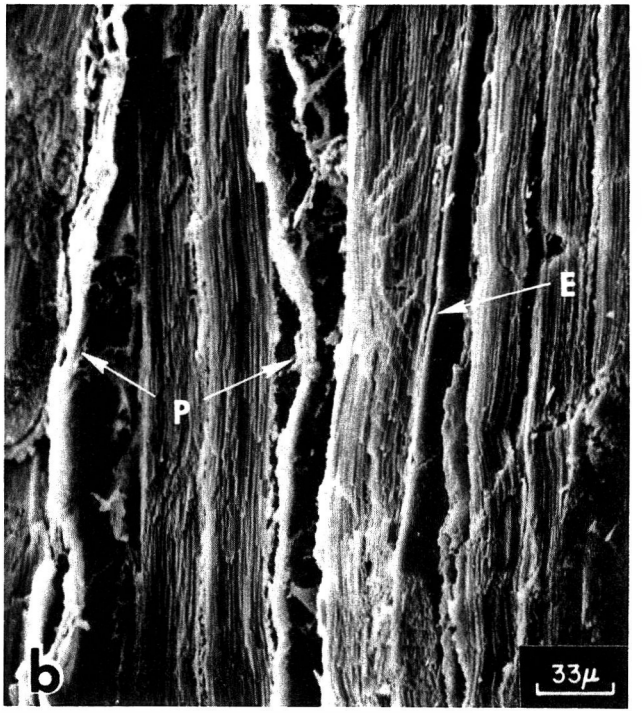


Fig. 3(b)—Heated (90°C) semitendinosus fractured parallel to fiber axis. The endomysium (E) is congealed. Perimysium (P) can be seen running down both sides of a fiber bundle. The fibrils making up the fiber have been exposed by the fracture.

bundles. The fiber bundle is composed of individual muscle fibers, each surrounded by the endomysium sheath (E). This sheath is internally connected within the fiber bundle and coalesces at the periphery of the bundle with the coarse fibers of the perimysium. In Figure 2b, muscle tissue fractured parallel to the fiber axis is observed. The perimysium (P) and the endomysium connective tissue (E) are clearly discernible.

This organizational structure is still evident in cooked samples (Fig. 3), although a number of significant changes have taken place. As seen in cross-section (Fig. 3a), the fine fibrous perimysial network has become more congealed (P) with particulate material enmeshed within it. The muscle fibers are in tight contact with the endomysium (E). When viewed parallel to the fiber axis (Fig. 3b), the endomysium (E) in this instance appears nonfibrous and the perimysium (P) borders both sides of a muscle fiber bundle.

In Figure 4 are shown time-sequence light photomicrographs taken from a video recording of raw muscle tissue stressed parallel to the fiber axis. In 4a, initial stress is being applied; in 4b and 4c, the muscle fibers begin to rupture and thin strands begin to appear. As more stress is applied (4d), a number of fibers are definitely ruptured and the stranded material bridges the gap. The strands appear to originate from the perimysial connective tissue. In 4e and 4f, the muscle fibers have completely ruptured and only the strands remain intact. The force required to rupture the strands is approximately twice the force required to rupture the muscle fibers.

In Figure 5, both light microscopy and scanning electron microscopy observations of the same sample area in raw tissue are depicted. Figure 5a, taken from a video playback, shows the strands remaining before the complete rupture of the sample. The bright spots on the strands are moisture droplets reflecting the light. The same specimen carried through fixation and processed for the SEM is depicted in Figure 5b at higher magnification. Many of the strands are aligned in the direction of the stress. Some have ruptured and have sprung back to coil in a random fashion. The lower half of the micrograph shows the ruptured ends of muscle fibers. The large fiber diameters of the strands as shown in Figure 5b give additional support for the perimysial origin of the strands, since the endomysial connective tissue appears to be composed of much finer fibers.

Efforts were made to identify these strands by staining techniques (Orcein, Weigert's, and Pollock's Trichrome stains). Positive results were obtained for collagen but no evidence was found for the presence of elastin. The strands, when viewed under polarized light, were seen to be birefringent, this result lending additional corroborative evidence for the collagenous nature of the strands.

Raw and heated (90°C) meat samples were stressed parallel and perpendicularly to the fiber axis almost to the point of rupture. Some samples did in fact break and these were discarded. In some other samples, especially for the parallel stress experiments, the overall structures remained intact, but portions of the myofibers had ruptured. The samples were removed in the stressed position, fixed in the clamping holder, carried through ethanol dehydration, and cryofractured.

In Figure 6a is shown interior surfaces of raw meat stressed parallel to the fiber axis. The strands appear taut and aligned in the direction of the applied stress. A similar orientation of the perimysium fibers is obtained with the heated muscle when stressed.

In Figure 6b is shown interior surfaces of heated meat stressed perpendicularly to the fiber axis. The rupture occurs first at the perimysium-endomysium junction. This is in contrast to parallel stressed tissue for which the muscle fibers rupture first. The muscle fibers in 6b appear undistorted, but the connective tissue has been aligned in the direction of the applied stress. Analogous results are obtained with the uncooked muscle.

For the parallel stressed samples, the aligning of the connective tissue in the direction of the applied stress as illustrated in the preceding micrographs begins to take place only after considerable stress is applied. At elongations of 50% of the rest length, for example, very little orienting of the con-

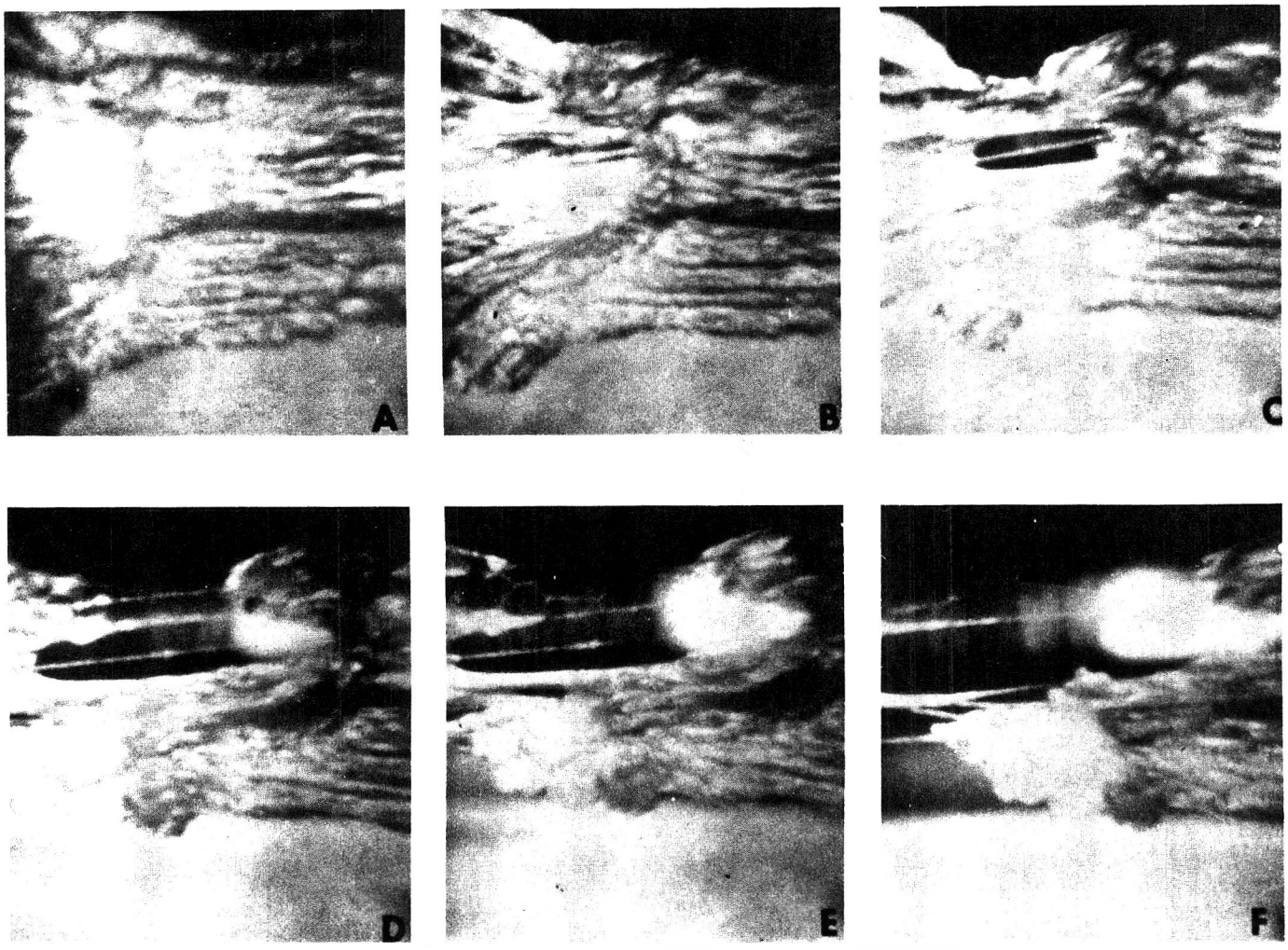


Fig. 4—A series of time sequence photographs taken from video tape of light microscopy of semitendinosus muscle under dynamic tensile stress. (Magnification ∿ 10X) The series of rings observed in the background of D, E, and F is the worm drive of the stage: (A, B) Muscle fibers begin to rupture; (C) Strand material is observed. (D, E) Network of strand material appears as more muscle fibers rupture; (F) Strand network intact after muscle fibers have ruptured.

nective tissue strands is evident. Consistent strand orientation is obtained only when the elongation reaches about 100%. On the other hand, for the perpendicularly stressed samples, consistent strand orientation is evident at about 50% elongation.

To explore in more detail the contribution of the perimysial connective tissue to meat structure, we carefully dissected the perimysium from raw and heated (90°C) meat tissue under a stereo microscope. Light microscopic examination of stained sections of this tissue showed some striated muscle and collagen of the perimysium. When viewed under polarized light the birefringence of unheated collagen fibers was quite evident. Small amounts of elastin were observed in association with occasional blood vessels. No elastin was observed in the perimysial tissue. In the heated perimysium, collagen fibers were found, though the birefringent appearance was somewhat less intense.

The isolated tissue, when observed in the scanning electron microscope, appeared as in Figure 7. In both instances, an interwoven fibrous network is observed, with fibers of various diameters similar to those of the intact tissue in Figure 3. The raw connective tissue, Figure 7a, displays an extensive network with a small amount of amorphous material with little structural detail. The perimysium from heated tissue, Figure 7b, still retains a fibrous appearance but with a significant amount of sharply defined particulates found on, around, and trapped within the fibrous network. Some of these particulates are presumed to be formed by coagulation of the sarcoplasmic proteins. The fine fibers observed in the raw perimysium are not observed in the heated tissue, Figure 7b.

A portion of the isolated perimysium was dispersed in a Waring Blendor and examined with the transmission electron microscope. Figure 8a shows a network of dispersed fibrils displaying the banding pattern associated with collagen fibrils. Some electron dense material is also observed in this micrograph but is unidentifiable. In the heated perimysium (Fig. 8b), most of the sample appears as electron dense material in the transmission electron microscope. However, several collagen fibrils displaying typical banding pattern enmeshed within electron dense material can be observed. A rough estimate would indicate ∿ 10% of the observed specimen contained collagen fibrils showing the band pattern.

DISCUSSION

Although the minitensile stage, upon which the muscle tissue was stressed, is a crude device compared to Universal tensile testers, a rough estimate of rate of extension can be obtained from video tape playback. In our experiments, the rate of extension was approximately 1.0–1.5 cm/min, which was much slower than the 30 cm/min rate employed by Stanley et al. (1972) in their study on muscle tensile properties. We selected this slow extension rate so that we could pinpoint the sequence of events on the various muscle components and to

stop action as quickly as possible for observation in the scanning electron microscope.

Video playback of the stressing experiments shows that with the force applied parallel to the fiber axis (Fig. 4), the initial rupture occurs at the muscle fiber level, concomitant with the appearance of strand material. As the stress is increased, the muscle fibers completely rupture and only the stranded material remains intact. Approximately twice the force is necessary to rupture the strands as is required to rupture the muscle fibers.

On the other hand, when the force is applied perpendicularly to the fiber axis, the rupture occurs at the endomysial-perimysial connective tissue junction. The muscle fibers with their network of connecting sheaths remain relatively undisturbed.

Scanning electron microscopy of stressed but intact muscle shows the events taking place within the muscle structure prior to actual rupture. At elongations of 50% of rest length, very little change in the ultrastructure of the connective tissue is evident. At elongations of 100%, however, the individual fibers of the perimysial connective tissue are seen to align themselves in the direction of the applied stress. This is true for the raw as well as the cooked muscle (Fig. 6).

Since the major component of the perimysial connective tissue, collagen, is known to be relatively inextensible (Bailey, 1972), any apparent elasticity of the connective tissue must arise from reorienting individual fibers of the perimysial network. The perimysium of unstressed samples exhibits a multitude of randomly oriented fibers. Evidently, as the muscle tissue is elongated, the randomly oriented fibers become aligned in the direction of the applied stress (Stanley et al., 1972).

Eventually, as the muscle tissue is stretched, the fibers of the perimysium become fully aligned. Increased stress will cause them to rupture.

The orienting of the connective tissue fibers seems to take place for the cooked as well as the raw muscle. Heating to 90°C apparently does not completely destroy the fibrous nature of the perimysium. Even though the perimysium of the 90°C heated samples gives a more congealed appearance than that of the raw samples, well defined fibers remain and are subject to reorientation under stress.

SEM observations of the isolated perimysium yield results similar to those on the connective tissue in unstressed intact muscles for both raw and cooked samples. Fine fibrils, evident in the raw tissue, are absent in the cooked perimysium. However, the large connective tissue fibers persist even at the high temperatures despite the appearance of coagulated and amorphous material.

The transmission electron micrographs of the isolated perimysium reveal more fully the nature of the fibrous material. Figure 8a shows the typical banding pattern of native collagen in the uncooked material. For the heated sample, however, most of the fine collagen fibrils have been replaced by large amorphous and undefined masses (Fig. 8b). Nevertheless, some of the material retains the banding pattern of native collagen (roughly 10% of the total) which is indicative of the persistence of undenatured collagen even at 90°C.

This result is unexpected in view of the much lower shrink temperature of collagen. Intramuscular collagen has been reported to undergo shrinkage at 61–63°C as determined by differential scanning calorimetry (McClain et al., 1970; McClain, 1971) and has been found to undergo a precipitous drop in shear resistance in the interval 50–60°C attributable to collagen shrinkage (Draudt et al., 1964). Despite the uncertainty of the relationship of these measurements to those on intact collagen, it appears that intramuscular collagen undergoes a thermal transition comparable to that observed in skin and tendon collagen which is indicative of the unfolding of the collagen triple helix in the range about 60°C

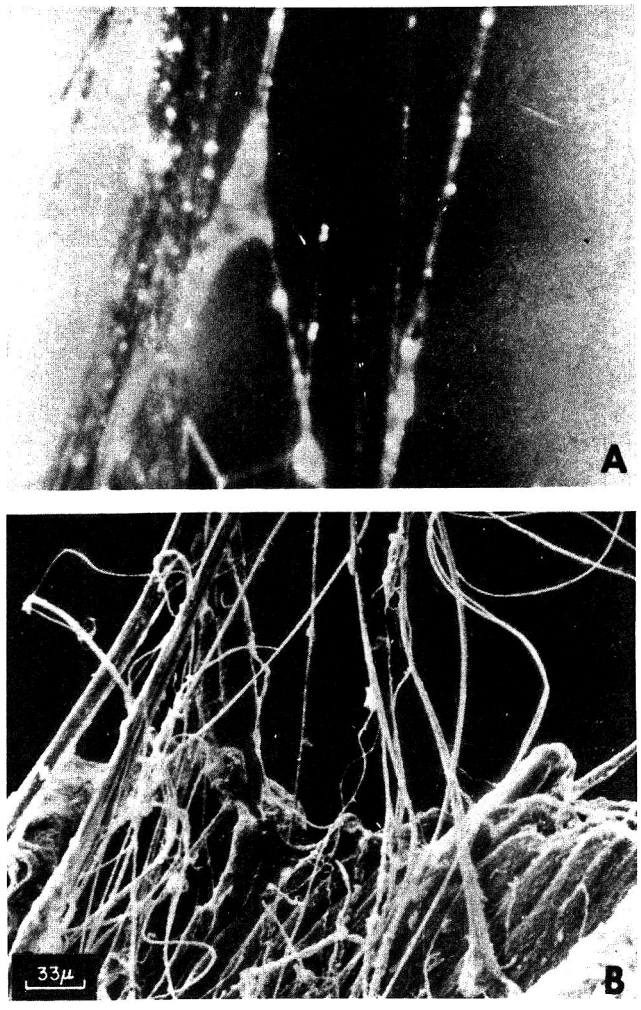


Fig. 5(a)—Light micrograph of strands under stress as observed on video playback. (B) Same area of (A) of the strands under stress and the ends of ruptured muscle fibers as observed in the scanning electron microscope.

(Ramachandran, 1967). By this reasoning, no collagen native structure should exist much above 60°C. Some native structure does remain at as high as 70°C as shown by Giles (1969) in transmission electron microscopy studies of 70°C heated muscle. However, the persistence of native structure in samples heated to 90°C for 45 min is very difficult to interpret.

There is evidence that intramuscular collagen is much more extensively cross-linked (some 10–14 times) than tendon collagen and that the cross-links are much more thermostable (Mohr and Bendall, 1969). It is possible that the collagen showing the banding pattern of the native structure represents a small portion of the collagen network that is sufficiently cross-linked to withstand these high temperatures without denaturing. The small quantity seen ($\sim$ 10%) would not be expected to alter significantly the shrink temperatures of the bulk of the material.

It is evident from these studies that the tensile properties of bovine muscle tissue depend very strongly on the nature, extent, and reaction to thermal stress of the perimysial connective tissue. These tensile properties, in turn, have a significant effect on both the subjective and objective evaluations of meat tenderness. Electron microscopy offers a powerful method of determining the relative roles of muscle tissue components in their reaction to a variety of mechanical, aging, and thermal stresses. Through a synthesis of the knowledge derived from studies of this kind extended to other stresses, an understanding of the factors which control meat tenderness as

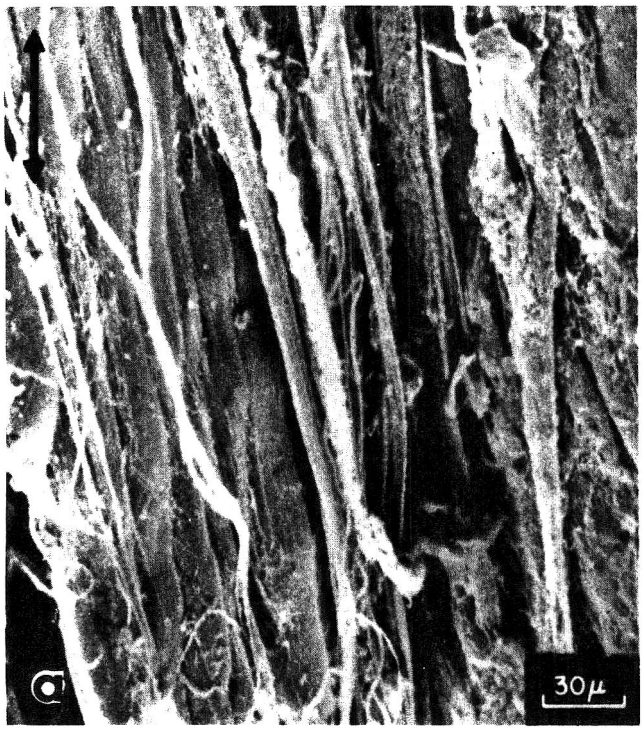


Fig. 6(a)—Fractured interior surface of raw semitendinosus stressed parallel to fiber axis showing the perimysium fibers oriented in the direction of applied stress. The arrows indicate the direction of the applied stress.

Fig. 6(b)—Fractured interior surface of heated (90°C) semitendinosus stressed perpendicularly to the fiber axis. The orienting effect of the stress on the perimysium fibers is evident; the muscle fibers appear undisturbed.

well as the development of objective tests to measure them should be forthcoming.

REFERENCES

Bailey, A.J. 1972. The basis of meat texture. J. Sci. Food Agr. 23: 995.

Bouton, P.E. and Harris, P.V. 1972. The effects of cooking temperature and time on some mechanical properties of meat. J. Food Sci. 37: 140.

Bouton, P.E., Harris, P.V., Shorthose, W.R. and Radclift, D. 1974. Changes in the mechanical properties of veal muscles produced by myofibrillar contraction state, cooking temperature and cooking time. J. Food Sci. 39: 869.

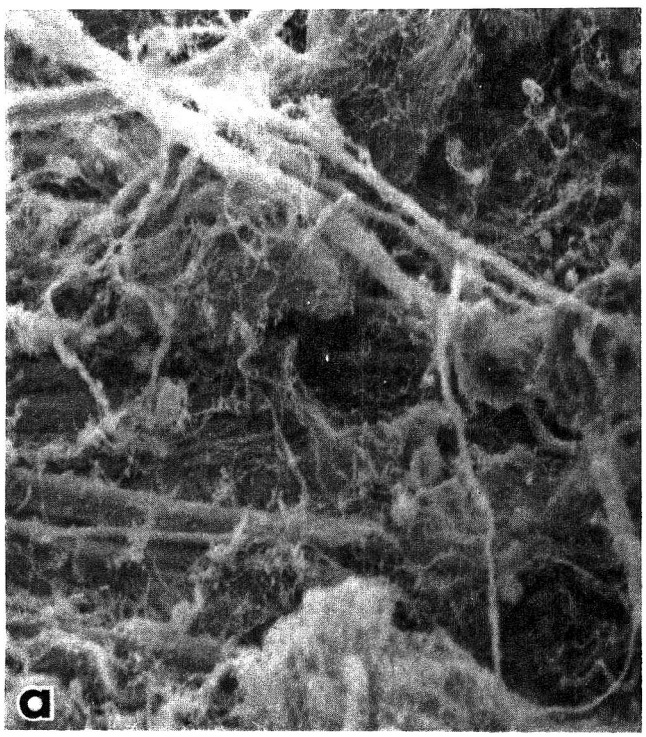


Fig. 7 (a)—Isolated intact perimysium from raw semitendinosus. The random distribution of the fibers is shown with many fine fibers dispersed within the fibrous network.

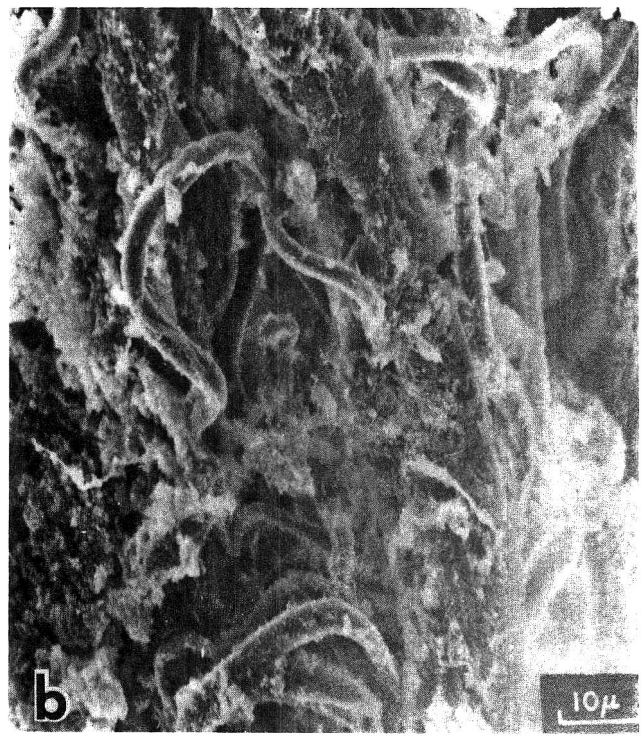


Fig. 7(b)—Isolated intact perimysium from heated (90°C) semitendinosus. The fibrous network is retained with a large amount of amorphous material present. The fine fibers have disappeared.

Fig. 8(a)—TEM of dispersed perimysium from raw semitendinosus shows a random network of collagen fibrils with the characteristic band pattern. Some electron dense particulates are present.

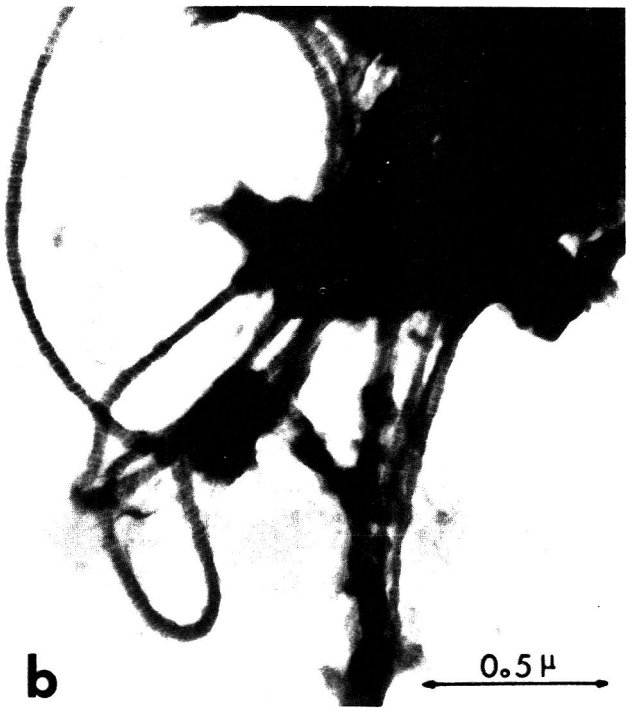


Fig. 8(b)—TEM of dispersed perimysium from heated (90° C) semitendinosus with some collagen fibrils remaining enmeshed in a large amount of amorphous material.

Draudt, H.N., Machlik, S.M. and Rimstidt, C.D. 1964. Effect of cooking time and temperature on tenderness and papain action in beef. Amer. Meat Inst. Found. No. 76, p. 115.
Dutson, T.R. 1974. Connective tissue. Proceedings of the Meat Industry Research Conference, p. 99.
Giles, B.G. 1969. Changes in meat produced by cooking. Proc. 15th European Meeting of Meat Research Workers, Helsinki, Finland, Aug. 1969, p. 289.
Hamm, R. and Deatherage, F.E. 1960. Changes in hydration, solubility and charges of muscle proteins during heating of meat. Food Res. 25: 587.
Hegarty, P.V.J. and Allen, C.E. 1975. Thermal effects on the length of sarcomeres in muscle held at different tension. J. Food Sci. 40: 24.
Herring, H.K., Cassens, R.G. and Briskey, E.J. 1965. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. J. Food Sci. 30: 1049.
Herring, H.K. 1976. Evaluation of beef texture. Objective methods for food evaluation, "Proceedings of a Symposium," p. 7. National Academy of Science, Washington, DC.
Humphreys, W.J., Spurlock, B.O. and Johnson, J.S. 1974. Critical point drying of enthanol-infiltrated, cryofractured biological specimens for scanning electron microscopy. In "Scanning Electron Microscopy," Proceedings of the 7th Annual Scanning Electron Microscope Symposium, Ed. Johari, O. and Corvin, I., p. 275. IIT Research Institute, Chicago, IL.
Jones, S.B., Carroll, R.J. and Cavanaugh, J.R. 1976. Muscle samples for scanning electron microscopy: Preparative techniques and general morphology. J. Food Sci. 41: 867.
Jones, S.B., Carroll, R.J. and Cavanaugh, J.R. 1977. Structural changes in heated bovine muscle: A scanning electron microscopy study. J. Food Sci. 42: 125.
L'Hirondelle, P.J. and Martin, A.H. 1975. Evaluation of methods of assessing tenderness of raw and cooked beef muscle. Can. J. Anim. Sci. 55: 519.
Laakkonen, E. 1973. Factors affecting tenderness during heating of meat. Advances in Food Res. 20: 257.
McClain, P.E. 1971. In vitro aging-like changes in the cross-linking characteristics of collagen from intramuscular connective tissue. Gerontologia 17: 139.
McClain, P.E., Creed, G.J., Wiley, E.R. and Hornstein, I. 1970. Effect of postmortem aging on isolation of intramuscular connective tissue. J. Food Sci. 35: 258.
Mohr, V. and Bendall, J.R. 1969. Constitution and physical chemical properties of intramuscular connective tissue. Nature 223: 404.
Paul, P.C., McCrae, S.E. and Hoffer, L.M. 1973. Heat induced changes in extractability of beef muscle collagen. J. Food Sci. 38: 66.
Pearson, A.M. 1963. Objective and subjective measurements for meat tenderness. Proceedings Meat Tenderness Symposium, Campbell Soup Co., p. 135.
Ramachandran, G.N. 1967. "Treatise on Collagen," Vol. 1. Academic Press, New York, NY.
Schaller, D.R. and Powrie, W.D. 1972. Scanning electron microscopy of heated beef, chicken, and rainbow trout muscles. J. Int. Can. Sci. Technol. Aliment 5: 184.
Schmidt, J.G. and Parrish, F.C. Jr. 1971. Molecular properties of postmortem muscle. 10. Effect of internal temperature and carcass maturity on structure of bovine longissimus. J. Food Sci. 36: 110.
Stanley, D.W., McKnight, L.M., Hines, W.G.S., Usborne, W.R. and Deman, J.M. 1972. Predicting meat tenderness from muscle tensile properties. J. Text. Stud. 3: 51.

Ms received 9/19/77; revised 2/4/78; accepted 2/8/78.

Research supported in part by the U.S. Army Natick Development Center Project No. AMXRED 73-161.

The authors thank Mrs. Ruth Zabarsky for excellent photographic assistance during the course of this research.

The mention of commercial items is for your convenience and does not constitute an endorsement by the Department of Agriculture over other items of a similar nature not mentioned.

EFFECTS OF pH, CALCIUM IONS AND ATP ON RIGOR CONTRACTION IN GLYCERINATED RABBIT PSOAS MUSCLE FIBER

K. IZUMI, T. ITO and T. FUKAZAWA

ABSTRACT

Isometric tension development of glycerinated rabbit skeletal muscle fibers due to rigor contraction in the presence or absence of calcium ions was estimated at varying pHs from 6.2–9.0. The onset and extent of rigor contraction were faster at alkaline pHs than at acidic pHs. Both of the onset and the extent of rigor contraction became faster and greater respectively as calcium ions in the medium increased from 10^{-9} to 10^{-5} M. At a constant pH value of 7.0 and in the absence of calcium ions, alteration in ATP concentrations in a relaxing solution from 5.0–1.0 mM did not influence the magnitude of the tension developed in rigor contraction.

INTRODUCTION

STIFFENING OF MUSCLE during onset of rigor mortis is the most obvious postmortem changes in the physical characteristics of muscle (Bendall, 1973). The obligatory condition for the development of rigor is the loss of ATP, a plasticizer in muscle cells which makes the muscle in a flexible and easily extensible state. Rigor and muscle contraction are essentially the same process having the same mechanism of myosin heads attaching themselves to actin filaments at numerous points (Bendall, 1951; Marsh, 1954; Locker, 1959; Mauriello and Sandow, 1959), except that rigor is irreversible and develops without external stimuli (Nauss and Davies, 1966) and calcium ions (Bremel and Weber, 1972). Bendall (1973) has distinguished three types of rigor in postmortem muscle; acid rigor, alkaline rigor and an intermediate type. The type of rigor is strictly determined by the initial levels of creatine phosphate, ATP and glycogen in the muscle cell. In addition, it has been demonstrated that the amount of the work done by a muscle during rigor increases as ultimate pH of the muscle increases (Bendall, 1951). Rigor occurs in glycerinated muscle fibers when the fiber is moved from a relaxing solution containing ATP to a rigor solution without ATP. This was termed as "rigor contraction" by White (1970).

In the present study we have investigated the effects of pH and the concentrations of calcium ions and ATP on the speed and extent of rigor contraction in a glycerinated single fiber.

MATERIALS & METHODS

GLYCERINATED FIBERS of rabbit psoas muscle were prepared according to the procedure of Huxley (1963). Preparation of single fibers, measurement of the length of its sarcomere and isometric tension measurements were made using the apparatus previously described (Izumi et al., 1977).

The fibers having the length of a sarcomere (2.08–2.15μ) and the width (50–70μ) were immersed in solutions held in a thermostated water bath at 25°C. The solutions which were prepared on the basis of a relaxing solution containing phosphate buffer (Huxley, 1963) and Tris-maleate buffer (Kitazawa, 1975) are given in Table 1. Rigor solutions containing varying concentrations of Ca^{2+} were prepared by adding definite amounts of Ca^{2+}. Concentration of free calcium ion (pCa) was calculated by assuming the apparent binding constant of ethylene glycol-bis-(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) for Ca^{2+} as 5×10^5 M^{-1} at pH 6 8 (Ogawa, 1968). The pCa of the rigor solutions containing Tris-maleate buffer was 9.0, 8.0, 7.0, 6.0 and 5.0. The pH of the rigor solutions containing phosphate buffer was 9.0, 8.6, 7.8, 7.0, 6.6 and 6.2, whereas that of the rigor solutions containing Tris-maleate buffer was 9.0, 8.6, 7.8, 6.8, 6.6 and 6.2 in the presence of 0.1 mM Ca^{2+}. Effects of the concentrations of ATP in the relaxing solution were examined at 5, 3. 2 and 1 mM in phosphate buffer and 4, 2, 1 mM in Tris-maleate buffer.

Experiment on each of the factors tested in this study was repeated three to five times with separate single fibers.

Table 1—Composition of chemicals in relaxing, contracting and rigor solutions employed in this study.

	ATP	$MgCl_2$	EGTA	KCl	$CaCl_2$	Phosphate
Relaxing	5	10	2	50	–	6.7
Contracting	5	10	–	50	0.1	6.7
Rigor	–	10	2	50	–	6.7
						Tris-maleate
Relaxing	4	4	2	120	–	20
High Ca^{2+} rigor	–	4	–	120	0.1	20

[a] Solutions (all concentrations in mM)

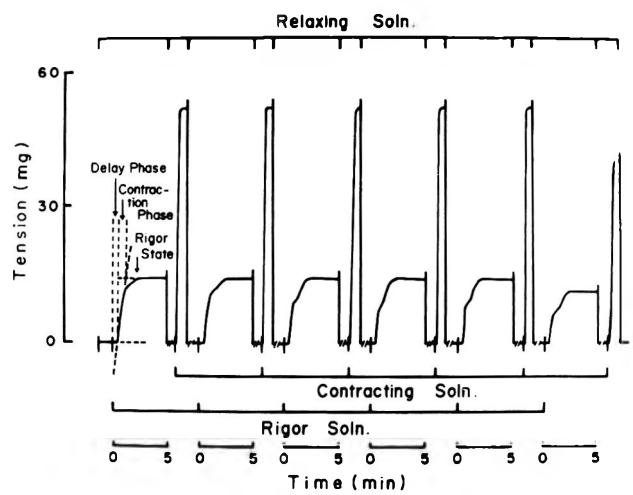


Fig. 1—Repeated records of isometric rigor and active tension development by a glycerinated fiber. All solutions used were phosphate buffer (pH 7.0). The short vertical lines in the record indicate when the solutions were changed. The first tension curve is labelled three stages of rigor contraction.

RESULTS

A CYCLE of rigor contraction, active contraction and relaxation with a single fiber can be repeated several times without impairing initial contractile potency. The magnitude of the tension induced by the contracting solution was more than three times greater than that induced by the rigor solution containing no Ca^{2+} (Fig. 1). In the absence of Ca^{2+}, the tension decreased as the pH of the rigor solution decreased (Fig.

Authors Izumi, Ito and Fukazawa are with the Lab. of Chemistry & Technology of Animal Products, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

0022-1147/78/0004-1188$02.25/0
© 1978 Institute of Food Technologists

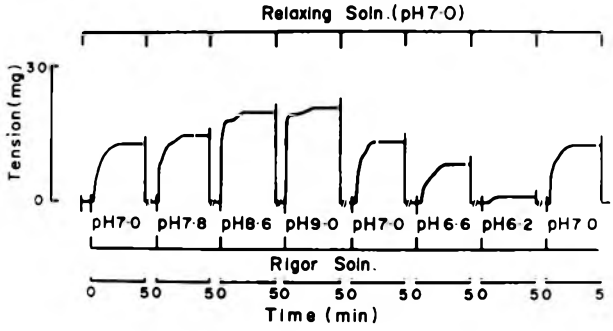


Fig. 2—Isometric tension development by a glycerinated fiber in rigor solutions having various values of pH. No calcium ions were added to the rigor solutions. All solutions used were phosphate buffer. The short vertical lines indicate when the solutions were changed.

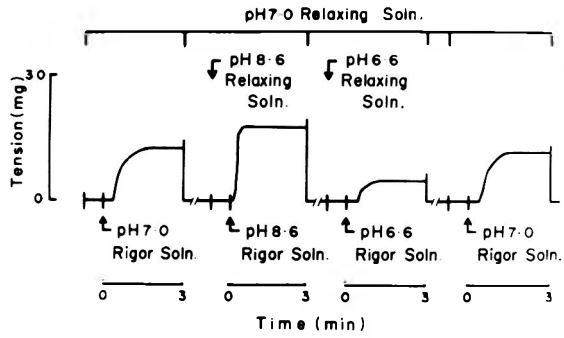


Fig. 3—Isometric tension development by a glycerinated fiber in a rigor solution having the same value of pH as a relaxing solution. All solutions used were phosphate buffer. The short vertical lines indicate when the solutions were changed.

2). In this case, the development of rigor contraction can be described in terms of the three stages defined by White (1970); delay phase, contraction phase and rigor state as illustrated in Figure 1. The duration of the delay phase depended upon the pH values of the rigor solutions (Fig. 2). At pH 7.0 or below there was a 16–20 sec time lag, while at alkaline pHs the delay phase became short; 12 sec at pH 7.8, 9 sec at pH 8.6 and 6 sec at pH 9.0, respectively, with the same single fiber.

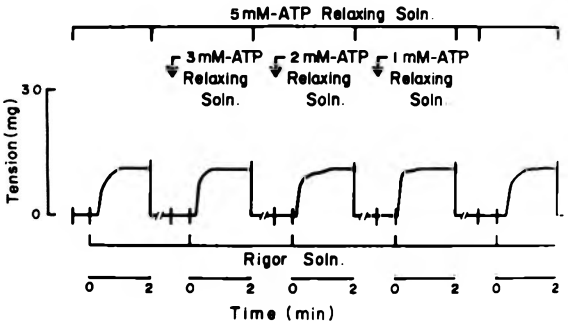


Fig. 4—Effect of ATP concentrations in the relaxing solution on the isometric tension development by a glycerinated fiber in a rigor solution without Ca^{2+}. All solutions used were phosphate buffer (pH 7.0). The short vertical lines indicate when the solutions were changed.

As shown in Figure 3, exchanging the relaxing solutions with the rigor solutions having the same pH values had no effect upon the pattern of rigor contraction versus the pH of the rigor solution illustrated in Figure 2.

On the other hand, it was found that the concentrations of ATP in the relaxing solutions could affect on the delay phase: the time lag of 16, 12, 9 and 6 sec referred to 5, 3, 2 and 1 mM ATP in the relaxing solutions, respectively (Fig. 4). This also indicates that in the absence of Ca^{2+} the differences in the concentration of ATP in the relaxing solutions did not affect on the magnitude of the tension induced by the rigor contraction; muscle undergoes into rigor at a constant level of ATP which may depend upon the pH values.

In the presence of Ca^{2+}, the extent of rigor contraction also depends upon the concentrations of Ca^{2+} in the medium as demonstrated by Mobley (1977): the tension developed rapidly and the magnitude of the tension became greater as the concentrations of Ca^{2+} in the rigor solutions increased (Fig. 5). Figure 6 shows the effect of pH of rigor solution on the development of rigor contraction. When a fiber was transferred from the relaxing solution to the rigor solution containing high concentrations of Ca^{2+} (0.1 mM), the maximum tension was much greater at alkaline pHs than at acidic pHs. The maximum tension of rigor contraction increased as the concentration of ATP in the relaxing solution increased (Fig. 7). In addition, initial rapid force spikes of the tension induced by the rigor solutions containing high concentrations of Ca^{2+} are possibly

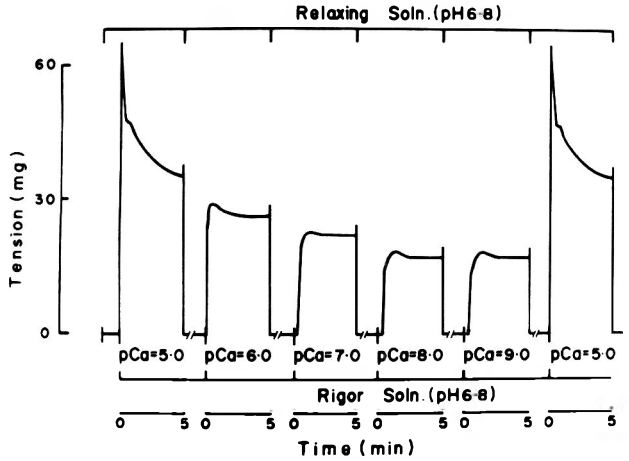


Fig. 5—Isometric tension development by a glycerinated fiber in rigor solutions containing varying concentrations of Ca^{2+}. All solutions used were Tris-maleate buffer. The short vertical lines indicate when the solutions were changed.

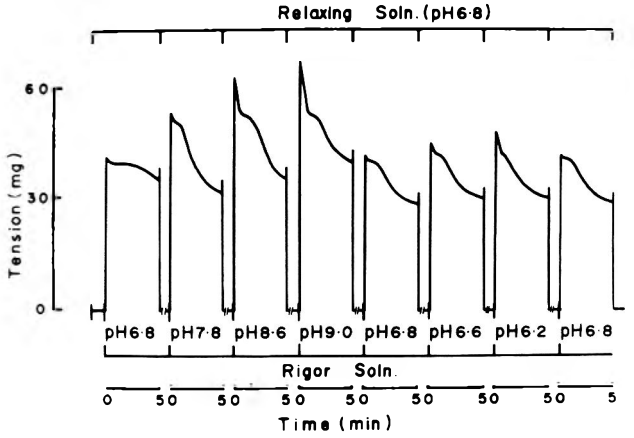


Fig. 6—Effect of pH values of the rigor solution containing 0.1 mM Ca^{2+} on the isometric rigor tension development by a glycerinated fiber. All solutions used were Tris-maleate buffer. The short vertical lines indicate when the solutions were changed.

a reflection of active contraction, because the extent of the force spikes depends upon the concentration of ATP in the relaxing solutions (Fig. 7). Although the concentration of ATP in the fiber is quickly diluted in the rigor solution, it is likely that Ca^{2+} which can diffuse more quickly than ATP molecules make thin filament "turn on" before rigor complexes are formed. This infers that active contraction becomes prevalent to rigor contraction as Ca^{2+} in the rigor solution increase to the level which sufficiently remove the depressive effect of troponin, although true concentration of Ca^{2+} in the fiber was not examined.

DISCUSSION

BENDALL (1960) has suggested that the critical level of ATP at onset of rigor is fixed in each case by the combined effect of ATP level and pH which determines the sensitivity of the actomyosin system to ATP. In fact, the threshold of ATP concentration at which muscle goes into rigor is probably determined by pH alone in the medium without Ca^{2+}, because the onset and extent of rigor contraction is only influenced by the pH value (Fig. 2, 3 and 4). On the other hand, in the presence of low concentration of Ca^{2+} below about 10^{-6}M, Ca^{2+} is able to control the development of rigor contraction due to the cooperative action between rigor complexes and calcium binding affinity of troponin molecules (Bremel and Weber, 1972; Mobley, 1977). In the presence of sufficiently high concentration of Ca^{2+} above 10^{-5}M, the maximum tension depends upon the concentration of ATP and pH in the medium (Fig. 6 and 7). It appears that active tension forms a major part of the tension induced by the rigor solution containing high concentration of Ca^{2+}. Thus, the pattern of the maximum tension versus pH values (Fig. 6) was quite similar to the pH/activity curve for myofibrillar ATPase activated by Mg^{2+} and Ca^{2+} where the activity increases as an S-shaped curve from a minimum at pH 6.0 to a maximum at 8.5 (Bendall, 1969).

The present results indicate that pH and the concentrations of Ca^{2+} and ATP determine the pattern of rigor contraction: the onset and extent of rigor are modified by the combined effect of these factors.

REFERENCES

Bendall, J.R. 1951. The shortening of rabbit muscles during rigor mortis: its relation to the breakdown of adenosine triphosphate and creatine phosphate and to muscular contraction. J. Physiol. 114: 71.

Bendall, J.R. 1960. Postmortem changes in muscle. In "The Structure and Function of Muscle," Vol. 3, p. 227. Academic Press Inc., New York.

Bendall, J.R. 1969. "Muscles, Molecules and Movement," p. 37. American Elsevier Publishing Company, Inc., New York.

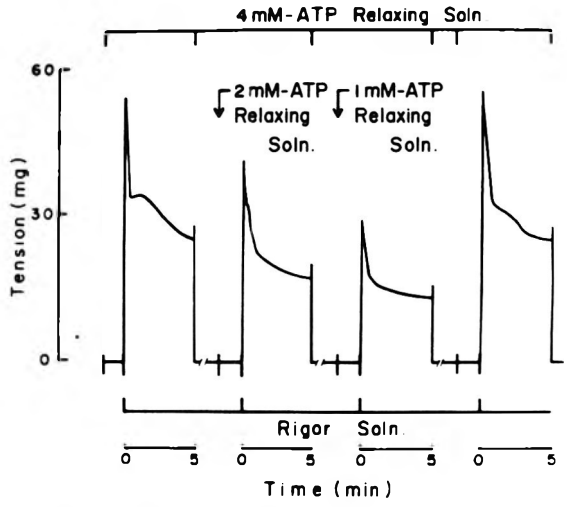


Fig. 7—Effect of ATP concentrations of the relaxing solution on the isometric tension development by a glycerinated fiber in the rigor solution containing 0.1 mM Ca^{2+}. All solutions used were Tris-maleate buffer (pH 6.8). The short vertical lines indicate when the solutions were changed.

Bendall, J.R. 1973. Postmortem changes in muscle. In "The Structure and Function of Muscle," Vol. 2, p. 243. Academic Press Inc., New York.

Bremel, R.D. and Weber, A. 1972. Cooperation within actin filament in vertebrate skeletal muscle. Nature 238: 97.

Huxley, H.E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. J. Mol. Biol. 7: 281.

Izumi, K., Ito, T. and Fukazawa, T. 1977. Isometric tension development of glycerinated fibers prepared from normal and PSE porcine muscles and the effect of myosin irrigation on the tension development of "ghost" fibers. J. Food Sci. 42: 113.

Kitazawa, T. 1975. "Kin-niku," Ed. Ebashi, S. and Maruyama, K., p. 281. Tokyo Kagaku Dojin Co., Tokyo. (In Japanese).

Locker, R.H. 1959. Striation patterns of ox muscle in rigor mortis. J. Biophys. Biochem. Cytol. 6: 419.

Marsh, B.B. 1954. Rigor mortis in beef. J. Sci. Food Agr. 5: 70.

Mauriello, G.E. and Sandow, A. 1959. Active state of muscle in iodoacetate rigor. J. Gen. Physiol. 42: 865.

Mobley, B.A. 1977. Effect of calcium ions on rigor contractions in skinned frog muscle fibers. Biochim. Biophys. Acta 459: 325.

Nauss, K.M. and Davies, R.E. 1966. Changes in phosphate compounds during the development and maintenance of rigor mortis. J. Biol. Chem. 241: 2918.

Ogawa, Y. 1968. The apparent binding constant of glycoetherdiaminetetraacetic acid for calcium at neutral pH. J. Biochem. 64: 255.

White, D.C.S. 1970. Rigor contraction and the effect of various phosphate compounds of glycerinated insect flight and vertebrate muscle. J. Physiol. 208: 583.

Ms received 9/8/77; revised 1/3/78; accepted 1/10/78.

We thank Dr. S. Watanabe, Faculty of Science, Tokyo Institute of Technology, for his reading of the manuscript and useful suggestions.

MECHANISMS OF WATER LOSS OF BOVINE SEMITENDINOSUS MUSCLE DRY COOKED FROM THE FROZEN STATE

C. C. HUNG, E. A. DAVIS, J. GORDON and H. T. DAVIS

ABSTRACT

The mechanisms of water loss and their relation to muscle shortening were investigated by dry cooking in a controlled environment oven. Air was pumped vertically upward in the oven at a rate of 13.7 m^3/hr. Cylindrical muscle samples were cooked at 121°C, 149°C and 177°C. The axes of these samples were in vertical, 45° and horizontal directions with respect to the oven. The muscle fibers were parallel to the axes of these samples. It is observed that total weight loss is almost independent of sample orientation, implying that the draining of fluid by gravity (a mechanism which is believed to be closely related to sample orientation) is not an important mechanism of mass transfer. The main mechanism is possibly fiber shrinkage which squeezes fluid out during cooking. Linear relationships between remaining water content and sample length were observed both before and after protein denaturation. The slopes of these two linear relations are different, suggesting that the way fluid is squeezed out is different before and after protein denaturation. Also, these slopes are almost independent of oven temperature in the 121–177°C range, suggesting that the remaining moisture content depends primarily on the shortening during cooking of a sample and only weakly on the oven temperature and initial condition (i.e., frozen or frozen-thawed) of the sample.

INTRODUCTION

FOR THE PURPOSE of understanding temperature-time-weight loss (shrink) relationships, heat and mass transfer in bovine semitendinosus (ST) muscle cooked in a dry atmosphere has been the subject of research in this laboratory. Godsalve et al. (1977a, b) recently examined water loss rates of bovine ST muscle samples as affected by varying oven temperature, sample size, rate of air flow past the cooking muscle, the orientation of muscle fibers relative to direction of air flow, and postmortem treatment of the muscle prior to cooking in a controlled environment oven. The moisture loss rate curves possessed the general characteristics of two constant rate periods (these constant rate periods in some samples manifest themselves as maxima rather than plateaus) followed by a falling rate period. The different periods correspond to surface boiling, protein denaturation and progressive dehydration of the sample. Equilibrium of heat and mass transfer cannot be reached until total dryness of the meat sample. This is in contrast to the results of Hwang et al. (1977) that showed equilibrium of heat and mass transfer can be reached within two hours when meat samples are moist cooked in a water bath at temperatures ranging from 30–80°C.

In this study, the mechanisms of water loss and their relation to muscle shortening during cooking and protein denaturation were investigated by dry cooking in the controlled environment oven built by Godsalve et al. (1977a). Cylindrical muscle samples were studied by vertical, 45° and horizontal directions and were cooked at 121°C, 149°C and 177°C.

Authors Hung and H.T. Davis are with the Dept. of Chemical Engineering & Materials Science, University of Minnesota, 421 Washington Avenue S.E., Minneapolis, MN 55455. Authors E.A. Davis and Gordon are with the Dept. of Food Science & Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

0022-1147/78/0004-1191$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Muscle samples

Commercial bovine semitendinosus muscles were obtained from USDA Choice carcasses after a 5-day postmortem aging period at 4°C. These muscles were cut into cylindrically-shaped samples with fibers parallel to the axis of the cylinder. Two sample sizes were used, 100g and 600g. The respective dimensions (length × diameter) of these samples were 10.8 cm × 3.49 cm and 19.1 cm × 6.35 cm. A small hole (about 3.5 mm diameter) through the axis of every sample was drilled for insertion of a glass rod that suspends the muscle sample in the air. Muscle samples were kept frozen throughout the procedure of sample preparation. Unless otherwise stated, the samples were cooked from frozen state.

Apparatus

The controlled environment oven used in this experiment was the one used by Godsalve et al. (1977a) with several modifications. This oven is illustrated diagrammatically in Figure 1. In the cases where the axes of the cylindrical samples were horizontal or 45° from horizontal, the components enclosed in dashed lines in Figure 1(a) were replaced by those shown in Figure 1(b) and 1(c), respectively.

Atmospheric air was pumped through the pipe system (11) to the lower chamber (15) of the oven where the air was heated by an electric heater (10). The air then moved to the upper chamber (2) where a meat sample (7) was being cooked. The cylindrical meat sample, being hung in the air by a glass rod (8) and heat-insulated at both ends by two pieces of ¼ in. Teflon disks (9), was connected to a balance (1) on top of the oven by aluminum bars (13) and a glass rod. A meter (6) graduated in 1/8 in. divisions was hung next to the meat sample to measure the length of the sample continuously during cooking. A tray (4) was placed underneath the sample to collect the cooked out liquid drops (i.e., drip) during cooking. A temperature controller (12), kept the oven temperature constant.

For the purpose of estimating the amount of drip during cooking, spots of different sizes were drawn on a piece of tape (5) which was placed on the wall of the tray (4). These spots were used as a reference to estimate the sizes and, hence, amount of drip drops. For these experiments, four spots (0.40, 0.36, 0.34 and 0.32 cm diam) were drawn on the tape.

Admittedly, there would be some errors in estimating the amount of drip. Firstly, it is difficult to draw spots exactly the sizes wanted. Secondly, it is not easy to tell whether the size of a drip is closer to the size of the 0.36 cm spot or to that of the 0.34 cm spot, because the diameters of the two spots are very close. It is, however, believed that this error will not influence the comparative results obtained from this experiment.

Experimental procedure

(1) 100g frozen samples in vertical, 45° and horizontal directions were cooked at 121°C, 149°C and 177°C oven temperature. One sample was cooked in each run. The weight of the sample and the number of drip droplets formed per minute were measured throughout the experiments. From these data, the weight loss and rate of weight loss as a function of time can be calculated. Each experimental run was terminated at the onset of the falling rate period following the second peak of the drying rate curve. This end point is chosen because the important changes of muscles are believed to reach completion by this time. (It is interesting to note that the second peak always appears at the time when the centerline temperature indicates that the meat is in the range of doneness).

The rate of air flow through the oven was set at 13.7 m^3/hr in all runs.

All of the nine treatments (i.e., horizontal, 45°, and vertical cooked at 121°C, 149°C and 177°C) were triplicated.

The phenomena of mass transfer on the sample surface were also observed visually. These phenomena and the facts they reveal are discussed in the next section. *–Text continued on page 1192*

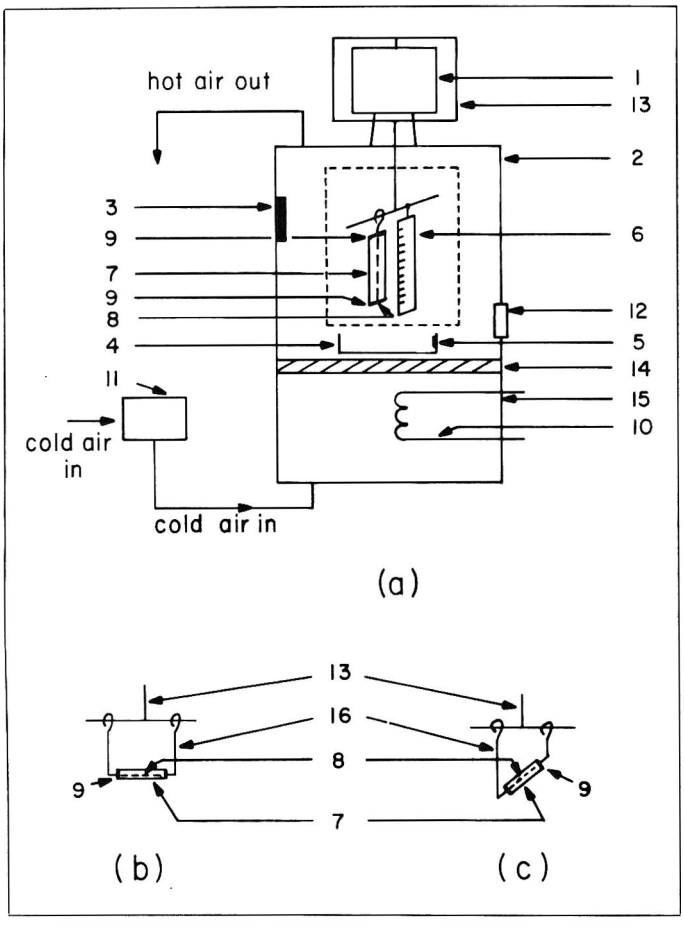


Fig. 1—Diagrammatical description of the equipment used in dry cooking: (a) vertical sample component enclosed in dashed line was replaced by (b) or (c) if horizontal or 45° sample were used. Component 5 and 6 were used only when 600g samples were used. (1) balance; (2) upper chamber of the oven; (3) window; (4) tray; (5) tape on which spots were drawn; (6) ruler; (7) meat sample; (8) glass rod; (9) Teflon plate; (10) heater; (11) pipe system and pump; (12) heat controller; (13) aluminum bars; (14) wire to separate upper and lower chamber; (15) lower chamber of the oven.

(2) 600g frozen samples, with axes vertical, were cooked in a 121°C and a 177°C oven. One sample was cooked in each run. The rate of air flow through the oven was set at 13.7 m^3/hr in all runs. The weights of the samples, the sizes of the drip droplets, the number of drip droplets formed per minute and the lengths of the samples were measured throughout the experiments. From these data, the weight loss, the rate of weight loss, the rate of drip in g/min (assuming density of drip is 1 g/cm^3), the rate of evaporation (difference between the rate of total loss and the rate of drip) and the weight ratio of water to dry solid in meat can be calculated. The approximation that the density of the drip is 1 g/cm^3 is based on the assumptions that under the cooking conditions used, the majority of the drip is water, and that the densities of protein and lipids are not making a major contribution.

The ratio of water to dry solid in meat can be obtained by assuming (1) 75% water and 25% solid in raw meat, and (2) negligible nonaqueous loss as compared to water loss. These assumptions can be justified by the facts that (1) actual composition of raw bovine ST muscle is 74.2% water, 2.10% fat and 21.9% protein (Swift and Berman, 1959) and (2) the nonaqueous weight loss of a nearly dried sample, calculated as the difference between total weight loss (Fig. 5 and Fig. 6) and water loss (Godsalve et al., 1977a), is about 1.3% of the weight of the original sample. Although, the exact value of this ratio may be in some error, the comparative results obtained in the next section are believed not to be distorted.

Both treatments (i.e., 600g samples, 121°C and 177°C oven temperature) were repeated four times.

(3) One 600g freeze-thawed sample was cooked in a 177°C oven. The procedure of this experiment is the same as that described in the last section. It is believed that some of the effects of thawing can be determined qualitatively by comparing the results obtained from these two sections. Data of internal temperature histories on 600g and 100g samples were obtained from the results by Godsalve et al. (1977a) and Godsalve (1976).

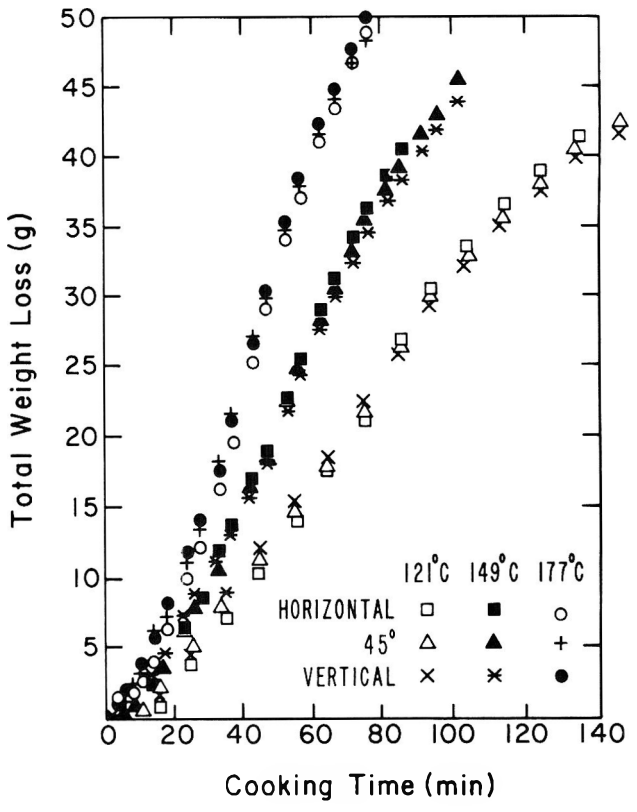


Fig. 2—Relation between total weight loss of 100g samples and cooking time (avg values from three experiments; data on some of the horizontal samples are omitted).

RESULTS & DISCUSSION

FIGURE 2 shows the effect of cooking time, cooking temperature and sample orientation on the weight loss of the 100g samples (original weight). It is clear that, as expected, temperature and time are important to the amount of weight loss. However, the amount of weight loss is not statistically dependent on the sample orientation ($P > 0.05$ for all time and oven temperatures, $P > 0.2$ for all oven temperatures at time $t > 35$ min). Since the fiber and, hence, sample orientation is believed to be closely related to the amount of moisture drained by gravity, it is suggested that the majority of weight loss is not due to the effect of gravity.

In most cases, two drip periods were observed. These phenomena are described as follows:

First drip period

(a) The amount of drip for vertical or 45° samples was more than that for horizontal samples (Fig. 3). This phenomenon is especially true for 121°C where P (vertical – horizontal) <0.001 and P (45° – horizontal) = 0.05, despite small number of replications (degree of freedom = 4) and great variations of number of drip drops in the first drip period. Recalling that the fibers were parallel to the axis of the cylindrical samples, it is suggested that it is more difficult for water to migrate across the fibers than along the fibers and that gravity played a role in determining the amount of drip in this period.

(b) In a few cases, only one drip period existed in the cooking processes (i.e., the one corresponding to the second

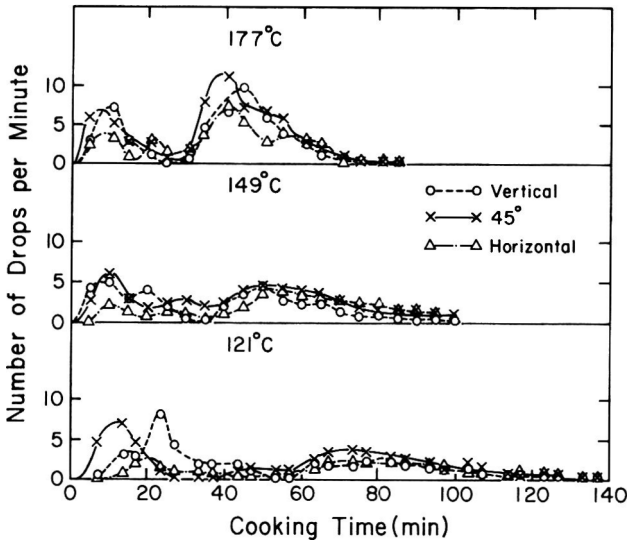


Fig. 3–Rate of drip from 100g samples as function of cooking time, oven temperature and sample orientation (avg values from three experiments).

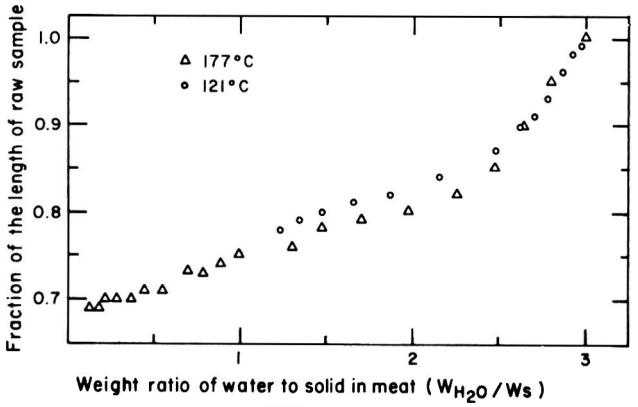


Fig. 4–Relation between length of meat sample and water content during cooking (75% water and 25% solid are assumed in the sample to prepare this graph; average values from four experiments).

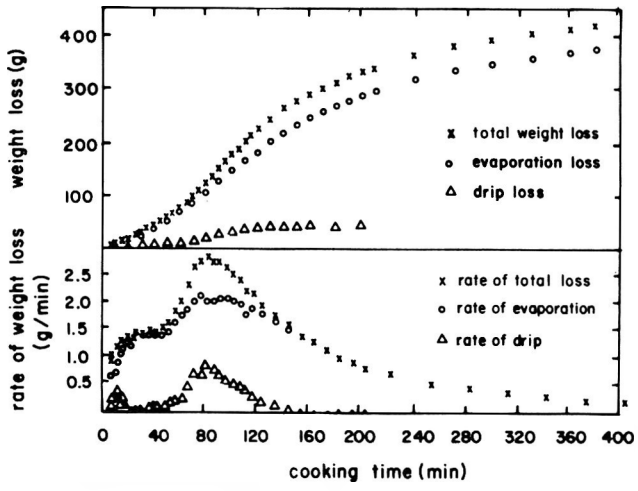


Fig. 5–Weight losses and rates of weight losses of 600g samples in 177°C oven; air flow rate 13.7 m^3/hr; average values from 4 experiments.

drip period in regular cases). In all such cases, the frozen samples to be cooked were exposed to room temperature for an extraordinary period of time and were believed to be partially thawed.

(c) Although the drip is small in this period, it was red, looked turbid and viscous. It dried slowly in the pan, possibly because it was cold and high in protein.

(d) According to Ramsbottom and Koonz (1939) and Wang et al. (1954), if meat samples are rapidly frozen (e.g., −150°C) both interfiber and intrafiber ice crystals occur. If the meat samples are slowly frozen (e.g., −17°C) only interfiber freezing takes place and the muscle fibers are partially dehydrated. It is believed here that the first drip period occurs when the interfiber ice crystals are thawed. Part of the liquid thus formed is absorbed by muscle fibers. The rest of it migrates parallel to the direction of the fibers, reaches the bottom end (or both ends if samples are horizontal) of the cylindrical sample where it either forms drip or is evaporated. If the rate of thawing is slow compared to the rate of absorption, it is possible that absorption is sufficiently effective to pick up most of the thawed liquid. The remainder of it adheres to the bottom of the sample or on the teflon disk (Fig. 1) and remains there until evaporated. In such cases, the drip in this period vanishes. It is believed that the intrafiber fluid will be retained by the fibers except those near the cutting ends where the fibers are severely damaged.

Second drip period

The drip in this period was clear. It evaporated explosively at the time when it dropped to the pan. Thus it was considered to be composed mostly of water. The surface of the meat samples appeared to be dry, or at least partially dry, after the time corresponding to the first peak of the drying curve. Therefore, it is believed that the sample cannot act as a wet bulb thermometer and, therefore, can exceed wet bulb temperature. However, as the cooking process continues, liquid drops started to be formed on the previously dried sample surface. Thus the surface was wet again. The sizes of these liquid drops grew with time and, finally, they flowed and dropped to the tray. This phenomenon was observed in all sample orientations. Therefore, it is implied that the liquid drops were squeezed out due to the cooking shortening of the meat sample and not drained out due to the effect of gravity, otherwise sample orientation should have some effect on this phenomenon and more liquid drops should be formed at the lower part of the samples.

Because the cooking shortening of the meat samples is an important mechanism in causing weight loss during cooking, a detailed study of this phenomenon is needed to facilitate further modeling of the heat and mass transfer in meat. A plot (Fig. 4) of sample length vs moisture content was thus made for this purpose. This graph shows linear relations between sample length and water content at high and low values of water content. The slopes of these linear functions change when the water content in meat is 2.25 W_{H_2O}/W_s 2.50 for a 177°C oven and is 2.15 W_{H_2O}/W_s 2.45 for a 121°C oven, where W_{H_2O} denotes the weight of moisture remaining in the sample and W_s the dry weight of the sample. The data obtained from the frozen-thawed sample (unpublished) gave the same results (within experimental error). This may indicate that the initial condition of the sample has little effect on the weight loss and muscle shortening behavior during cooking. However, further experiments need to be done in order to confirm this. The cooking time corresponding to these ranges of water content can be calculated from the data of weight losses and rates of weight losses given in Figures 5 and 6 (60–75 min for 177°C oven and 120–150 min for 121°C oven). These are the ranges of time corresponding to the rising parts of the second peaks of the overall drying curves and of

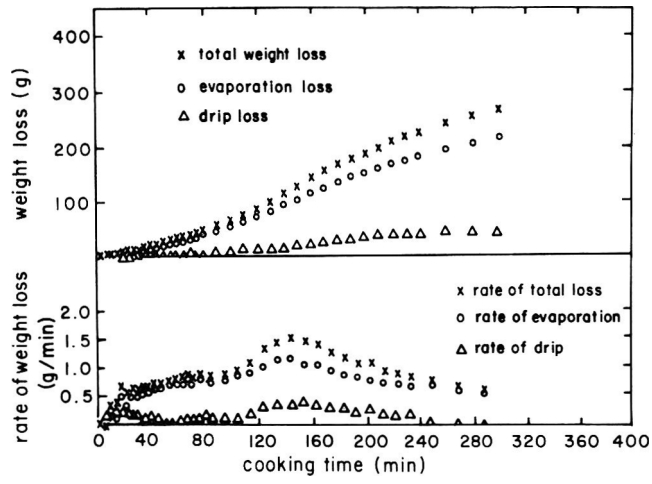


Fig. 6—Weight losses and rates of weight losses of 600g samples in 121°C oven; air flow rate 13.7 m^3/hr; average values from four experiments.

the drip curves (Figures 4 and 6), implying that the transition from one linear region to the other is related to the onset of protein denaturation. Thus it is suggested that the two linear functions at high and low water content correspond to two different mechanisms of shortening during cooking and the sharp curve in between corresponds to the changes of one mechanism to another. At an early cooking stage, ($W_{H_2O}/W_s \geqslant 2.6$ in Fig. 4) before protein denaturation is significant, the major route of mass transfer is along the fibers. As protein is denatured, the rate of mass transfer across the fiber increases greatly, possibly due to (1) cracks across muscle fibers as illustrated by Paul (1963) and Schaller and Powrie (1972), and (2) heat damage of sarcolemmae, the major barrier of mass transfer (Locker and Daines, 1974; Davey and Gilbert, 1975). Thus the shrinkage of the sample can force the intracellular water to migrate through the cracking points (or points of damaged sarcolemmae), across as well as along the fibers. As a result, the resistance to mass transfer decreases. A certain amount of shortening during cooking can therefore force more water out after protein denaturation ($W_{H_2O}/W_s < 2.3$ in Fig. 4).

Godsalve et al. (1977a) suggested that collagen shrinkage is the main driving force of mass transfer. This suggestion was based on the fact that the center part of the meat ($O < r < 2/3$ R, where r is the distance from the axis of the cylindrical sample and R is the radius of the sample) was in the temperature range of collagen shrinkage at the time corresponding to the second peak of the drying curve. While collagen shrinkage may be important, it is unlikely to be the only major driving force because the outer part of the meat ($2/3$ R $< r <$ R) constitutes 5/9 of the volume of the whole meat sample and cannot be neglected. Other mechanisms such as shrinkage and cracks of muscle fibers and damage to sarcolemmae may also be important.

Figures 5 and 6 show the total loss, drip loss, evaporation loss and the rates of these losses as functions of time at 177°C and 121°C oven temperature, respectively. Data from samples of 600g were used to prepare these figures. From these figures and the temperature histogram obtained by Godsalve et al. (1977a), it is possible to calculate the fraction of total loss contributed by drip. The results are given in Table 1.

From the table, it is clear that drips contribute a significant part of total weight loss and cannot be neglected. For the 600g cylindrical sample, cooking losses were smaller at lower temperature.

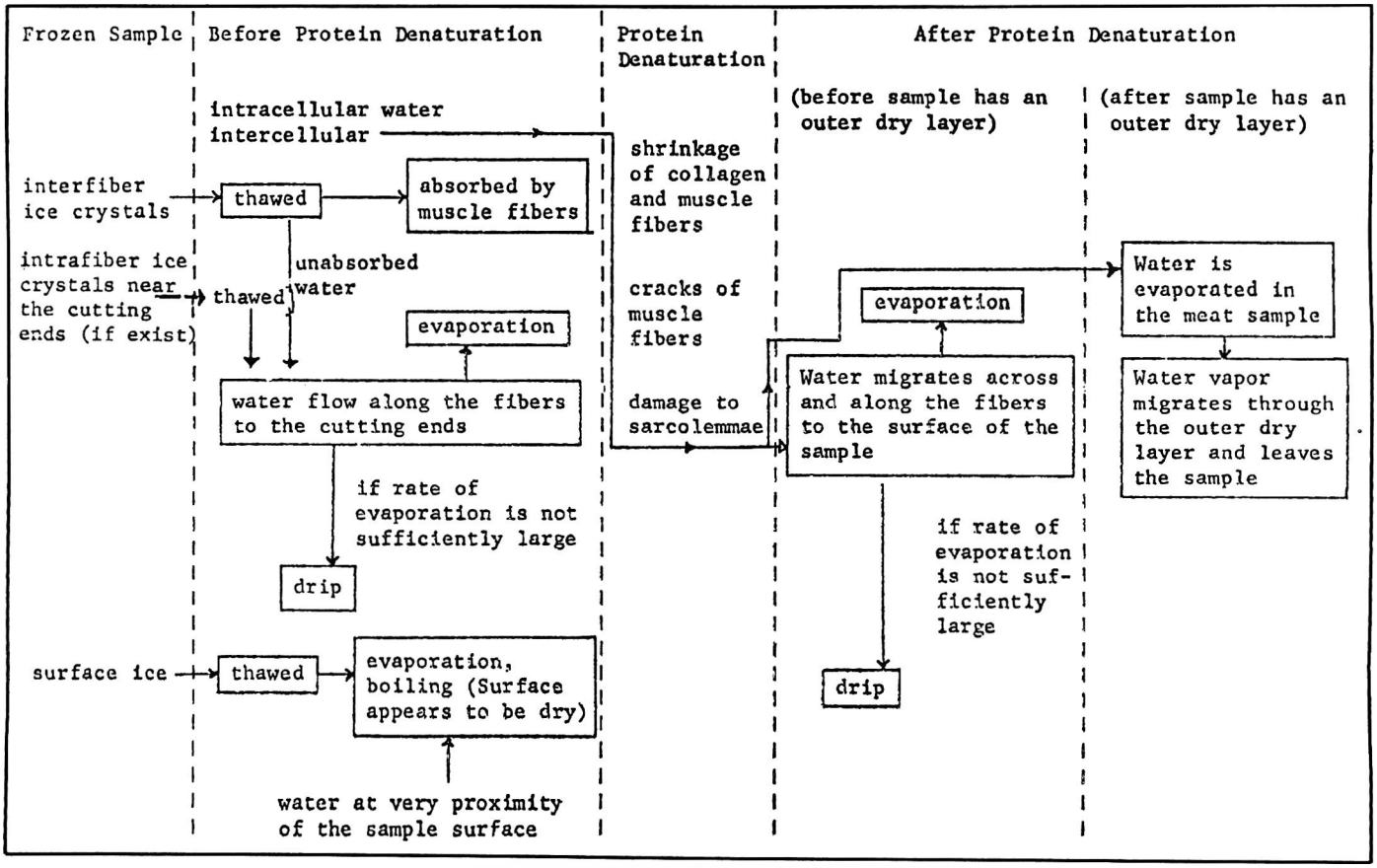


Fig. 7—Flow chart of water migration in meat during cooking.

Table 1–A comparison of total weight loss and drip loss at different temperature end points. Sample size: 600g; Air flow rate: 13.7 m^3/hr

Factor	Rare		Medium rare		Medium		Well done	Reference
Centerline temp (°C)	59	59	67	67	74	74	81	
Oven temp (°C)	177	121	177	121	177	121	177	
Cooking time (min)	75	120	87	150	99	180	107	Godsalve et al. (1977a)
Total wt loss (g)	112	86	146	128	180	169	198	Fig. 4 Fig. 5
% Yield	81	86	76	79	70	72	67	
Drip loss (g)	13.5	10	22.5	19	30.9	30	34.6	Fig. 4 Fig. 5
Drip Loss X 100% / Total wt loss	12	12	15	15	17	18	17	

One of the largest energy requirements in cooking meat is the evaporation of water. Thus, from the data presented herein, it is concluded that energy saving can be obtained by (1) thawing meat and letting surface water drain before cooking, (2) designing an oven that lets drip pass out of the cooking space without evaporation and (3) cooking at lower temperatures.

The mechanism of water migration suggested by the work of Godsalve et al. (1977a, b) and the present work has been summarized in the form of a chart illustrating mass transfer in meat during cooking (Fig. 7). By examining Table 1, Figures 5 and 6, it can be seen that regular cooking processes end approximately at the time corresponding to the second peak of the drying curve. Thus, the resulting cooked sample does not have an outer dry layer and both evaporation and drip mechanisms are still operative and contributing to cooking losses.

REFERENCES

Davey, C.L. and Gilbert, K.V. 1975. Cold shortening and cooking change in beef. J. Sci. Food Agr. 26: 761.

Godsalve, E.W. 1976. Heat and mass transfer in cooking meats. Ph.D. thesis, University of Minnesota, Minneapolis, MN.

Godsalve, E.W., Davis, E.A., Gordon, J. and Davis, H.T. 1977a. Water loss rates and temperature profiles of dry cooked bovine muscle. J. Food Sci. 42: 1038.

Godsalve, E.W., Davis, E.A. and Gordon, J. 1977b. The effect of oven conditions and sample treatment on water loss of dry cooked bovine muscle. J. Food Sci. 42: 1325.

Hwang, P.T., Addis, P.B., Rosenau, J.A., Nelson, D.A., Thompson, D.R. 1977. Use of a rapid muscle protein solubility technique to predict beef muscle yield as a function of time, temperature, salt and phosphate. J. Food Sci. 42: 590.

Locker, R.H. and Daines, G.J. 1974. Cooking loss in beef. The effect of cold shortening, searing and rate of heating; time course and histology of changes during cooking. J. Sci. Food Agr. 25: 1411.

Paul, P.C. 1963. Influence of method of cooking on meat tenderness. In "Proceedings Meat Tenderness Symposium." Campbell Soup Company, Camden, NJ.

Ramsbottom, J.M. and Koonz, C.H. 1939. Freezing temperature as related to drip of frozen-defrosted beef. Food Res. 4: 425.

Schaller, D.R. and Powrie, W.D. 1972. Scanning electron microscopy of heated beef, chicken and rainbow trout muscle. Can. Inst. Food Sci. Technol. J. 5: 184.

Swift, C.E. and Berman, M.D. 1959. Factors affecting the water retention of beef. 1. Variations in composition and properties among eight muscles. Food Technol. 13: 365.

Wang, H., Aubreach, E., Bate, V., Doty, D.M., Kraybill, H.R. 1954. A histological and histochemical study of beef dehydration. 4. Characteristics of muscle tissues dehydrated by freeze drying techniques. Food Res. 19: 543.

Ms received 8/25/77; revised 12/14/77; accepted 12/20/77.

Scientific Journal Series Article No. 10005, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

This study was supported in part by the University of Minnesota Agricultural Experiment Station Projects No. 18—27 and No. 18—63, and Contract No. ENG 76-09808, National Science Foundation, Washington, DC.

BACTERIAL FLORA OF ANTARCTIC KRILL (Euphasia superba) AND SOME OF THEIR ENZYMATIC PROPERTIES

M. D. KELLY, S. LUKASCHEWSKY and C. G. ANDERSON

ABSTRACT

Four fresh and two frozen samples of the krill (*Euphasia superba*) were examined bacteriologically. Coryneform like organisms were predominent in the bacterial flora of fresh krill along with strains of *Pseudomonas*, *Moraxella*-like taxa, *Alcaligenes*, and *Flavobacterium*. Bacterial counts were low and ranged from 650/g–1,100/g at 25°C. Studies of the spoilage flora developing during cold storage show that, similar to other fishery products, bacteria of the *Pseudomonas*, *Alcaligenes* and *Moraxella*-like taxa groups predominate. The bacterial count was low, 9,800/g at 25°C, on krill processed and stored at –20°C for 45 days. Enzymatic evaluation showed that krill bacteria have strong proteolytic and lipolytic activity.

INTRODUCTION

SINCE THE DEMISE of whales, due to heavy exploitation, scientists from various countries have suggested that the krill, on which the whales feed, could be enjoying a population boom. Krill (*Euphasia superba*) is a small Antarctic crustacean with an average length of 4–6 cm and average weight of 0.7–1.2g. A conservative estimate of krill stock puts the total at over 3 billion metric tons. Conceivably, krill could help fill a gap in the world's available food supply and is possibly the largest animal protein resource left in the world today.

As with many other fishery products, quality standards are important, especially for exportation purposes, since microbial deterioration would have a deleterious effect upon quality. Information on the type and extent of microbial flora and changes which occur during refrigeration can be important in the prevention of spoilage.

The microbiology of fish and fishery products was reviewed by Shewan (1971) and Shewan and Hobbs (1967). More recent complementary work has been done by Horsley (1973), Trust (1975), Gilespie and Macrae (1975), and Gilmour et al. (1976). Research on the initial and subsequent spoilage flora of Crustacea has been published by Walker et al. (1970), Cann et al. (1971), Cobb et al. (1976), and Vanderzant et al. (1970).

It was concluded from the published work that the most common bacteria associated with fish and Crustacea in their natural environment comprise mainly the coryneform group, *Pseudomonas*, *Alcaligenes*, *Micrococcus*, *Flavobacterium*, and *Moraxella*-like taxa. The spoilage of iced fishery products generally results from increased growth of Gram negative bacteria of the *Moraxella*-like taxa, *Pseudomonas*, and *Alcaligenes* genera.

Some of the taxonomy of microorganisms found associated with fish and crustacea in the marine environment is still indefinite, and some of the organisms found in the Antarctic waters may be new species. Using the methods and materials available, it was not possible to attach species names to the bacteria isolated. This paper presents a preliminary investigation of the flora of freshly caught and frozen krill plus the results of some studies on the subsequent enzymatic activity of this flora.

Authors Kelly and Lukaschewsky are with the Instituto de Fomento Pesquero, Jose Domingo Canas 2277, Casilla 1287, Santiago de Chile. Author Anderson formerly with the Instituto de Nutricion Y Tecnologia de los Alimentos Universidad de Chile, Casilla 15138, Santiago-11, Chile, is now located at 1132 EAst 8th South, Salt Lake City, UT 84102.

0022-1147/78/0004-1196$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Sampling

Owing to the long distance between the Antarctic fishing grounds and the laboratory in Santiago, Chile, four samples were taken directly from the water and cultivated on board the fishing vessel. The two frozen samples were processed from krill transferred from the freezer compartment of the ship to storage freezers at –20°C in the laboratory.

Microbiological procedures

Total viable counts. For aerobic plate counts Plate Count Agar (PCA) with 1.5% NaCl was used. Both fresh and frozen krill samples were prepared for plating by blending for 2 min, 50g of sample and 450 ml of sterile 0.1% w/v peptone in M/15 phosphate buffer, pH 7.0. After the particulate matter settled, decimal dilutions of the homogenate were then prepared. Duplicate inoculated plates were incubated for 3 days at 25°C.

Bacterial flora. To determine bacterial types 20 colonies were picked at random from plates containing between 30–100 countable colonies. The colonies picked from the plates on board the fishing vessel were streaked for purification and stored on PCA agar slants containing 1.5% salt at 5°C. They were kept at this temperature for 30 days until reaching the laboratory for identification. A modified identification scheme incorporating the methods of Hendrie et al. (1968), Shewan (1971), Gilmour et al. (1976), and using the taxonomic names found in the 8th edition of *Bergey's Manual* (1974) was used.

The criteria and tests employed were as follows:

(1) Morphology, colony appearance, Gram reaction, acid-fast reaction, pigment production, temperature tolerance at 0, 5, 15, 25 and 35°C, and the oxidase test (Kovacs, 1956) were performed on cultures growing on Difco Nutrient Agar (NA) plus 1.5% salt. Pigment production was also demonstrated on Kings A & B media. Colony appearance and color were noted on agar containing casein and tellurite. The ability to use poly-β-hydroxybutyrate as an intracellular carbon reserve was used to help differentiate pseudomonads (Stainer et al., 1966).
(2) Motility was based on the movement observed in phase contrast microscopy and the use of motility test medium. Bacteria were gently washed with sterile water from 24–48 hr NA slants for phase contrast examination.
(3) The glucose utilization test of Hugh and Leifson (1953) with added 1.5% (w/v) NaCl was used for the oxidation-fermentation examination of Gram negative rods.
(4) The composite Arginine Glucose Medium (AG) of Stewart (1971) was used for the arginine dihydrolase and acid and/or gas production from glucose of Gram negative rods. Arginine and lysine broths were also used for testing sole carbon source utilization.

Extracellular enzymatic activity

All the bacteria samples from both fresh and frozen krill were tested for proteolysis, lipolysis, carbohydrate hydrolysis, and cellulytic activity. A basal medium of NA was used for the incorporation of the appropriate substrates. Casein, gelatin, and "Azocoll," a degraded form of collagen (Wellcome Research Laboratories, Beckenham) were used for testing proteolysis, tributyrin was used for lipolysis, starch for carbohydrate hydrolysis, commercially produced chitin for chitin hydrolysis, and filter paper for cellulose breakdown. The methods of Barnes and Melton (1971) for demonstrating enzymatic activity were used.

RESULTS

Bacterial counts

The total viable counts for fresh and frozen krill were low.

Table 1—Bacteriological examination of krill (Euphasia superba)

Sample	Total viable count/g 25°C	Percentage numbers of bacteria											
		Pseudomonas I[a]	Pseudomonas II[a]	Pseudomonas III-IV[a]	Moraxella-like taxa	Acinetobacter	Alcaligenes	Flavobacterium	Coryneform types	Micrococcus	Vibrio	Bacillus	Enterobacter
Fresh krill	650–1,100	1	5	19.5	13	1	9	4	40	2.5	2.5	2.5	
45 day frozen krill	9,800	4	9	30	17	4	13	9		13			
6 mo frozen krill	5,000	9	4.5	27	27	4.5	14	9					4.5

[a] The pseudomonads were tentatively grouped into three of their four classes according to Stainer et al. (1966).

Table 2—Substrate utilization of krill bacteria

	Fresh krill	Frozen krill	
		45 day	6 mo
Total no. of isolates	80	23	22
% giving:			
Casein hydrolysis	48.75	33	36
Gelatin hydrolysis	56.25	43	41
"Azocoll" breakdown	57.50	52	–
Lipolysis of tributyrin	57.50	74	41
Starch hydrolysis	15.00	4	4.5
Chitin decomposition	1.25	4	0
Cellulose breakdown	0	0	–

At 25°C the bacterial count of fresh krill ranged from 650/g to 1,100/g, while krill processed and frozen for 45 days averaged 9,800/g and krill frozen 6 mo, 5,000/g (Table 1).

Bacterial flora

Samples of fresh krill were examined from four separate locations in the Antarctic. Two frozen krill samples from Santiago were examined and compared with fresh samples. Eighty isolates were taken from fresh samples on board ship and 45 from frozen krill. The bacterial flora of the samples are presented in Table 1. Coryneform type organisms comprised 40% of the flora associated with fresh krill. Two sub-groups could be identified among the coryneform isolates. One subgroup exhibited traits described in Bergey's Manual for *Arthrobacter*, except for appearing motile, while the other resembled a *Corynebacterium*. After the coryneform types, *Pseudomonas*, *Moraxella*-like taxa, and *Alcaligenes* made up the majority of flora present in fresh krill samples.

Frozen samples were kept at −20°C in the freezer compartments of the ship until being transferred to the storage freezer in the laboratory approximately 30 days later. The predominant bacteria of these samples were *Pseudomonas*, *Moraxella*-like taxa, *Alcaligenes*, *Flavobacterium*, and *Micrococcus* with a complete absence of the coryneform types.

Enzymatic activity

The results obtained for substrate utilization by the krill bacteria are given in Table 2. The strains showed proteolytic activity for casein, gelatin, and "Azocoll," lipolytic activity for tributyrin, little activity towards starch, none against cellulose, and with the exception of one pseudomonad organism, did not attack chitin.

DISCUSSION

TABLE 1 shows that the total viable counts of bacteria on fresh krill are low. This is probably due to the low temperature of the water (−1.6°C) and to an environment relatively free from contamination. As to be expected from a cold marine environment, the majority of the organisms grew best between 5 and 25°C and quickly died at temperatures over 30°C. The low viable counts found associated with fresh krill point to the possibility that more spoilage will be due to indigenous enzymatic breakdown than to bacterial.

The flora analysis agrees well with the reports mentioned in the introduction showing that in fresh Crustacea coryneforms, *Pseudomonas*, *Moraxella*-like taxa, and *Alcaligenes* predominate. Almost all the bacteria isolated were psychrophilic, and no organisms of the enteric coliforms were found, which is to be expected from the Antarctic waters. The shift to more *Pseudomonas* and elimination of coryneforms is common in frozen fishery products. Any differences in microbial flora of krill compared to other Crustacea can be attributed to differences in species, marine environment, time and temperature of ice storage, and handling practices.

Enzymatic evaluation shows that most of the psychrophilic spoilage organisms have strong proteolytic and lipolytic activity. This could be important in the development of rancidity on prolonged storage of krill.

REFERENCES

Barnes, E.M. and Melton, W. 1971. Extracellular enzymatic activity of poultry spoilage bacteria. J. App. Bact. 34: 599.

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

Cann, D.C., Hobbs, G., Wilson, B.B. and Horsley, R.W. 1971. The bacteria of scampi (Nephrops norvegicus). 2. Detailed investigation of the bacterial flora of freshly caught samples. J. Fd. Technol. 6: 153.

Cobb, B.F. III, Vanderzant, C., Hanna, M.O. and Yeh, C-P.S. 1976. Effect of ice storage on microbiological and chemical changes in shrimps and melting ice in a model system. J. Food Sci. 41: 29.

Gillespie, N.C. and Macrae, I.C. 1975. The bacterial flora of some Queensland fish and its ability to cause spoilage. J. Appl. Bact. 39: 91.

Gilmour, A., McCallum, M.F. and Allan, M.C. 1976. A study of the bacterial types occurring on the skin and in the intestines of farmed plaice (Pleuronectes plateris L.). Aquaculture 7: 161.

Hendrie, M.S., Mitchell, T.G. and Shewan, J.M. 1968. "Identification Methods for Microbiologists," Part B. Ed. Gibbs, B.M. and Shapton, D.A., p. 67. Academic Press, London and New York.

Horsley, R.M. 1973. The bacterial flora of the Atlantic Salmon (Salmo salar L.) in relation to its environment. J. Appl. Bact. 36: 377.

Hugh, R. and Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. J. Bact. 66: 24.

Kovacs, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature 178: 703.

Shewan, J.M. 1971. The microbiology of fish and fishery products—A progress report. J. Appl. Bact. 34: 299.

Shewan, J.M. and Hobbs, G. 1967. "Progress in Industrial Microbiology," Vol. 6, Ed. Hockenhull, D.J.D., p. 169. Hiffe Books Ltd., London.

Stainer, R.Y., Palleroni, N.J. and Doudoroff, M. 1966. The aerobic Pseudomonads: a taxonomic study. J. Gen. Microbiol. 43: 159.

Stewart, (1971).

Trust, T.J. 1975. Bacteria associated with the gills of salmonid fishes in freshwater. J. Appl. Bact. 38: 225.

Vanderzant, C., Mroz, E. and Nickelson, R. 1970. Microbial flora of Gulf of Mexico pond shrimp. J. Milk & Food Technol. 33: 346.

Walker, P., Cann, D.C. and Shewan, J.M. 1970. The bacteriology of "Scampi" (Nephrops norvegicus). 1. Preliminary bacteriological, chemical and sensory studies. J. Fd. Technol. 5: 375.

Ms received 8/19/77; revised 11/28/77; accepted 12/1/77.

The authors acknowledge the assistance of Mr. R. Toro in obtaining and maintaining the samples on board ship in the Antarctic. Our thanks also, to Dr. Kenneth L. Simpson for his help in the preparation of the final manuscript. Dr. Anderson extends his gratitude to the United States Peace Corps who made it possible for him to take part in this research.

FLAVOR COMPOUNDS RELATED TO THE WARMED–OVER FLAVOR OF TURKEY

E. L. RUENGER, G. A. REINECCIUS and D. R. THOMPSON

ABSTRACT

Taste panel results indicated that the warmed-over flavor of turkey meat was readily detectable and was equally detectable in both white and dark meat. Statistical analysis of gas chromatographic flavor profiles comparing fresh and reheated turkey meat selected three components of the profile as being correlated to the warmed-over flavor. The three components were found to increase due to reheating. Two of the three were identified as heptanal and n-nona-3,6-dienal. The remaining compound could not be identified. The two known compounds are typical end-products of lipid oxidation which further supports the hypothesis that warmed-over flavor is due to lipid oxidation.

INTRODUCTION

CHANGING SOCIAL PATTERNS and eating habits have greatly increased the demand for precooked foods. The acceptance of some precooked foods, especially meat products, is diminished due to a flavor defect which develops during reheating. The off-flavor is often described as warmed-over, stale, reheated, or institutional. The problem often makes nutritionally acceptable products unpalatable. Turkey, is especially subject to the development of a warmed-over flavor (WOF) (Wilson et al., 1976).

WOF is generally accepted to be the result of autoxidation of tissue lipids. Initial workers (Hirano and Olcott, 1971; Kendrick and Watts, 1969; Younathan and Watts, 1960) suggested that the oxidation was heme catalyzed; however, more recent data (Love and Pearson, 1974; Sato and Hegarty, 1971) have shown nonheme iron to be the major proxidant. Wilson et al. (1976) suggested that the phospholipids play a major role in the formation of WOF in turkey, beef and chicken while total lipids were most important to WOF in pork.

The purpose of our study was to determine if a relationship existed between taste panel responses to WOF and volatile flavor constituents in turkey.

METHODS

Source of turkeys

Turkeys were purchased from a commercial processor (Richland Foods, Faribault, MN) as frozen, unadulterated birds. Twelve to fourteen-pound hen turkeys were used. Each was halved, wrapped and stored at −29°C to allow paired comparisons.

Cooking method

One-half of a bird was thawed at room temperature and wrapped in aluminum foil. It was then placed in a preheated oven (177°C) until an internal temperature of 82°C was reached. If the meat were to be reheated, it was then cooled at room temperature followed by refrigeration at 4°C. After storage for 48 hr, the bird was reheated in an oven preheated to 177°C until an internal temperature of 82°C was reached.

Author Ruenger is with the Dept. of Environmental Health & Safety, University of Minnesota, Minneapolis, MN 55455. Author Reineccius is with the Dept. of Food Science & Nutrition, University of Minnesota, St. Paul, MN 55108. Author Thompson is with the Dept. of Agricultural Engineering, University of Minnesota, St. Paul, MN 55108.

0022-1147/78/0004-1198$02.25/0
© 1978 Institute of Food Technologists

Organoleptic evaluation

A flavor panel was held weekly for 12 wk to compare freshly cooked to reheated turkey meat. The panel consisted of 6 male and 9 female untrained members, 10 of which took part in each testing session. A triangle test was used to compare fresh to reheated turkey meat. The order of sample examination and sample numbering was randomized. Samples were served warm (60°C), and apple juice was used to rinse the mouth between samples.

Extraction methods

Samples were evaluated within 20 min of finish cooking of the samples. Three hundred grams of white meat (or 275g of dark meat) were ground in a Waring Blendor in 3L of distilled water. The slurry was poured into a 5-L round bottom flask and distilled using steam vacuum distillation. Ice water was used to cool the condensers and liquid nitrogen was used in three Dewar traps. A vacuum (50 mm Hg) was drawn by the vacuum pump, and the steam generator was kept between 40° and 45°C. After about 2 hr, 180 ml of distillate had been collected and the distillation stopped. The distillate was then transferred to a lighter-than-water continuous extractor (Ace Glass Inc., Vineland, NJ) for extraction with pentane which had been purified by the method of Murray and Keller (1969). The Dewar traps were rinsed with pentane before and after distillation. The pentane rinse containing flavor compounds was poured into the distillation reservoir of the continuous extractor. Enough pentane was added to have a reservoir of 20 ml in the side flask. The extraction was conducted for 3½ hr after which the side flask was removed and the pentane was dried with 0.2g of anhydrous $MgSO_4$. The pentane was filtered and filtrate placed into a screw capped test tube. The volume of the sample was then reduced to 0.1 ml under a stream of N_2 gas.

Gas liquid chromatography

A Hewlett-Packard model 7620A research gas chromatograph equipped with a hydrogen flame ionization detector was used in this study. Separation of the concentrated volatile material was accomplished using a 3m by 0.32 cm o.d. stainless steel column packed with 10% Carbowax 20 M on 80/100 Gas Chrom P. The column was programmed from 50–195°C at 4°/min with a 2-min post injection hold and a 15 min hold at final limit. A Hewlett-Packard 3370B electronic integrator was used to determine peak areas.

Mass spectrometry

A LKB model 9000 combined gas chromatograph-mass spectrometer was used to identify unknown flavor compounds. Separation of the flavor compounds was obtained using the same Carbowax column as described. The LKB 9000 has, as a detection system, a total ion current recording of the gas chromatographic eluants. Flash heater and molecular separator temperatures were 280°C. Mass spectra were obtained with a constant accelerating voltage of 3,500 volts with 70 ev energy, and a scanning time of 5 sec over a m/e range of 10–300. Compounds were considered identified when mass spectra and gas chromatographic retention times of known compounds were compatible with those of the unknown compounds.

Statistical analysis

Fractional values for each chromatographic peak were obtained by dividing each peak area by total chromatogram peak area. Statistical analysis was conducted in two steps. First, meats were grouped into categories: fresh white meat, reheated white meat, fresh dark meat and reheated dark meat. The mean and standard deviation of each fractional value (6 trials) were determined for each category. No attempt was made to compare the bird half which was fresh to the corresponding half which was reheated. The average fractional value ($\bar{X}_F$) for 6 fresh birds was compared to the average fractional value of 6 warmed-over birds ($\bar{X}_R$). (H_o: $\bar{X}_F = \bar{X}_R$, H_a: $\bar{X}_F \neq \bar{X}_R$). The results were analyzed using the student's T test. Those peaks which had statistically significant differences in their averages were analyzed in the second phase. In this second phase of analysis, a comparison was made between fractional values of peaks in the individual fresh birds to their corre-

sponding reheated halves. The differences between the individual fractional values (d) were analyzed for whether there was (H_o:d = 0) or was not (H_a:d ≠ 0) a significant difference in fractional value.

Identification of flavor compounds

An LKB model 9000 combined gas chromatograph-mass spectrometer was used to identify unknown compounds for which a significant difference was found between the gas chromatogram of freshly cooked and reheated turkey meat.

Separation of the flavor compounds was obtained using the same Carbowax column and temperature program as described earlier. Compounds were considered identified when mass spectra and gas chromatographic retention times of known compounds were compatible with those of the unknown compounds.

Table 1—Organoleptic detection of freshly cooked and reheated turkey meat

Sample	No. of samplings	No. Correct	Significance
White meat	76	41	$p < 0.001$
Dark meat	77	42	$p < 0.001$

Table 2—Paired comparison student's t-test[a] of turkey flavor components which were found to be significantly different (α = 0.15) following reheating

Peak[b]	Average difference[c]	Standard deviation	t Value	
4	−0.1149	0.0792	5.025	$p < 0.001$
5	−0.1282	0.1272	3.491	$p < 0.01$
6	+0.0800	0.1534	1.806	
8	−0.1020	0.1476	2.394	$p < 0.05$
11	−0.5733	1.302	1.525	
15	−0.0692	0.1660	1.444	
16	−0.1761	0.3095	1.971	

[a] The paired comparison student's t-test was computed as follows:

$$t_{n-1} = \bar{D} / \left(S_d / \sqrt{n} \right) \quad \text{where} \quad S_d^2 = \frac{\left[\left(\sum_{n=1}^{i=12} D_i^2 \right) - \frac{\left(\sum_{n=1}^{i=12} D_i \right)^2}{n} \right]}{n - 1}$$

where S_d = standard deviation, n = number of samples, D = difference in fractional value.

[b] See Fig. 1

[c] Average difference, fresh meat fractional value minus reheated meat fractional value.

RESULTS & DISCUSSION

TASTE PANEL DATA (Table 1) indicate that the panel could distinguish between fresh and reheated turkey samples ($p < 0.001$).

Wilson et al. (1976) reported that red muscle consistently had higher TBA numbers than white muscle. Since several workers have shown a correlation between TBA and WOF, their data would suggest that the WOF is more intense and easier to detect in the red muscle than the white muscle. Our results do not support this hypothesis for our panel had equal success in selecting the WOF in dark and light muscle.

A typical gas chromatogram of the volatile constituents of turkey is presented in Figure 1. Visual inspection of the chromatograms of freshly roasted and reheated turkey yielded no obvious differences between the two samples. Peaks were labeled and area computed for use in statistical analysis. In the first phase, computer analysis, comparing by groups (white meat or dark) each peak of the chromatograms from freshly cooked turkey to its corresponding peak in chromatograms from reheated turkey meat, found peaks 4, 5, 6, 8, 11, 15 and 16 (Fig. 1) to be significantly different (α = 0.15). Paired comparison (Student's t-test) was conducted on these seven peaks. Peaks 4, 5 and 8 were found to be statistically significant (Table 2) using α = 0.05.

A paired analysis (Student t-test) of white meat (individual fresh half vs corresponding reheated half) and dark meat (individual fresh half vs corresponding reheated half) was conducted (Table 3). Statistically significant differences (α = 0.10) were observed for peaks 4, 5 and 8 in dark meat, but only for peak 4 in white meat.

All three compounds increased in concentration in the reheated sample (Table 3). This would suggest that the WOF is

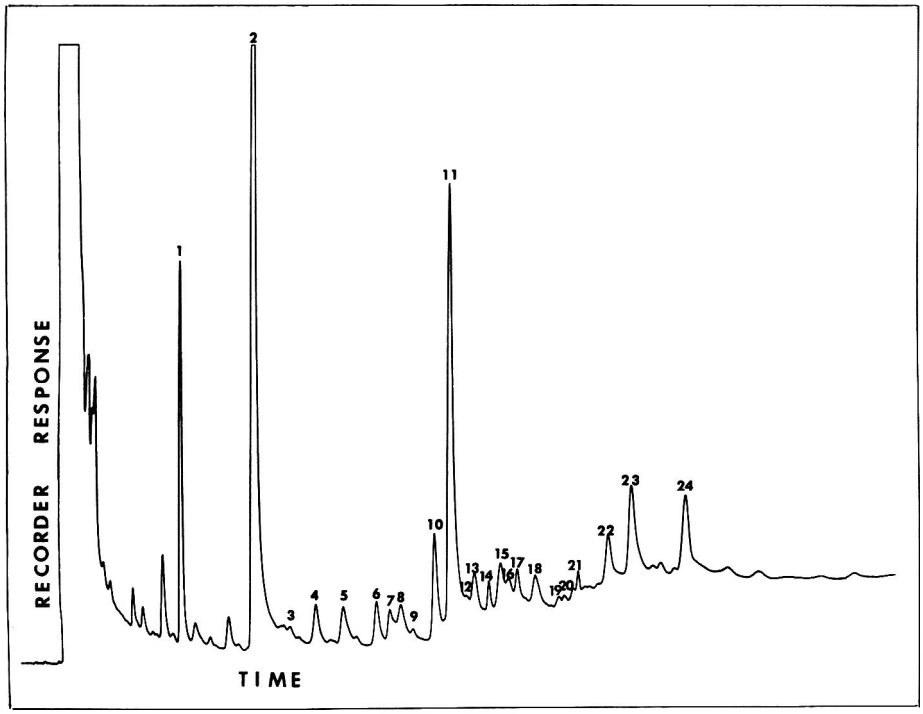


Fig. 1—Gas chromatographic profile of the volatile constituents in turkey.

Table 3—Comparison of paired analyses of individual fresh to reheated white meat halves and individual fresh to reheated dark meat halves.

Peak[a]	White meat	Dark meat
4	$\bar{b} = -0.1236$	$\bar{b} = -0.1062$
	$S_d = 0.0991$	$S_d = 0.0619$
	$t = 3.056$	$t = 4.203$
	$p < 0.05$	$p < 0.01$
5	$\bar{b} = -0.1109$	$\bar{b} = -0.1456$
	$S_d = 0.1753$	$S_d = 0.0641$
	$t = 1.55$	$t = 5.565$
		$p < 0.01$
8	$\bar{b} = -0.0865$	$\bar{b} = -0.1176$
	$S_d = 0.1760$	$S_d = 0.1278$
	$t = 1.204$	$t = 2.254$
		$p < 0.10$

[a] Fig. 1

[b] $\bar{b}$ = Average differences, fresh meat fractional value minus reheated meat fractional value.

S_d = Standard deviation

t = t-test value

Table 4—Mass spectral data of significant peaks compared with standard compounds

Heptanal (Peak 4)[a,d]			n-Nona-3,6-dienal[b] (Peak 5)[a]			1-Nonene (Peak 8)[a]		
m/e	Standard	Isolate	m/e	Standard	Isolate	m/e	Standard[c]	Isolate
27	35.7	42.9	27	32.5	13.7	27	46.4	39.6
29	50.0	53.6	29	14.8	11.5	29	48.3	48.1
41	69.0	69.6	39	27.0	10.0	39	36.8	24.5
42	54.8	42.9	41	42.4	16.1	41	81.8	69.8
43	83.3	77.7	43	11.5	5.2	42	38.7	46.2
44	100.0	100.0	53	11.8	15.0	43	100.0	100.0
55	55.6	44.6	54	10.7	2.8	55	65.4	61.3
57	50.8	42.0	67	16.9	4.3	56	77.8	93.4
70	90.5	59.8	81	100.0	100.0	69	34.4	27.4
71	27.0	24.1	82	10.7	23.5	70	36.3	7.5
			55		9.1			
			138		14.1			

[a] Fig. 1
[b] Literature values for n-nona-2,4-dienal obtained from *Complication of Mass Spectral Data, Supplement II,* A. Cornu and R. Massot
[c] Literature values obtained from the American Petroleum Institute Research Project 44, Serial Number 240.
[d] Compound co-chromatographed with standard.

due to the formation of additional volatile material rather than a reduction in the "fresh turkey" flavor components.

Two of the three significant peaks were identified, peak 4 as heptaldehyde and peak 5 as n-nona-3,6-dienal (see Table 4). Flavors attributed to heptaldehyde are characterized as "very strong, fatty, harsh, pungent odor, and unpleasant fatty taste" (Furia and Bellanca, 1975). Nonconjugated dienals ($C_8 - C_{10}$) have a "trainy-fishy" odor (Badings, 1960). Both of these compounds are typical products of lipid oxidation. This further supports the contention that WOF is the result of lipid oxidation.

REFERENCES

Badings, H.T. 1960. Principles of autoxidation process in lipids with special regard to the development of autoxidation off-flavors. Neth. Milk & Dairy J. 14: 215.

Furia, T.E. and Bellanca, N. 1975. "Fenaroli's Handbook of Flavor Ingredients," 2nd ed. CRC Press, Inc., Cleveland, OH.

Hirano, Y. and Olcott, H.S. 1971. Effect of heme compounds on lipid oxidation. J. Am. Oil Chem. Soc. 48: 523.

Kendrick, J. and Watts, B.M. 1969. Acceleration and inhibition of lipid oxidation by heme compounds. Lipids 4: 454.

Love, J.P. and Pearson, A.M. 1974. Metmyoglobin and nonheme iron as proxidants in cooked meat. J. Agric. Food Chem. 22: 1032.

Murray, E.C. and Keller, R.N. 1969. Purification of hydrocarbon solvents with a silver nitrate column. J. Org. Chem. 34: 2234.

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

Wilson, B.R., Pearson, A.M. and Shorland, F.B. 1976. Effect of total lipids and phospholipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. J. Agric. Food Chem. 24: 7.

Younathan, M.T. and Watts, B.M. 1960. Relationship of meat pigments to lipid oxidation. Food Res. 25: 538.

Ms received 8/17/77; revised 1/14/78; accepted 1/18/78.

Scientific Journal Series Paper No. 9980, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

EFFECT OF COOKING METHOD ON TBA NUMBERS OF STORED BEEF

WENN HSU HUANG and BARBARA E. GREENE

ABSTRACT

Conditions favoring the development of TBA retarding activity (TBRA) in cooked meat were studied. Beef semitendinosus muscle was heated by different home cooking methods (roasting, braising, pressure cooking, pressure canning, and electric slow-cooking). Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) test after storing meat for 5 days. Meat which was subjected to high temperatures and/or long periods of heating developed lower TBA numbers than did samples subjected to lower temperatures for shorter periods of time. TBRA was postulated to have resulted from antioxidant substances produced from browning reactions which progress as the meat is heated.

INTRODUCTION

PHENOLIC ANTIOXIDANTS such as butylated hydroxanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate have been shown to retard lipid oxidation in meat (Greene, 1969; Sato and Hegarty, 1971). These antioxidants have been approved for use in several foods, but are not allowed presently in fresh meat except for pork sausage (Stuckey, 1972). The need to protect foods from oxidative flavor changes has prompted researchers to seek alternative antioxidant sources which might be approved for use in a wider variety of foods. A number of studies have shown that antioxidant compounds can be produced through nonenzymatic browning reactions. Zipser and Watts (1961) and Sato et al. (1973) presented evidence for an apparent antioxidant activity in retorted beef. They attributed this activity to browning reactions taking place during the heating process. Extremely high temperatures were required to produce this activity under the conditions of their studies (meat sealed in cans and heated either in a water bath or retort). The possibility of developing such activity through modification of common home and institutional cooking procedures has not been investigated.

Fluorescing compounds are produced in the course of a browning reaction (Hodge, 1953). Adhikari and Tappel (1973) found a direct relationship between fluorescence and browning intensity. Studies which have demonstrated production of antioxidant substances from browning reactions have utilized color intensity measurements to follow browning progression more often than any other method. Little attention has been given to a probable relationship between fluorescence and antioxidant activity.

The purpose of the present study was (1) to test the capability of various meat cooking methods to induce antioxidant activity in the cooked product and (2) to determine if a relationship exists between fluorescence production and antioxidant development in cooked meat.

EXPERIMENTAL

THE FOLLOWING COOKING methods were tested: (1) *Dry heat*, in which 300g roasts were heated in a 163°C oven, a 92°C oven and a microwave oven to internal temperatures (IT) of 60–90°C; (2) *Moist heat*, in which roasts were placed on racks in covered casserole dishes with 360 ml water in the bottom of the dish and heated in a 163°C oven to IT's of 86, 94, or 99°C (braising); or in an electric slow-cooker (Wear-ever) for 13 hr to an 84°C IT; and (3) *Pressure heating*, in which meat was cooked in either a pressure saucepan or home pressure canner under 15 lb of pressure. For pressure cooking, 300-g roasts were placed on a rack in the pressure saucepan with 360 ml of water in the bottom of the pan. For pressure canning, 150-g roasts were sealed into 6.5 × 7 cm "C" enamel cans with no added water and placed on a rack in the canner. IT of oven and slow cooker heated samples was measured by copper-constantan thermocouples or thermometers inserted into the meat center. Microwave-heated meat was tested with thermocouples after removing meat from the oven. A maximum thermometer was inserted into meat cooked under pressure.

Beef semitendinosus muscle was used exclusively. Antioxidant activity was tested by assessing for extent of lipid oxidation after 5 days of storage. With dry heat cooked samples roasts were cut into 2.5 × 2 × 1 cm slices, covered with oxygen-permeable meat film and stored at 4–6°C. With moist heat and pressure-heated samples two additional methods were incorporated. One-half of the slices were dipped in the cooking liquid for 5 sec prior to storage; and 2 ml cooking liquid were mixed into raw ground beef which was then stored as 50-g patties. Cooking liquid was either concentrated at 60°C or diluted with distilled water to make a constant volume of 35 ml.

Lipid oxidation was assessed by the 2-thiobarbituric acid test for malonaldehyde (Tarladgis et al., 1960). Results are expressed as mg malonaldehyde per kg tissue (TBA number). The expression "TBRA" (TBA retarding activity) is used when interpreting data from this study to avoid discrepancies which may result through the use of the TBA test as the only objective measure of lipid oxidation.

Color intensity of cooking liquids was estimated by measuring absorbance at 430 nm (Tomita, 1970) in a Bausch & Lomb Spectronic 20 Colorimeter. Routine fluorescence measurements were made on a Turner Model III Fluorometer, using a No. 110-811 (360 nm) primary filter and a No. 110-817 (485 nm) secondary filter. Selection of filters was made after determining excitation and emission peaks on a Turner Model 430 Spectrofluorometer. Peaks were found at approximately 380 nm excitation and 470 nm emission.

A 6–9 member sensory panel composed of trained and untrained testers rated samples for oxidized flavor. Coded samples were placed randomly (Cecil, 1957) on white plates and served mid-afternoon. Panelists tasted in individual booths located in a climate-controlled room that was isolated from outside noise and odors. Samples were served warm. An intensity score sheet was used with adjectives assigned numerical values of 1–4 (i.e., no oxidized flavor = 1, strong oxidized flavor = 4). Data were evaluated statistically by the Wilcoxin Matched-Pairs Signed Ranks test (Siegal, 1956) and correlation coefficients (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Effect of dry heat cooking on TBA retarding activity (TBRA)

Combined data from samples cooked with dry heat are presented in Table 1. Roasts heated in the slow (92°C) oven showed a decrease in TBA numbers as internal temperature (IT) of the meat increased. This did not occur in samples cooked in either the 163°C or microwave ovens. Browning does not occur readily under the conditions of microwave heating (Copson et al., 1955). If TBRA is produced from browning reactions, then it would not be expected to develop during microwave heating. The longer heating time needed to reach a high IT in the 92°C oven appeared to be the factor

Author Greene is with the Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602. The current address for author Huang, formerly with the Univ. of Georgia, is 1610-D Cripple Creek Drive, Birmingham, AL 35209.

0022-1147/78/0004-1201$02.25/0
© 1978 Institute of Food Technologists

Table 1—Effect of dry-heat cooking time and temperature on TBA numbers of sliced beef after 5 days of aerobic storage (4–6° C)

Internal temp (° C)	92° C Oven			163° C Oven			Microwave		
	Cooking rate (g/min)	TBA no.		Cooking rate (g/min)	TBA no.		Cooking rate (g/min)	TBA no.	
		Cent.[a]	Surf.[b]		Cent.[a]	Surf.[b]		Cent.[a]	Surf.[b]
< 70	6.9	7.0	–	8.8	8.2	–	48.7	5.5	–
70–85	1.0	6.3	–	6.5	6.2	–	35.9	6.5	5.9
> 80	0.6	4.4	1.1	3.0	8.0	5.0	33.7	7.6	6.8

[a] Center portion of slices
[b] Outside portion of slices (browned surface)

Table 2—Effect of pressure heating on TBA numbers of beef samples stored aerobically—5 days, 4–6° C

Testing medium	TBA numbers					
	Minutes in pressure cooker			Minutes in pressure canner		
	20	30	40	20	30	40
Slices[a]	6.7	7.8	4.2	4.9	1.1	0.6
Slices[a] + CL[b]	3.8	3.4	0.9	3.4	0.9	0.4
Gr. Beef[c] + CL[b]	2.7	2.5	0.4	2.7	2.1	2.1

[a] Sliced from pressure-cooked or pressure-canned roasts prior to storage
[b] CL = cooking liquid from pressure-cooked or pressure-canned beef
[c] Raw ground beef; Average TBA, untreated ground beef control = 3.2

Table 3—Effect of moist heat cooking on TBA numbers of beef samples after 5 days aerobic storage at 4–6° C

Testing medium	TBA numbers			
	Cooked in oven			Cooked in "Slow-Cooker"
	46 min (86° C IT)	61 min (94° C IT)	142 min (99° C IT)	13 hr (84° C IT)
Slices[a]	3.7	3.8	1.8	1.0
Gr. Beef[b] + CL[c]	2.8	2.1	1.8	3.9

[a] Sliced from cooked roasts prior to storage
[b] Raw ground beef (average TBA, untreated control = 2.9)
[c] CL = Cooking liquid from roasts

conducive to TBRA with dry heat cooking. The 92°C oven, >85°C IT roasts, were also more visibly browned throughout the meat than were any of the other samples. TBA numbers for these samples approached those obtained with phenolic antioxidant treatment (Greene, 1969) and are close to the 0.5–1.0 range for rancid odor detection established by Tarladgis et al. (1960). The combined effects of greater exposure of surface to heat and greater surface dehydration would promote nonenzymatic browning (Reynolds, 1963; Cole, 1967).

TBRA in pressure-heated samples

A series of samples was heated under pressure to provide conditions similar to those of Zipser and Watts (1961) and Sato et al. (1973). Strong TBRA was evident in the pressure cooker liquid and the pressure canned meat after 40 min of heating (Table 2). TBA numbers for these two components were the lowest observed for any cooking method tested and were similar to those obtained on meat treated with commercial antioxidants (Greene, 1969). These two components also exhibited greater visible evidence of browning then did the pressure canning liquid or pressure cooked meat.

These data agree with those of Zipser and Watts (1961) and Sato et al. (1973) in that heating meat to extremely high temperatures produced TBRA in the product. The difference in location of strong TBRA (liquid vs meat) may be a result of differences in heat intensity to which the samples were subjected. The liquid in the pressure cooker was subjected to harsher heating conditions because it was in direct contact with the bottom of the pan which, in turn, was in direct contact with the heating element. The liquid was spread over a large surface area and considerable evaporation took place during heating. Liquid was somewhat charred in appearance. This did not occur in the pressure-canning process. Meat was sealed in cans and cans were placed on a rack with water between the rack and pan bottom. This liquid has less opportunity to scorch and no chance to evaporate. On the other hand, meat in the pressure canner was subjected to a higher temperature for a longer time. It had reached an IT of 121°C after 20 min of heating. The highest IT reached by the pressure-cooked meat was 112°C after 40 min of heating. There was no available means of measuring cooking liquid temperature while heating the meat, but distilled water tested separately measured 121°C in both pressure cooker and canner after 20 min of heating.

Effect of moist heat cooking on TBRA

Sato et al. (1973) found the antioxidant factor in their retorted beef to be in the water-soluble fraction of the meat. In the present study, steam may have extracted the TBRA components from the pressure-cooked sample allowing these compounds to collect at the bottom of the pan. In the pressure-canned samples, the cooking liquid remained in contact with the meat and may have kept the TBRA contained within the muscle portion. To determine if the TBRA of the pressure-heated samples was more closely associated with steam extraction of TBRA substances or high cooking temperatures, two moist heating methods which utilized lower cooking temperatures were tested: (1) braising and (2) electric " slow cooker" heating. Only the higher IT's were tested. Mean data are presented in Table 3.

TBRA developed in the meat slices as IT and cooking time increased. The mean TBA number of 1.0 for the slow-cooker slices is close to the range in which an oxidized flavor might not be perceived. TBA's for individual roasts cooked this way ranged from 1.8–0.5. Cooking liquid from these roasts exhibited no evidence of TBRA. Liquid from the braised roasts exhibited some TBRA but less than that found in the liquid from the 40-min pressure-cooked meat. Apparently water extraction of TBRA compounds was not the critical factor responsible for observed TBRA differences in the pressure-heated samples. Cooking time and final product temperature still appear to be the major factors in TBRA induction.

The figures given in Table 3 are mean values for several tests. Additional variables were included in some of the individual runs. With one of the slow-cooker tests a roast cut from the same muscle was dry-heat roasted in a 163°C oven to 70°C IT. The TBA number for the stored dry heat-cooked slices was 10.7 while that for the slow-cooked slices was 0.5. When test-

Table 4—Effect of fluorescence and color intensity of cooking liquid[a] from pressure heated beef on TBA numbers of raw ground beef[b]

	Minutes in pressure cooker			Minutes in pressure canner		
	20	30	40	20	30	40
Fluorescence units	26.6×10^3	31.5×10^3	21.3×10^4	16.5×10^3	25.5×10^3	28.3×10^3
Optical density (430 nm)	40	40	130	50	70	90
TBA number (Raw ground beef + 2 ml cooking liquid)	2.7	2.7	0.5	2.7	2.1	2.1

[a] Cooking liquid diluted 1000X before taking readings. Readings then multiplied by 1000.
[b] Average TBA number of raw ground beef control was 3.2.

ing TBRA of cooking liquids from slow-cooked samples, a raw ground beef variable containing 0.01% BHA was included in one set of tests. TBA numbers for these ground beef patties were 3.3, 3.9 and 0.3; for the untreated control, cooking liquid-treated and BHA-treated samples, respectively. The comparative effectiveness of slow-cooking became more evident with the inclusion of these variables.

Relationship of fluorescence and color intensity to TBRA

Table 4 gives fluorescence and color intensity readings for the pressure cooking and canning liquids. Both color intensity and fluorescence increased with increased sample heating time. Corresponding TBA numbers show that the very high fluorescence exhibited by the 40-min pressure-cooker liquid was associated with a very low TBA number in ground beef treated with this liquid. Fluorescence readings for this liquid were higher by almost a factor of 10 that those for liquid from the pressure canner counterpart. All liquids were brown. The 40-min pressure-cooker liquid also registered the highest of all samples in color intensity, but this difference was less striking than was the difference in fluorescence.

Fluorescence and color intensity measurements were made on the liquids from the braised and slow-cooked samples also. Fluorescence readings from these liquids were about 1000 times lower than those from the pressure cooked liquid; color intensity, about 100 times lower. Work is needed to determine if the fluorescence-TBRA relationship can be further quantified.

Relationship of observed TBRA to meat flavor

Preliminary sensory evaluation tests were conducted on some of the ground beef patties and on the slow-cooked samples. Variables for the ground beef patties included: (1) untreated control (stored raw), (2) untreated control (stored cooked), (3) 2 ml pressure-cooking liquid mixed with raw beef (stored raw), and (4) 2 ml pressure-cooking liquid added to raw beef (stored cooked). All samples were stored for 5 days and were heated (2 min) or reheated (0.5 min) in a microwave oven prior to evaluation. Results are presented in Table 5. A significant difference was found between scores for variable (4) and the two untreated variables (1) and (2). The r value of 0.9062 for TBA vs sensory scores was not significant for the small N in this test. Although variable (3) had a lower TBA number than variable (4), panelists rated it as being somewhat more oxidized. This may be because more of the liquid remained in the meat patty as a result of only one heating treatment. The strong browned or somewhat burned flavor imparted by the cooking liquid may have been more dominant in this sample and interfered with panelists' judgments of oxidized flavor.

Slow-cooked samples were judged against their 163°C oven-70°C IT dry heat-cooked counterparts after 3 days of storage. Mean panel scores of 1.0 for the slow-cooked vs 2.5 for the dry heat-cooked samples again showed that panelists were able to discriminate between the samples. However, they did not rate them with the magnitude of difference exhibited by the TBA numbers (1.2 and 10.1, respectively). Tarladgis (1960) found a TBA number of 0.5–1.0 to be the threshold for oxidized odor detection with the distillation method.

Table 5—Effect of liquid from pressure cooked beef on flavor scores and TBA numbers of ground beef (stored aerobically 5 days at 4–6°C)

Variable	Mean panel score[a]	TBA number
(1) Untreated control (stored raw)	3.1	5.2
(2) Untreated control (stored cooked)	3.4	9.6
(3) 2 ml pressure cooking liquid mixed with raw beef (stored raw)	2.1	0.5
(4) 2 ml pressure cooking liquid mixed with raw beef (stored cooked)	1.6[b]	1.3

[a] Based on 1–4 intensity scale (i.e., no oxidized flavor = 1, strong oxidized flavor = 4).
[b] Significantly different from (1) and (2) at $p \leq 0.01$.

Zipser and Watts (1961) had panelists evaluate heat-sterilized samples for odor but not for flavor. Odor was judged to be pleasant and meaty with little or no detectable oxidized odor. A flavor threshold for this TBA method has not been clearly established. A thorough investigation of all the sensory characteristics of these meat samples will be necessary to determine the practical value of extended cooking procedures.

CONCLUSIONS

BEEF COOKED to high internal temperatures or for extended cooking times was found to result in lower TBA numbers in the stored product. Browning reactions occurring in the meat during the heating process appear to be responsible for the TBA lowering activity. According to Hamm (1966), the Maillard reaction in meats begins at about 90°C and increases with increased temperature and heating time. This appears to be supported by the present study. The more visibly browned products were those that had either attained a very high IT, as with pressure heating, or those that had been subjected to excessively long heating times. These were also the products that had the lower TBA numbers after storage.

Evidence for a relationship between TBRA and fluorescence was obtained. This further supports the hypothesis that browning reaction products are responsible for the TBRA. Adhikari and Tappel (1973) found that the fluorogens could be useful predictors of browning potential in food products. Data from the present study suggest that fluorogen production may also be a useful predictor of potential antioxidant activity (or TBRA) in cooked beef if the relationship can be quantified.

From a practical standpoint, it was usually necessary for the meat to be heavily heat processed, or overcooked, before strong TBRA was developed. However, the appearance of this activity in samples subjected to somewhat less harsh heat treatments (for example, braising, slow-cooking) suggests that it may be possible to induce TBRA through further modification of some cooking methods. The slow cooking and the "cook-hold" methods employed in some food service operations may be especially suited for investigation into this possibility.

—Continued on page 1209

EFFECTS OF HEAT TREATMENT AND PROTEIN CONCENTRATION ON THE RHEOLOGY OF BOVINE PLASMA PROTEIN SUSPENSIONS

J. P. HARPER, D. A. SUTER, C. W. DILL and E. R. JONES

ABSTRACT

Changes in rheology during the heating of bovine plasma protein suspensions were examined at temperatures between 50°C and 90°C and for concentrations ranging from 6–12% protein isolate over a 3-hr period. An annular pumping device was used to determine a viscosity index for the suspensions. A model was developed to explain the data. The viscosity index increased exponentially with time at a particular temperature while being linearly dependent upon the concentration. The Arrhenius Equation described the effect of temperature on the rate of change of the viscosity index during heating.

INTRODUCTION

THE PREPARATION of protein isolates from the plasma of bovine blood has been described by Vickery (1968), Pals (1970) and Tybor et al. (1975). These isolates represent promising sources of food grade proteins. Aside from their dietary value, these protein isolates possess important functional characteristics such as their ability to act as emulsifiers (Breyer and Goodwin, 1962; Tybor et al., 1973).

Another significant property of plasma protein isolates is their capacity to form colloidal suspensions. Upon heating, mixtures of the isolate and water produce colloidal suspensions whose rheology can vary from viscous fluid to semi-solid paste or gel. Many factors affect the texture of the colloid. Important experimental variables include the concentration of the protein isolate in solution, the temperature at which the suspension is maintained, and the period of time the suspension is at a particular temperature.

The purpose of this study was to ascertain the effects of protein concentration, temperature and heating time on the extent of gelation of the suspensions. The extent of gelation was determined by estimating the viscosity of the suspension.

EXPERIMENTAL

Materials

A spray-dried plasma protein isolate was prepared from whole bovine blood (Tybor et al., 1975). The blood was mixed (9:1v:v) with a 0.85% NaCl solution containing sufficient sodium citrate to produce a level of 0.02% in the final mixture. The temperature was then lowered to 5°C and within 24 hr, the mixture was centrifugally separated into plasma and red blood cell fractions. The aqueous plasma stream was desalted by ultrafiltration, then spray-dried to produce the protein isolate.

The protein isolate was analyzed for nitrogen using the Kjeldahl procedure (AOAC, 1970), and results were converted to protein using the factor 6.25. The isolate preparation used contained 86.7% Kjeldahl nitrogen. For testing, the protein isolate was rehydrated with physiological saline to provide mixtures at concentrations of 6%, 8%, 10% and 12% protein isolate. For example, 10g of protein isolate were mixed with 90 ml physiological saline to obtain the 10% concentration by weight. The resultant mixture was refrigerated overnight and 5 ml aliquots were pipetted the following morning into test tubes for the mechanical loading tests.

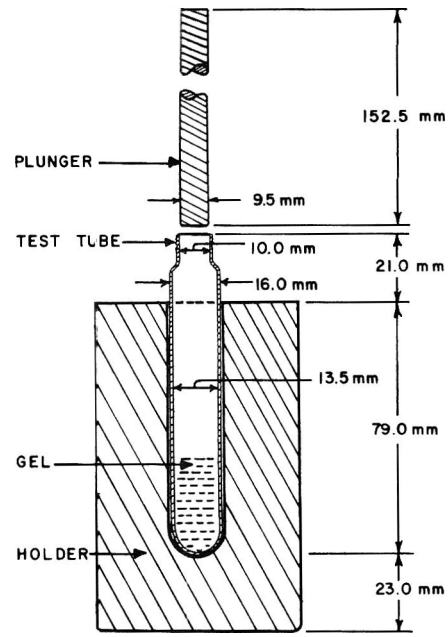


Fig. 1—Diagram of annular pump used in determination of viscosity index.

Methods

Mechanical tests to determine the rheological behaviors of viscoelastic materials are classified either as constant strain rate tests or as constant load rate tests. The method employed here was a constant strain rate approach. An annular or concentric cylinder pumping device, pictured in Figure 1, was used in determining the maximum shear stress required to move the plasma protein suspension through the annulus between the plunger and test tube wall.

The procedure followed was to first heat 5 ml of the sample solution in a 16 × 100 mm screw-top glass test tube by placing the sample in a constant temperature water bath fitted with a reciprocating shaker which agitated the tubes at 100 cycles per minute. After heating for a designated time, the tube was removed and placed in the plexiglass holder for mechanical loading. This arrangement was centered and the stainless steel plunger forced at a constant rate of 100 mm/min through the material to a distance 30 mm below the original surface of the sample. The force required to move the plunger was monitored on an Instron (Model 1122) universal load testing instrument. Figure 2 illustrates the typical pentration-force plot obtained in the Instron. The maximum force was utilized in determining the shear stress.

The temperature effects of gelling were investigated using 12% protein isolate suspensions. Based on prior observations of the gelling phenomena, a temperature range of 50–90° was selected as the most interesting region for examining the temperature dependent rheological changes. Test runs were conducted at 50°C, 60°C. 65°C, 70°C, 75°C, 80°C, 85°C and 90°C. Effects of protein concentration (6%, 8%, 10% and 12% protein isolate).

A typical run spanned a 3 hr heating period with tests being done after 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150 and 180 min of

Author Suter is with the Agricultural Engineering Department, Author Dill is with the Animal Science Department, and Author Jones is with the Institute of Statistics, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843. Author Harper, formerly with the Bioengineering Div. of Texas A&M University, is now with the Energy & Environmental Systems Division, Argonne National Laboratory, 9700 S. Cass Avenue., Argonne, IL 60439.

0022-1147/78/0004-1204$02.25/0
© 1978 Institute of Food Technologists

heating at the specified temperature. On the average, 24 samples were tested for each run, the replicate times being randomly chosen.

Theory

Determination of viscosity. The rheology of the protein solutions shall be described by their viscosity. The device used to measure the viscosity of the protein isolate solutions is an annular or concentric cylinder pumping instrument similar to that described by Philippoff (1965) and Ferry (1970) for viscosity determination of viscoelastic fluids in oscillatory experiments. They defined the complex viscosity, η^* as:

$$\eta^* = \frac{F}{bv} \tag{1}$$

where F is force (dynes) required to oscillate the plunger, v is the velocity (cm/sec) and b is a geometric factor (cm) defined by:

$$b = \frac{2\pi L}{\log_e R_2/R_1 - \dfrac{(R_2^2 - R_1^2)}{(R_2^2 + R_1^2)}} \tag{2}$$

where L is the length of sample (cm) in contact with the plunger, R_2 is the inside radius (cm) of the retaining cylinder, and R_1 is the radius of the inner cylinder such that $R_2 > R_1 > 0$.

Using a similar approach, we shall define a viscosity index, η_I, for our system as:

$$\eta_I = \frac{F_{max}}{b_{max}v} \tag{3}$$

where F_{max} is the maximum force (dynes) required to move the plunger; b_{max} is the maximum value of the geometric factor. A single point, F_{max}, was used in constructing the viscosity index because it represented the maximum force required for flow to occur in the system for the specified conditions of protein concentration, temperature and heating time. Such an approach was taken because the inhomogeneity of the gelled materials gave rise to a fluctuating deformation force (as depicted in Fig. 2) and the extremum force value was thought to best characterize the rheology. The value for b_{max} is determined when the plunger is fully extended into the suspension. Assuming the material to be incompressible, the L value can be taken as constant since the fluid volume and maximum plunger penetration are invariant for all tests. Hence, L equals 5.943 cm and b_{max} is 2708 cm; v is in cm/sec which gives η_I the units of dynes sec/cm^2, or poises. The viscosity index defines the flow behavior of the system at a given shear stress and strain rate. This measure is similar to the apparent viscosity, which is defined as the ratio of shear stress to strain rate.

Kinetic model. The reason for the changing rheology of the protein isolate solutions is that a polymerization of the plasma protein constituents is occurring. The resultant polymer is called a polyamide and is usually formed as a result of amine-carboxylic acid condensation (Jones and White, 1972). The stoichiometry for such a linear condensation is given as:

$$P + M \rightarrow P \tag{4}$$

where P is the reactive polymer species (moles) and M is a reactive monomer (moles). The kinetics are typically second-order and the rate equation for the disappearance of the monomer species is:

$$\frac{-dM_c}{dt} = k_2 P_c M_c \tag{5}$$

where k_2 is the second-order rate constant (min^{-1} mole^{-1}) and the subscript c designates concentration of the particular chemical species.

The kinetics become pseudo first-order if it is feasible to assume that the concentration of one reactive species will remain essentially unchanged during the course of the reaction. Such as assumption is made with regard to the reactive polymer species. It is thought that only one type of plasma protein serves as the reactive polymer species, and as such stays constant during polymerization.

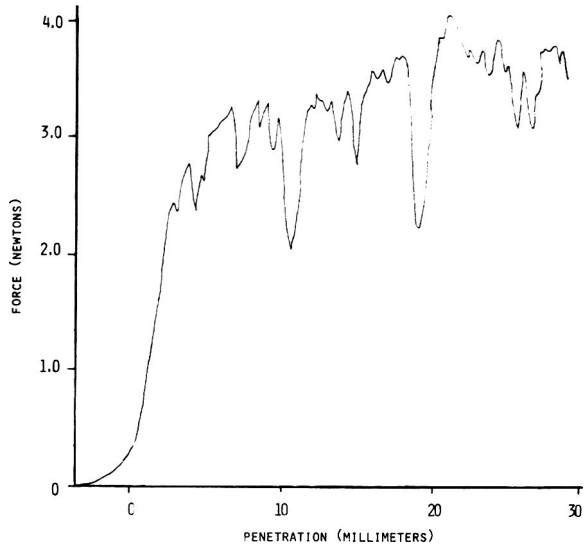


Fig. 2—Typical force-penetration plot obtained during experiment force (N), penetration (mm).

The rate equation for the pseudo first-order reaction is:

$$\frac{-dM_c}{dt} = k_1 M_c \tag{6}$$

where k_1 is the pseudo first-order reaction constant (min^{-1}). Integration yields an equation which gives the monomer concentration an any time t:

$$M_c = M_c^o e^{-k_1 t} \tag{7}$$

where M_c^o is the initial concentration of reactive monomer.

The number-average degree of polymerization (Williams, 1971) DP_n, may be estimated from the above equation:

$$DP_n = \frac{M_c^o - M_c}{P_c} = \frac{M_c^o}{P_c}(1 - e^{-k_1 t}) \tag{8}$$

The product of the number average degree of polymerication and the monomer molecular weight, W_m, approximates the molecular weight of the polymer, W_p. Correlation between the viscosity of a polymer solution and the molecular weight of the polymer is given by a power law relationship (Graessley, 1974):

$$\eta = k w_p^n = k(W_m DP_m)^n \tag{9}$$

where k is a proportionality constant (poises moles/gram).

By equating the true viscosity with our viscosity index and taking n equal to one, we can obtain an equation that describes the system's rheology as a function of the polymerization of the suspension:

$$\eta_I = \frac{kW_m M_c^o}{P_c}(1 - e^{-k_1 t}) \tag{10}$$

$$= A(1 - e^{-k_1 t})$$

where A is the asymptotic viscosity index in poises.

This modeling appraoch permits a causal explanation for the effects of the experimental variables on the changing rheology of the suspension. The asymptotic viscosity index, A, accounts for viscosity variations on the basis of different initial concentrations of plasma protein isolate. The dependence of the polymerization rate constant, k_1, on temperature is described by the Arrhenius Equation. The inclusion of time into the model demonstrates the effect of heating time on rheology. Thus, such a model can prove useful in interpreting the experimental results.

—Text continued on page 1206

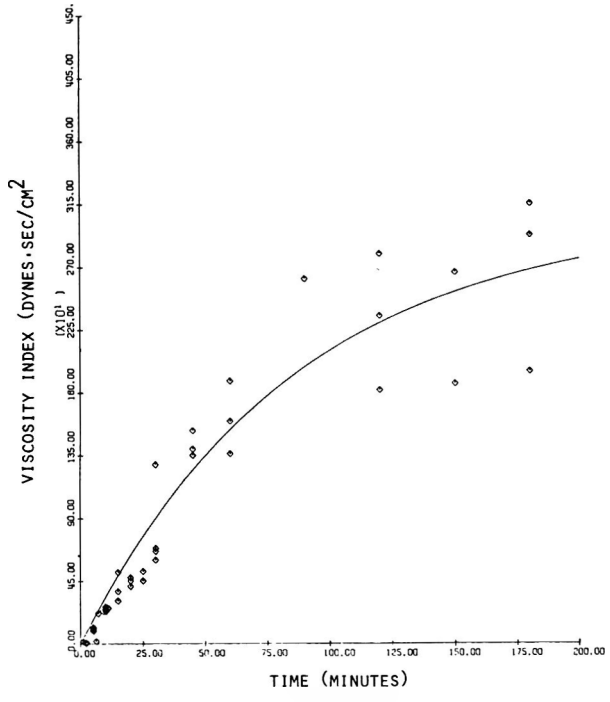


Fig. 3—Viscosity index vs heating time for a 12% protein suspension at 70°C.

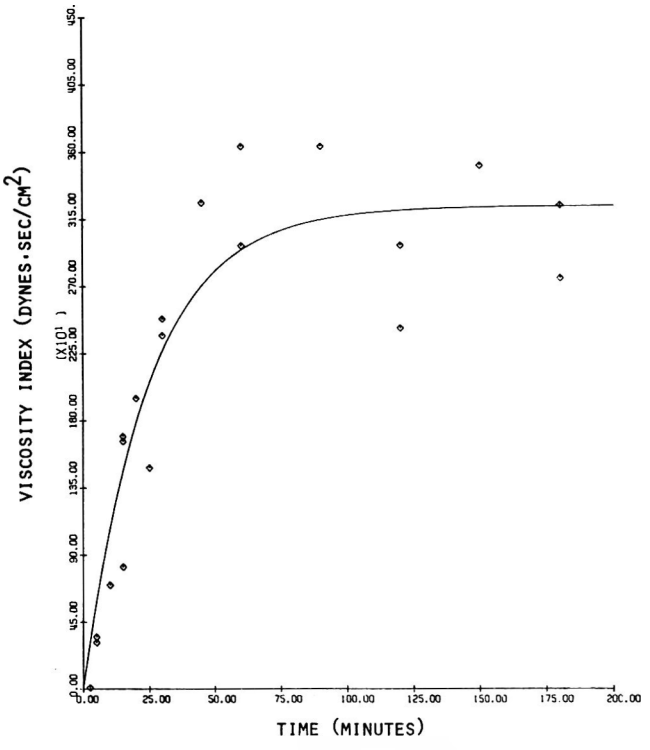


Fig. 4—Viscosity index vs heating time for a 12% protein suspension at 75°C.

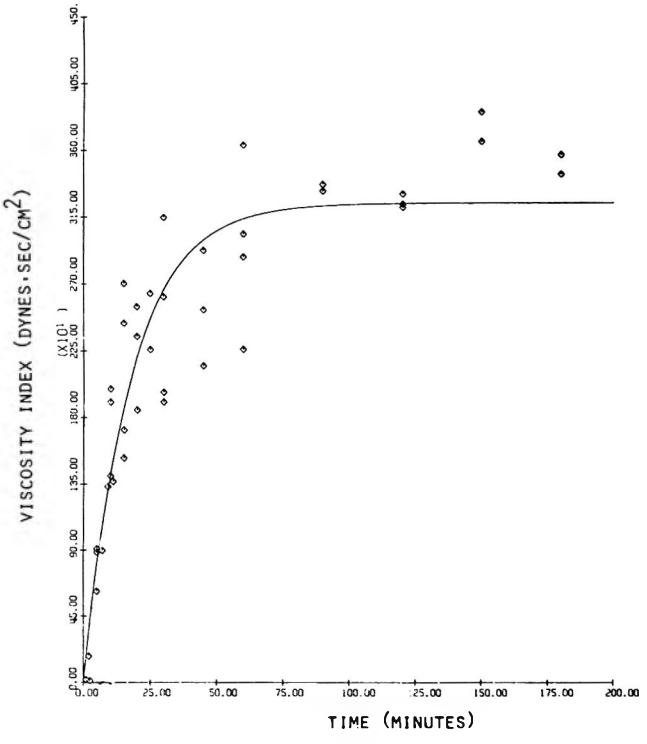


Fig. 5—Viscosity index vs heating time for a 12% protein suspension at 80°C.

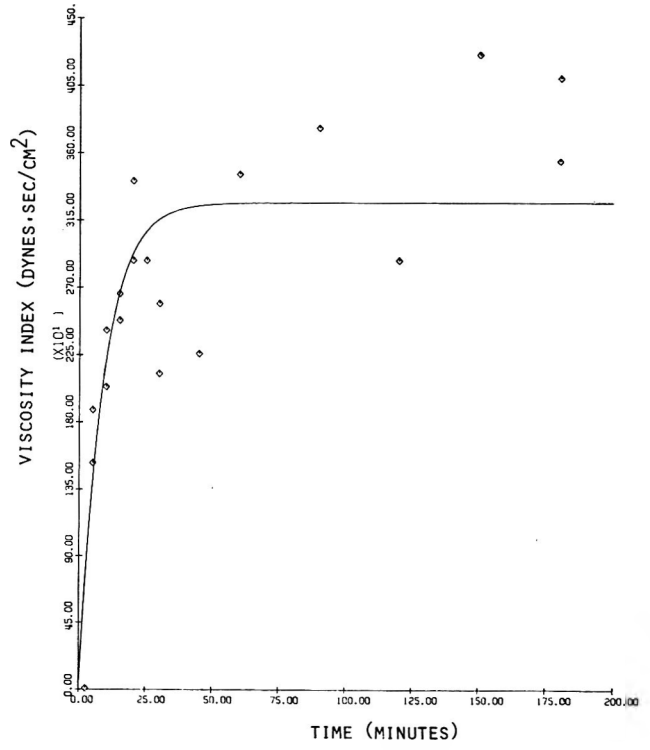


Fig. 6—Viscosity index vs heating time for a 12% protein suspension at 85°C.

RESULTS

Rheology, temperature, heating time correlations

The viscosity index versus the heating time data are presented in Figures 3, 4, 5, 6 and 7 for 12% protein isolate and temperature 70°C, 75°C, 80°C, 85°C and 90°C, respectively. A nonlinear least-squares approach developed by Marquardt (1963) was used to fit the data and estimate the rate constant, k_1, and asymptotic viscosity index, A. Both k_1 and A were specified to be greater than zero for the Marquardt analysis.

The solid lines on the above figures show the regression line

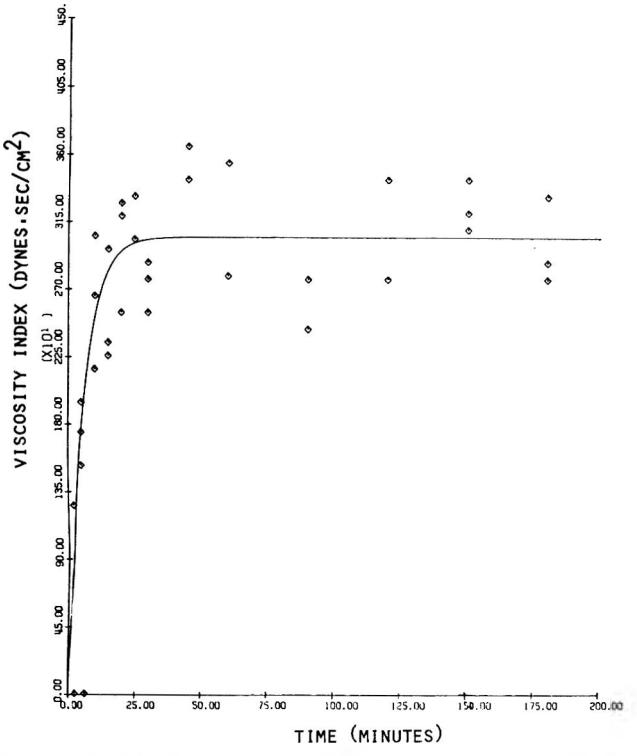


Fig. 7—Viscosity index vs heating time for a 12% protein suspension at 90°C.

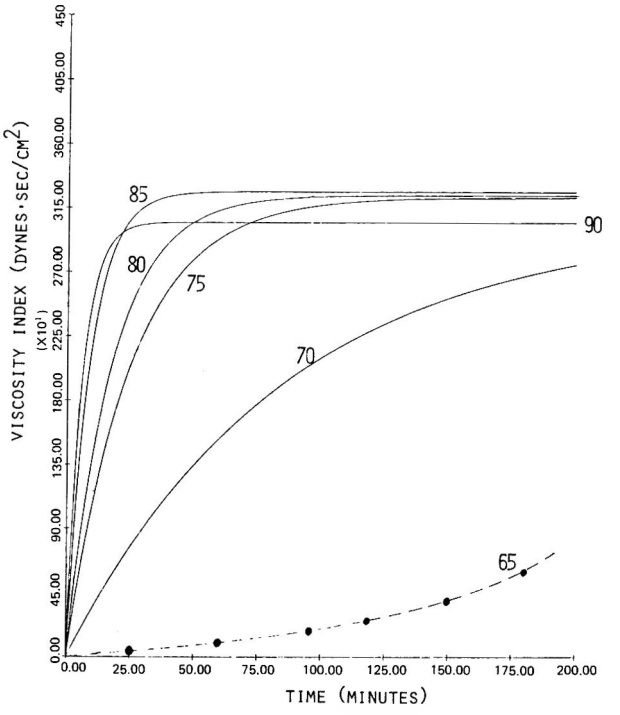


Fig. 8—Viscosity vs heating time curves for all temperatures.

Table 1—Rate constants and asymptotic viscosity indices at experimental temperatures for 12% protein isolate suspensions

Temp T(°C)	Rate constant k_1 (min^{-1})	Asymptotic viscosity index A (dynes sec/cm^2)
70	0.0117	3048
75	0.0403	3230
80	0.0571	3243
85	0.1130	3267
90	0.1739	3050

generated by the analysis with respect to the data. Table 1 lists the values of k_1 and A for the various temperatures.

No appreciable gelation occurred at 50°C and 60°C over the heating period studied. Changes did take place at 65°C but results did not conform to the proposed model. The researchers believe the lack of correspondence to the model for the 65°C data is due to the 3-hr heating time limitation during the experiment. At 65°C the exponential relationship of gelation to heating time required a longer period of observation. Figure 8 summarizes the heating effects at all temperatures.

Rheology, concentration, heating time correlations

The viscosity index versus heating time data are presented in Figures 9, 10 and 11 for a temperature of 80°C and protein isolate concentrations of 10%, 8% and 6%, respectively. The data are depicted in the same manner as above, with the solid line representing the fit of the Marquardt regression. The values of k_1 and A at the different concentrations are given in Table 2.

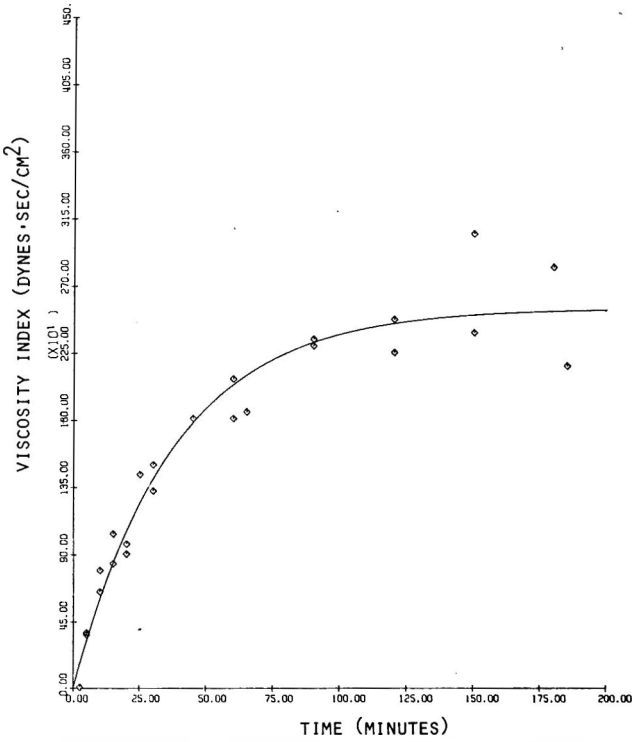


Fig. 9—Viscosity index vs heating time for a 10% protein suspension at 80°C.

DISCUSSION

Assessment of model

The model developed for relating viscosity, concentration, temperature and heating time indicates how these variables should be related. First, for a given concentration and temperature, an exponential relationship should hold between heating time and the viscosity index. The Marquardt regression analysis shows that the experimental results are well described by the model for any particular concentration and temperature.

Second, since the k_1's are rate constants, they should be related to temperature according to Arrhenius Equation.

—Text continued on page 1208

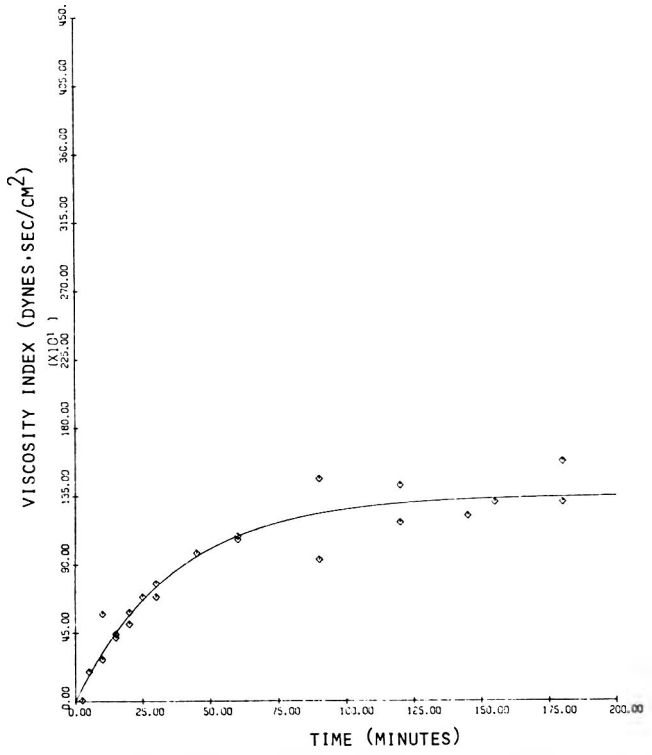


Fig. 10—Viscosity index vs heating time for an 8% protein suspension at 80°C.

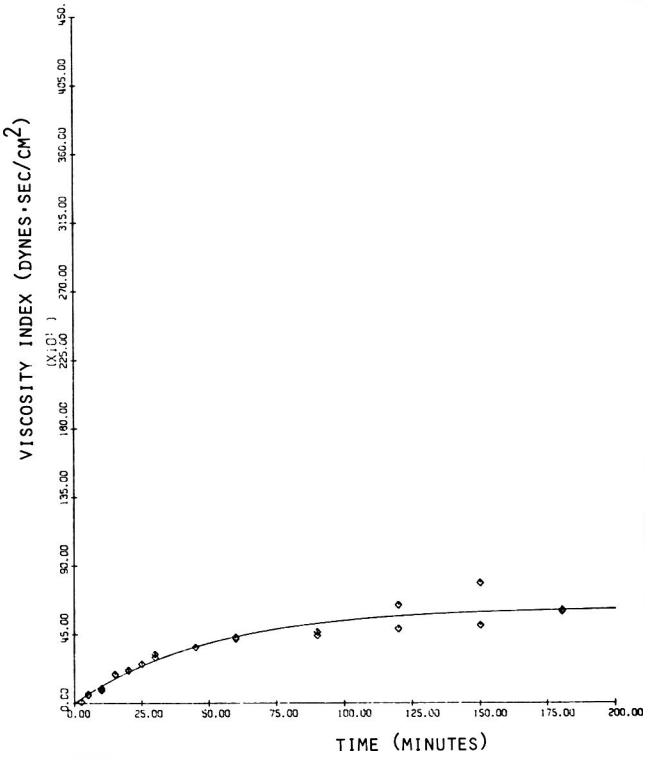


Fig. 11—Viscosity index vs heating time for an 6% protein suspension at 80°C.

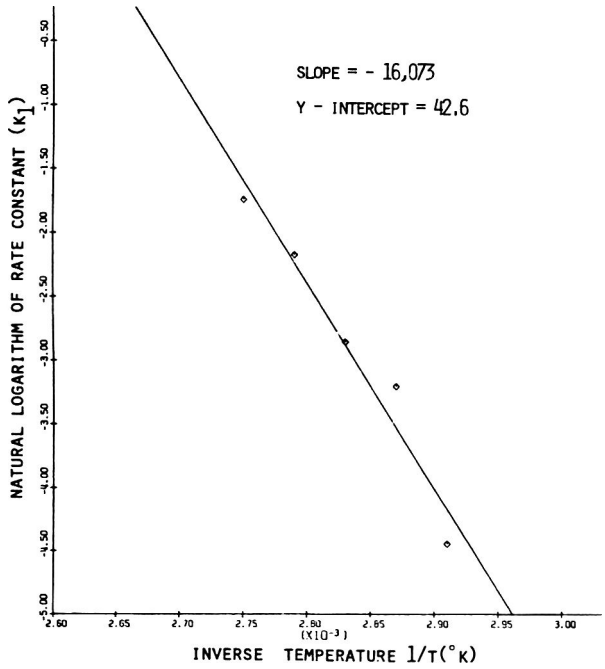


Fig. 12—Arrhenius plot of Ln K_1 vs 1/T.

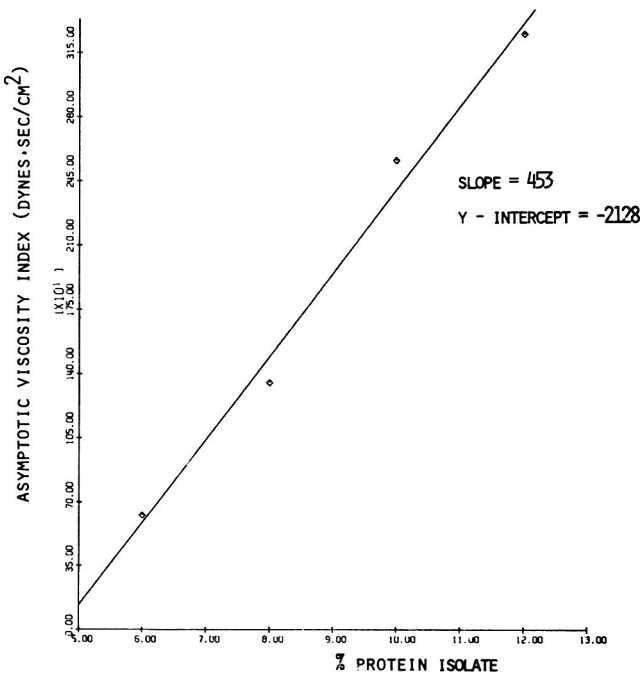


Fig. 13—Asymptotic viscosity vs % protein isolate concentration for 80°C data.

Figure 12 is an Arrhenius plot of the natural logarithm of the k_1's versus the inverse of the corresponding temperature (°K). Regression analysis (Richmond, 1964) indicates the plot is linear ($R = 0.98$ and $P < 0.01$), as one would expect from chemical kinetic theory.

Third, the asymptotic viscosity index, A should be a linear function of the protein concentration. Eq (10) defines A as equal to a constant factor times the ratio of the initial reactive monomer concentration to the reactive polymer concentration

Table 2—Rate constants and asymptotic viscosity indices at 80°C for experimental protein concentrations

Conc (% Protein isolate)	Rate constant k_1 (min^{-1})	Asymptotic viscosity index A (dynes sec/cm^2)
12%	0.0571	3243
10%	0.0267	2559
8%	0.0271	1352
6%	0.0197	628

necessitating linearity. Figure 13 plots A versus the percent concentration and verifies the linearity ($R = 0.99$ and $P < 0.01$) required by the model.

The fourth relationship which the model indicates is linearity between k_2 and concentration, which is implicit in the pseudo first-order assumption. A linear regression analysis significantly relates these parameters ($R = 0.87$ and $P < 0.02$).

Thus, experimental data appear to corroborate the model proposed to describe the rheological behavior of plasma protein gelation as a function of time, temperature and concentration.

Future implications of research

Heat treatment is an essential element in producing and controlling desirable textural characteristics of food products by the alterations effected in one or more of the protein constituents. The information for the plasma proteins presented herein can serve as the basis for the stepwise evolution of a model system which will accurately describe the textural properties of such food items as frankfurter, meatloaf, etc., as well as the effects of novel proteins, such as those isolated from soybeans, on these systems. The plasma protein isolate used is a mixture of several globular proteins, primarily albumin and the globulins (Tybor et al., 1973). Future investigations will delineate both the relative contribution of the individual protein components and the effects of salt concentration and composition on heat induced rheological changes in the plasma protein suspensions.

CONCLUSIONS

THE COMPLEX rheological changes in gelling suspensions of plasma proteins due to alterations of concentration, temperature and heating time, can be interpreted and even predicted by using the model given in this paper. Using Figure 13 one may determine the appropriate asymptotic viscosity for 6–12% protein isolate mixtures and with Figure 12 ascertain the appropriate rate constant for heating temperatures ranging from 70–90°C. The viscosity index may then be predicted as a function of time using the mathematical model given in Eq (10).

REFERENCE

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, D.C.
Breyer, B. and Goodwin, P.B. 1962. The emulsifying capacity of blood sera. Clin. Chim. Acto 7(5): 647.
Ferry, J.D. 1970. "Viscoelastic Properties of Polymers," 2nd ed. J. Wiley and Sons, New York.
Graessley, W.W. 1974. The entanglement concept in polymer rheology. Adv. Polymer Sci. 16: 49.
Jones D.C. and White, T.R. 1972. Polyamides. In "Step-Growth Polymerizations." Marcel Dekker, Inc.
Marquardt, D.W. 1963. An algorithm for least squares estimation of nonlinear parameters. SIAM J. 11: 431.
Pals, C.H. 1970. The practical aspects of blood component procurement. Proc. Meat Ind. Res. Conf., p. 17. American Meat Institute, Chicago.
Philippoff, W. 1965. Relaxations in polymer solutions, liquids and gels. In "Physical Acoustics," Vol. 2-B, Academic Press, New York.
Richmond, S.B. 1964. "Statistical Analysis." Ronald Press, New York.
Tybor, P.T., Dill, C.W. and Landmann, W.A. 1973. Effect of decoloration 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.
Vickery, J.R. 1968. The recovery and utilization of edible protein from blood and trash fish. Food Technol. in Australia July: 315.
Williams, D.J. 1971. "Polymer Science and Engineering." Prentice-Hall, Inc., Englewood Cliffs, NJ.
Ms received 6/20/77; revised 11/18/77; accepted 11/23/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

Technical Article No. 13440 of the Texas Agricultural Experimental Station, College Station, Texas.

Research supported in part by a grant from the Houston Livestock Show and Rodeo Association. Mr. E. C. "Dick" Weekly, Manager.

TBA NUMBER OF STORED BEEF . . . From page 1203

REFERENCES

Adhikari, H.R. and Tappel, A.L. 1973. Fluorescent products in a glucose-glycine browning reaction. J. Food Sci. 38: 486.
Cecil, S.R. 1957. Sample arrangements for taste panel scoring of number-coded items. Unpublished. Georgia Station, University of Georgia, Experiment, GA.
Cole, S.J. 1967. The Maillard reaction in food products: Carbon dioxide production. J. Food Sci. 32: 245.
Copson, D.A., Neumann, B.R. and Brody, A.L. 1955. Browning methods in microwave cooking. J. Agr. Food Chem. 3: 424.
Greene, B.E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food Sci. 34: 110.
Hamm, R. 1966. Heating of muscle systems. In "The Physiology and Biochemistry of Muscle as a Food," p. 363, Ed. Briskey, E.J., Cassens, R.G. and Trautman, J.C. Univ. of Wisc. Press, Madison, WI.
Hodge, J.E. 1953. Chemistry of browning reaction in model systems. J. Agr. Food Chem. 1: 928.
Reynolds, T.M. 1963. Chemistry of nonenzymic browning. 1. The reaction between aldoses and amines. Adv. Food Res. 12: 1.
Sato, K. and Hegarty, G.R. 1971. Warmed over flavor in cooked meat. J. Food Sci. 36: 1098.
Sato, K., Hegarty, G.R. and Herring, H.K. 1973. The inhibition of warmed-over flavor in cooked meats. J. Food Sci. 38: 398.
Siegal, S. 1956. "Nonparametric Statistics for the Behavioral Sciences." McGraw Hill Book Co., Inc., New York.
Steel, R.D.G. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw Hill Book Co., Inc., New York.
Stuckey, B.N. 1972. Antioxidants as food stabilizers. In "Handbook of Food Additives," 2nd ed., p. 210. CRC Press, Cleveland, OH.
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.
Tomita, Y. 1970. Studies on antioxidant activity of amino-carbonyl reaction products. Part 1. Antioxidant activity of amino acids and browning solutions of amino acids with glucose. Kagoshima University Bulletin of the Faculty of Agriculture (Japan) 21: 153.
Zipser, M.W. and Watts, B.M. 1961. Lipid oxidation in heat-sterilized beef. Food Technol. 15: 445.
Ms received 8/10/77; revised 1/30/78; accepted 2/8/78.

CHEMICAL CHARACTERIZATION OF EGG YOLK MYELIN FIGURES AND LOW-DENSITY LIPOPROTEINS ISOLATED FROM EGG YOLK GRANULES

T. D. GARLAND and W. D. POWRIE

ABSTRACT

Myelin figures (MF) and low-density lipoprotein (LDLg) in yolk granules were isolated from the subpellicle fraction formed during the supercentrifugation of a granule dispersion in 10% NaCl (Garland and Powrie, 1978). The MF and LDLg fraction were analyzed for total nitrogen, total phosphorous, total lipid, cholesterol and phospholipids. MF fraction possessed components which were similar to those for LDLg, but concentrations of the components were different. MF contained about 86% lipid whereas the lipid content of LDLg was around 84%. The amount of total nitrogen in MF and LDLg fractions was dependent on the age of hens from which eggs were obtained. With eggs from 36-wk-old hens, the MF and LDLg fractions had % total nitrogen values of 1.29 and 1.82, respectively whereas with hens from 39 wk to 1.5 yr of age, total nitrogen values were between 2.39 and 2.52% for MF and between 2.46 and 2.51% for LDLg. The total phosphorous content of the LDLg fraction was dependent on hen age. As a proportion of the total lipid, MF fractions were found to be rich in cholesterol, about 11.5% whereas LDLg fraction contained about 3.7%. Total phospholipid content of MF and LDLg from eggs of 39-wk-old hens was approximately 30 and 26%, respectively. Phosphatidyl choline was the predominant phospholipid in both MF and LDLg, and small amounts of phosphatidyl ethanolamine, lysophosphatidyl ethanolamine and sphingomyelin were present.

INTRODUCTION

WHEN EGG YOLK is centrifuged at high speeds, granules are sedimented and a clear fluid supernatant, called plasma, is formed. Granules make up about 19–23% of the yolk solids (Burley and Cook, 1961; Saari et al., 1964; Saito et al., 1965). According to Burley and Cook (1961), granules consist to 70% α- and β-lipovitellins, 16% phosvitin and 12% low-density lipoprotein.

The low-density lipoproteins in granules can be isolated by adding 1.71M NaCl to water-washed, sedimented granules and centrifuging the dispersion. Chang et al. (1977) showed by electron microscopy that the two floating fractions (FI and FII) contained most of the low-density lipoproteins (LDLg) along with large globules and myelin figures (MF). The sedimenting fractions (FIII, FIV and FV) consisted primarily of fluffy masses made up of long protein strands with electron-dense microparticles attached. The subpellicle (FII), composed of LDLg and MF, was used by Garland (1973) and Garland and Powrie (1978) for the isolation of these particles. Electron microscopy was used to assess the purity and structural integrity of the two FII components after using ultrafiltration, dextran sulfate lipoprotein complexing and gel filtration techniques for separation. Sepharose 2B gel filtration was found to be the best method for isolation of the two components.

Chang (1969) postulated that the MF were composed mainly of phospholipid since their structure was similar to that of myelin figures of phospholipids. However, electron micrographic studies of Garland (1973) and Garland and Powrie (1978) indicated that yolk MF have a more complicated structure than that proposed by Chang (1969). Low electron-dense and high electron-dense MF with different proportions of lamellae and grey centers were noted.

Previous research work on the composition of LDL fractions from granules has been carried out with the floating upper gel formed during centrifugation of granules dispersed in a sodium chloride solution. Research workers were apparently unaware of the presence of three different types of floating particles, namely MF, small globules (LDLg) and large granules. Cook (1968) has reported that the composition of granule LDL fraction (which undoubtedly contained the three types of floating particles) was similar to that of plasma LDL faction except it had a higher phosphorous content, presumably the result of phosvitin contamination. Gornall and Kuksis (1971a, b) reported detailed information on the composition of the low-density fraction of granules (LDLG) and two fractions (LDFG-1 and LDFG-2) which were separated by thin layer chromatography of LDFG. The types of particles in those fractions are unknown. The lipid contents of LDFG, LDFG-1 and LDFG-2 were 88, 85 and 92 percent, respectively (Gornall and Kuksis, 1971a). The remaining percentages were considered to be protein. According to Gornall and Kuksis (1971b), the lipid fraction of LDFG was composed of 7.3% cholesterol, 22.6% phospholipid and 70.2% triglyceride. Although the LDFG-2 fraction had a lipid composition similar to that of LDFG, LDFG-1, which remained at the origin during thin layer chromatography, contained less triglyceride (57.0%) and more phospholipid (29.7%) and cholesterol (13.3%).

The purpose of this study was to examine the chemical composition of LDLg and MF isolated from floating fraction (FII) of granules by the Sepharose 2B gel filtration technique (Garland, 1973; Garland and Powrie, 1978).

EXPERIMENTAL

Materials

Eggs from White Leghorns, less than 24 hr-old were obtained from the University of British Columbia Poultry Research Station. Eggs were taken from two flocks of birds. Eggs from each flock were pooled for representative sampling.

Seamless cellulose dialysis tubing, permeable to low molecular weight compounds (M.W. < 12,000), was used for desalting all MF and LDLg preparations. Brinkman Polygram Sil N-HR precoated 20 × 20 cm silica gel thin layer chromatography sheets were used for the separation of MF and LDLg polar and nonpolar lipids.

Egg phosphatidyl choline, bovine phosphatidyl ethanolamine, egg lysolecithin, bovine lysophophatidyl ethanolamine, bovine phosphatidyl serine, plant phosphatidyl inositol, and bovine sphingomyelin were obtained from Applied Science Laboratories Inc., State College, PA. Cholesterol was purchased from Aldrich Chemical Co. Inc., Milwaukee, OH and triolein from Nutritional Biochemical Corp., Cleveland, OH.

Methods

Preparation of MF and LDLg fractions. Fraction II (FII) was isolated from washed yolk granules by the method of Chang (1969) and Chang et al. (1977) with a slight modification. A 300-g portion of the yolk pooled from 6 doz eggs was centrifuged in a Beckman Model L2-65B ultracentrifuge at 78,500 × G at R_{av} for about 16 hr at 15°C to sediment insoluble yolk granules. The plasma was removed and the yolk granules were dispersed in 12 volumes of distilled water. The dispersion was slowly mixed with a magnetic stirrer in a sealed Erlenmyer flask at 5°C for 1 hr. Washed granules were centrifuged in a

Author Powrie is with the Dept. of Food Science, University of British Columbia, Vancouver, B.C., Canada. Author Garland, formerly with the University of British Columbia, is now affiliated with Swan Valley Foods Ltd., Richmond, B.C., Canada.

0022-1147/78/0004-1210$02.25/0
© 1978 Institute of Food Technologists

Sorvall superspeed RC2-B automatic refrigerated centrifuge at approximately 2,000 × G for 1 hr at 15°C. This washing procedure was repeated three additional times, with a spinning time of 1 hr for the first run and 30 min for subsequent runs. Wet granules were then disrupted in approximately 4.25 volumes of 1.71M sodium chloride (D_{4}^{20} = 1.068) to provide 300 ml of dispersion and centrifuged at 78,500 × G for about 16 hr at 15°C. The dispersion was fractionated into two floating fractions (FI, FII) and three sedimenting fractions (FIII, FIV, FV). The FII was removed by Pasteur pipet.

Separation of the FII into MF and LDLg fractions was accomplished by Sepharose 2B gel filtration as described by Garland (1973) and Garland and Powrie (1978). Collected and pooled samples were dialyzed (approx 25 ml/tube) in cellulose dialysis tubing against 5L of stirred distilled water for 36 hr at 5°C to remove the NaCl. Desalted samples were frozen at −20°C and subsequently lyophilized with a Virtis, Model 10-145-MRBA, freeze drier. Dried material was stored under nitrogen in sealed brown bottles at 5°C.

Moisture content of lyophilized samples was determined essentially by the AOAC method (AOAC, 1970). The average moisture content of lyophilized samples was 0.04%.

Analytical Methods. *Lipid extraction*. Approximately 100 mg of lyophilized powder were accurately weighed, placed in a porcelain mortar and ground with successive 2 ml portions of an appropriate lipid solvent system. For total lipid extraction of the MF samples, extractants 1 and 2 were used and for the LDLg fraction, extractants 1, 2 and 3 were employed:

Extractant 1. chloroform-methanol (2:1), 20 ml;
Extractant 2. chloroform-methanol (9:1), 10 ml;
Extractant 3. ethanol, 10 ml.

After each grinding with 2 ml of an extractant, the mixture was allowed to stand for a few minutes while insoluble material sedimented. The supernatant was removed by pipet to a centrifuge tube. The combined solvent extract was centrifuged at approximately 10,000 × G for 30 min to eliminate particulate matter by sedimentation and filtered through Whatman No.52 filter paper in a Buchner funnel. The volume of each combined extract was reduced by evaporation under a flow of nitrogen gas.

Thin-layer chromatography. Rectangular trough glass chambers (21 × 21 × 9 cm) lined with Whatman No. 1 filter paper were used throughout this study for the ascending development of thin-layer sheets. Prior to use, the thin-layer sheets were washed with chloroform/methanol/ammonium hydroxide (65:35:5) by ascending solvent development. The top contaminated area was cut off and discarded.

The following method was used to separate the major lipids of both MF and LDLg extracts. With its longest side (20 cm), the TLC sheet was spotted with 400 to 700 μg of lipid at the bottom right hand corner, 2 cm from each edge. Plates were developed with chloroform/methanol/ammonium hydroxide (65:35:5) to a distance 11 cm above the origin. The plate was dried and further developed in the same direction with hexane/ether (4:1) to a distance of 17.5 cm above the origin. This procedure facilitated the separation of polar lipids from nonpolar lipids. Whereas the cholesterol and neutral triglyceride lipids were completely separated from each other, the phospholipid spots overlapped. After drying, the chromatogram was cut to segregate the polar lipids from the non-polar lipids. The sheet containing the polar lipids was then developed at right angles to the previous solvent direction with n-butanol/glacial acetic acid/water (60:20:20) to a height of 8 cm above the origin to separate the phospholipids. As a step to confirmation of the presence of only cholesterol and triglycerides, the sheets with the nonpolar spots were developed with hexane/ether/glacial acetic acid (90:10:1) at right angles to the previous solvent direction.

The chromatographed lipids were detected by three different methods: (1) Exposure to iodine vapor in a closed glass tank; (2) Spraying with a solution of 55% (w/v) sulfuric acid and 0.6% (w/v) potassium dichromate with subsequent charring in an oven at 120°C; and (3) Spraying with 0.2%, 2,7-dichlorofluorescein ethanolic solution with subsequent exposure to UV light.

Identification of the separated lipids was accomplished using a number of methods:

Reference standards. Reference lipids were spotted and developed in an identical manner to the unknown lipids. Standards were employed singly and as mixtures, and were also added to the unknown lipid samples.

Phosphoric acid spray. A 15% phosphoric acid solution was heavily sprayed on the TLC sheets until they were saturated and visibly translucent (Waldi, 1965a). Plates were heated at 120°C for approximately 10 min and subsequently observed under UV light (365 nm). With this method various steroids display specific colors. The cholesterol spot turned pink.

Ninhydrin spray. A solution of 0.3g ninhydrin dissolved in 100 ml n-butanol and mixed with 3 ml glacial acetic acid was lightly sprayed over the chromatograms. TLC sheets were heated in an oven at 110°C for 10 min. Purple spots were indicative of free amino groups or amino phospholipids (Waldi, 1965b).

Molybdenum blue spray. A solution of 5 ml 60% perchloric acid, 10 ml 1N hydrochloric acid and 25 ml of 4% (w/v) ammonium molybdate was made up to 100 ml with distilled water and sprayed lightly over the chromatogram, which was then heated in an oven at 110°C for 5 min. Blue spots indicated phosphorus containing lipids (Johnston, 1971).

For the quantitative analysis of the chromatographed phospholipids, spots were detected with iodine vapor. Blank zones corresponding in size to the separated phospholipid spots were marked on the same chromatogram. Moreover, a known amount of a phospholipid was spotted on the developed chromatogram as a check. Adsorbent within each spot was scraped from the plastic sheet and aspirated into a 30 ml Kjeldahl flask containing 1 ml of 72% perchloric acid. The flask was then heated gently to boiling and refluxed for 1 hr. The contents of each flask along with 4 consecutive washings were placed in a centrifuge tube and spun at about 5,000 × G for 20 min to sediment the silica gel. The supernatant was placed in a 25 ml volumetric flask along with 3 further aqueous washings of the silica gel residue. The solution was brought up to volume with distilled water. All samples were analyzed for phosphorus with the Technicon Autoanalyser (details given later).

For the major phospholipids, lecithin (PC) and phosphatidyl ethanolamine (PE), 4 or 5 chromatograms were developed and each spot was separately analyzed. However, for lysophosphatidyl ethanolamine (LPE) and sphingomyelin (S), which were present only in minor amounts, spots from 2 chromatograms were combined.

For expression of results as a percentage phospholipid in the total lipid, phosphorus values were multiplied by the following factors: PC, 24.4; PE, 25.4; LPE, 16.0; and S, 24.8.

Cholesterol spots on a developed thin-layer plate were located by running cholesterol standards on outer sides of the plate. The two outer zones were cut off, sprayed with 15% phosphoric acid and heated at 110°C to develop the color. For quantitative analysis of cholesterol, silica gel was scraped off the plate into a centrifuge tube containing 2 ml of acetone-ethanol solution (1:1). The suspension was centrifuged at about 5,000 × G for 5 min and the supernatant collected in a test tube. This procedure was repeated 4 more times on the silica gel residue resulting in the complete extraction of cholesterol, as confirmed by thin-layer chromatography. The combined extracts were evaporated to dryness with nitrogen gas and the residue analyzed by the method of Zak et al. (1957).

Nitrogen and phosphorus determination. Percent phosphorus of samples were determined with a Technicon Autoanalyser II. The method is based on the reaction of ammonium molybdate, antimony potassium tartrate and orthophosphate (Technicon method No. 1SS-71W). Nitrogen was also determined by micro-Kjeldahl official AOAC method (AOAC, 1970). The percent protein content of the fractions was calculated by multiplying % N by 6.25 and percent phospholipid by multiplying % P by 25.

RESULTS & DISCUSSION

THE PERCENT N and P contents of LDLg and MF fractions from eggs laid by birds of various ages are presented in Table 1. As shown in the table, the older birds (39 wk to 1.5 yr) laid eggs with LDLg having % N and % P values respectively 33 and 40% higher than those for younger birds (35 and 36 wk). With regard to MF, the % N for the older birds was about 100% higher than that for the younger birds, but the difference in the %P values was not as great. Steer et al. (1968) found that LDL of the yolk plasma consisted of 2.05% total N and 0.87% total P which are roughly close to the values reported in the present study for LDLg from eggs of young birds. The % protein values were calculated to be about 8 and 15% for MF from eggs laid by young birds (35 and 36 wk) and by older (39 wk to 1.5 yr) birds, respectively. LDLg from eggs of young and older birds contained about 12 and 15% protein, respectively. The average phospholipid content of MF was 29.5% for eggs from birds of all ages (% P differed only slightly) and the LDLg of eggs from young and older birds contained 18.3 and

Table 1—Percent of nitrogen and phosphorus in MF and LDLg fractions

Age of hen[a]	% Nitrogen[b] Mean	A.D.[c]	% Phosphorus[b] Mean	A.D.[c]
MF fraction				
35 wk-Fk1	1.26	0.00	1.13	0.02
36 wk-Fk1	1.29	0.00	1.15	0.01
39 wk-Fk1	2.52	0.00	1.14	0.00
41 wk-Fk1			1.20	0.00
1 yr-Fk2	2.44	0.12		
1.5 yr-Fk2	2.39	0.03	1.20	0.01
LDLg fraction				
35 wk-Fk1	1.93	0.00	0.72	0.02
36 wk-Fk1	1.82	0.01	0.75	0.01
39 wk-Fk1	2.51	0.02	1.05	0.01
41 wk-Fk1			1.01	0.01
1 yr-Fk2	2.50	0.01		
1.5 yr-Fk2	2.46	0.01	1.03	0.01

[a] Fk1 = flock one, Fk2 = flock two.
[b] Average of duplicate determinations of two samples on a dry weight basis
[c] Average deviation

Table 2—Lipid composition of MF and LDLg fractions

Sample		Percent lipid Mean[a]	SD[b]
MF	39 wk	86.01b	0.36
MF	41 wk	85.59b	0.67
LDLg	39 wk	84.10c	0.35
LDLg	41 wk	83.06d	0.37

[a] Eight replications of eggs from flock one on dry weight basis. Means with different letters are significantly different ($P < 0.05$)
[b] Standard deviation

Table 3—Composition of MF and LDLg fractions isolated from yolk of eggs from 39-wk-old hens

Component (%)	MF Mean[a]	MF SD[b]	LDLg Mean[a]	LDLg SD[b]
Total nitrogen	2.53	0.04[c]	2.50	0.02[b]
Total lipid	86.0	0.4	84.1	0.4
Total phosphorus	1.24	0.00[c]	1.05	0.01[b]
Nonlipid residue	14.0	0.4	15.90	0.4
Nonlipid residue nitrogen	11.43	0.17[c]	10.34	0.02[b]
Nonlipid residue phosphorus	0.14	0.06[c]	0.15	0.01[b]
Lipid phosphorus % of lipid	1.44	0.01	1.23	0.03
Cholesterol % of lipid	11.53	0.20	3.73	0.11
Phospholipid	29.94	1.15	25.68	0.36
Phospholipid % of lipid	34.81	1.35	30.58	0.43
Phosphatidyl choline % of phospholipid	80.73	2.10	80.95	0.11
Phosphatidyl ethanolamine % of phospholipid	15.97	1.45	15.69	0.21
Lysophosphatidyl ethanolamine % of phospholipid	1.29	0.11	1.69	0.05
Sphingomyelin % of phospholipid	2.01	0.18	1.67	0.06

[a] Number of replicate samples was between 2 and 10 on dry weight basis
[b] Standard deviation
[c] Average deviation of duplicate determinations of two samples

25.8% phospholipid, respectively. It is of interest to note that the protein contents of MF and LDLg from the eggs of older birds were about the same but the phospholipid content of MF was slightly higher than that for LDLg (29.5% vs 25.8%).

Table 2 presents the lipid content of MF and LDLg obtained from eggs laid by birds 39 and 41 wk old. The MF fractions had mean lipid values in the vicinity of 86% and the LDLg fractions contained 83–84% total lipid. The lipid content of the LDLG fraction was reported by Gornall and Kuksis (1971a) to be 88% whereas Tattrie and Cook (1969) reported values between 84 and 85%. Gornall and Kuksis (1971a) obtained lipid values of 85% for LDFG-1 and 92% for LDFG-2.

The lipid content of LDLg is very similar to that for the low-density lipoprotein fractions from yolk plasma. The lipid content reported by Gornall and Kuksis (1971a), Steer et al. (1968) and Tattrie and Cook (1969), for plasma LDL fell within the range of 86–90%. Saari et al. (1964) isolated two ultracentrifugally-different lipoproteins, LPL_1 and LPL_2, from the plasma low-density fraction. Total lipid was about 89% for LPL_1 (LPL_1 consisted about one-fifth of the total plasma LDL) and 86% for LPL_2. Martin et al. (1964) found that their LDL_1 consisted of about 87% lipid and LDL_2 about 83% lipid, with molecular weights of 10.3×10^6 and 3.3×10^6, respectively.

The nonlipid residues of MF and LDLg were analyzed for the N and P contents. The results are shown in Table 3. The % nitrogen in the nonlipid residue for MF and LDLg was found to be 11.43 and 10.34, respectively. The value of LDLg residue was low compared to 14.8% and 13.7% for nonlipid residues of LDL_1 and LDL_2, respectively, from plasma (Saari et al., 1964). The low % N values of MF and LDLg may be attributed to the presence of a considerable amount of mineral matter and carbohydrates. Since the MF and LDLg nonlipid residues had P contents of 0.14 and 0.15%, it is assumed that phosphoproteins with esterifield phosphate groups were present in the residue. Saari et al. (1964) reported that nonlipid residues of LDL_1 and LDL_2 had % P values of 0.15 and 0.16, respectively.

Saari et al. (1964) was first to show conclusively that cholesterol was the major egg yolk steriod. Tu et al. (1970) confirmed this statement and also showed that egg yolk contained many other steriods in minor amounts. In this study, a steriod zone was developed by thin-layer chromatography (TLC) using MF and LDLg lipid extracts. The steriod has the same R_f value as pure cholesterol. Chromatograms of reference standards as well as of MF and LDLg lipid extracts are shown in Figures 1, 2 and 3, respectively. Upon spraying the chromatograms of MF and LDLg fractions with 15% phosphoric acid, a pinkish red spot developed, typical for cholesterol. Cholesterol esters did not appear to be present.

As shown in Table 3, the cholesterol contents of the two particles were markedly different. The cholesterol value for MF lipid (11.53%) is high compared to that content in LDLg lipid fraction. Gornall and Kuksis (1971a) indicated that LDFG-1 contained 13.3% cholesterol. It is interesting to note that soluble lipoproteins (chylomicrons, VLDL, LDL, HDL) of human blood plasma have compositions similar to that of MF particles and are particularly rich in cholesterol (Margolis, 1969; White et al., 1968). Many authors have determined the cholesterol content of LDLp. Saari et al. (1964) reported about 3.4% of the total lipid as cholesterol whereas in our studies, a cholesterol content for LDLg cholesterol was 3.73%. Gornall and Kuksis (1971b) found that their granule low-density lipoprotein (LDFG) contained about 7% cholesterol on a lipid basis.

It has been known for some time that egg yolk contains only a few major phospholipids. Rhodes and Lea (1956) have shown that egg yolk consists of the following phospholipids: phosphatidyl choline (lecithin), 73%; phosphatidyl ethanolamine, 15%; lysophosphatidyl choline, 5.8%; sphingomyelin,

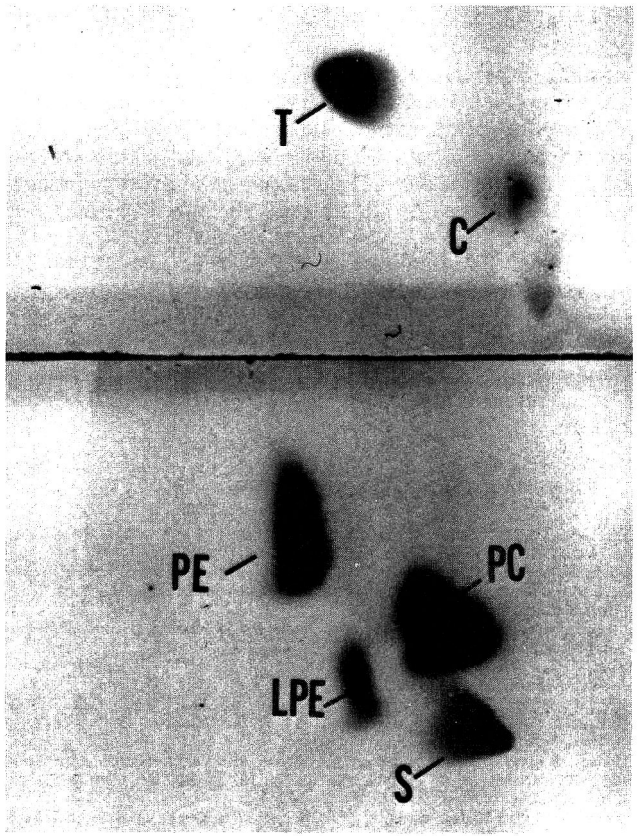


Fig. 1—Thin-layer chromatogram of reference lipids: T, triolein; C, cholesterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; LPE, lysophosphatidyl ethanolamine; S, spingomyelin.

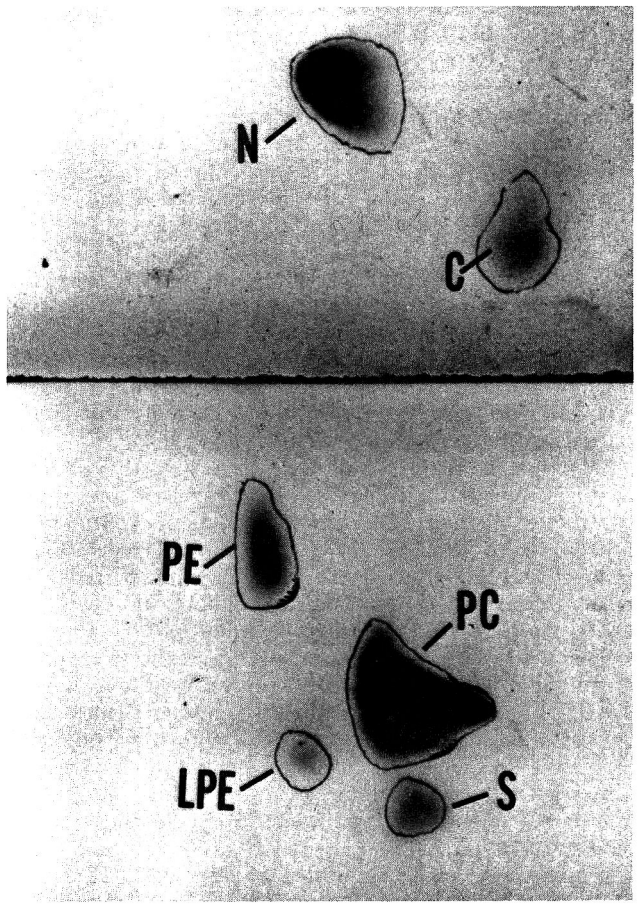


Fig. 2—Thin-layer chromatogram of MF lipids: N, neutral lipids; C, cholesterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; LPE, lysophosphatidyl ethanolamine; S, sphingomyelin.

2.5%; lysophosphatidyl ethanolamine, 2.1%; plasmogen, 0.9% and phosphatidyl inositol, 0.6%. Typical chromatograms of MF, LDLg and reference lipids are shown in Figures 1, 2 and 3. Chromatograms in Figures 2 and 3 show the presence of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), lysophosphatidyl ethanolamine (LPE) and sphingomyelin (S) in lipid fractions of MF and LDLg. Lysophosphatidyl choline was not detected in either MF or LDLg fractions. According to Gornall and Kuksis (1971b), PC, PE, S and lysolecithin were present in LDFG. Further, these researchers reported lysolecithin as the major lysophospholipid in egg yolk. Saari et al. (1964), using thin layer chromatography, found four phospholipids in the lipid extract of LPL_1 and LPL_2 from plasma. The two major phospholipids were identified as PC and PE and the other two, present in minor amounts, were presumed to be sphingomyelin and a lysophospholipid.

The amounts of the phospholipids of MF and LDLg fractions are presented in Table 3. Phosphatidyl choline (FC) was found to be the most prevalent phospholipid, comprising about 81% of the total phospholipid in both MF and LDLg structures. According to Gornall and Kuksis (1971b) the phosphatidyl choline content of LDLG was 69%. Saari et al. (1964) found that phosphatidyl choline made up 84% of the plasma LDL phospholipid. Tattrie and Cook (1969) reported somewhat lower values for plasma LDL phosphatidyl choline between 71 and 76%.

Phosphatidyl ethanolamine (PE), the next major phospholipid isolated from both MF and LDLg samples, comprised about 16% of the total phospholipid of each particle. Gornall and Kuksis (1971b) reported that the PE of LDFG was about 19%. The PE contents in the low-density lipoproteins isolated from plasma by various investigators range between 14 and

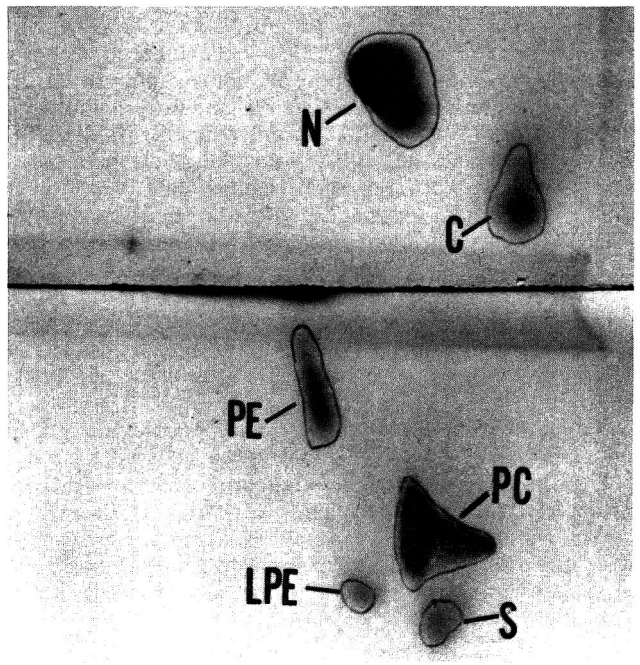


Fig. 3—Thin-layer chromatogram of LDLg lipids: N, neutral lipids; C, cholesterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; LPE, lysophosphatidyl ethanolamine; S, sphingomyelin.

23%. Saari et al. (1964) reported a PE value of 14%, Tattrie and Cook (1969) a somewhat higher range of 16 to 20% and Gornall and Kuksis (1971b) the highest reported value of 23%.

Lysophosphatidyl ethanolamine (LPE) and sphingomyelin (S) were isolated as minor phospholipids in MF and LDLg. The LPE content of 1.29% in the MF phospholipid fraction was somewhat lower than 2.01% for the content of sphingomyelin. With regard to the LDLg phospholipid, the S and LPE contents were about the same, approximately 1.7%. The data of Gornall and Kuksis (1971b) indicated that LDFG contained 6% S and 6% lysolecithin. Phospholipids termed "spot A" and "spot B" (presumably S and a lysophospholipid) by Saari et al. (1964) were found to be present at concentrations between 1.4 and 1.8% and between 1.5 and 1.9%, respectively, of the total phospholipid. Values of 1.69% for LPE and 1.67% for S from LDLg (Table 3) are almost identical in value and ratio with these values reported by Saari et al. (1964).

As shown in Figures 2 and 3, the triglyceride spots were prominent. On a total lipid basis, MF consisted of about 54% triglyceride whereas LDLg contained about 66%. The term "myelin figure" was originally coined by Chang (1969) and Chang et al. (1977) to classify the egg granule particles having concentric leaflet figures. The term myelin has been used to denote multilayered structures of brain and nervous tissue. Chang (1969) proposed that myelin figures were made up of phospholipids. The results of the present study show that, although phospholipids are a major component, they account for only about 30% of the total MF composition. Conceivably MF particles can be classed as low-density lipoproteins since these particles are made up of triglycerides, phospholipids, cholesterol and protein, and constitute a floating fraction of granules.

MF micelles can be envisioned as large particles consisting of an inner neutral lipid core stabilized with one or more lamellae composed of phospholipid, cholesterol and protein. Since osmium tetroxide interacts strongly with phospholipids to form an electron-dense area, it may be assumed that the electron-dense lamellae consists of phospholipids. The internal cores of MF are only weakly osmiophilic, suggesting the presence of neutral lipids. Pepsin treatment of a suspension of washed granules, although effective in disrupting the granules does not seem to alter the structure of the lamallae of the myelin figures (Chang et al., 1977). Presumably protein is intimately bound with surface phospholipids and exists in a protected configuration. Research by Garland (1973) and Garland and Powrie (1977) shows that two myelin species, "light" (low electron-dense lamellae) and "heavy" (high electron-dense lamellae) exist. The differences in electron density can be attributed to the number of lamellae in the MF micelles.

The LDLg micelles have a composition very similar to that for LDLp micelles. Presumably the organization of the constituents in each of these micelles is the same with the triglycerides existing as a core and the phospholipids, cholesterol and proteins being present on the core surface (Chang et al., 1977; Garland and Powrie, 1977). Chang et al. (1977) have hypothesized that proteins reside on the micelle surface as an interlinking component between the triglyceride core and phospholipid-containing particles called ultraparticles.

When these researchers treated yolk plasma with pepsin, distinct electron-dense peripheries of LDL_p micelles were absent and, indeed, the electron-dense ultraparticles were scattered throughout the plasma mass. Thus, the ultraparticles on the surfaces of native LDL_p micelles must have been released by proteolysis. According to Steer et al. (1968), about 80% of the protein in native low-density fraction of yolk plasma is accessible to proteolytic enzymes. This hydrolyzed portion of the polypeptides could be the link between the lipid core and the phospholipid-containing ultraparticle. The 20% of unhydrolyzed peptide could be buried in the lipid core and ultraparticles. Steer et al. (1968) proposed three possible models to explain the presence of residual unhydrolyzed peptide in Pronase-treated LDL_p: the backbone of the residual peptide is interdigitated between surface lipids; the residual peptide is located at a contact area between associated lipoproteins; and the residual peptide is buried in the lipid core. Experimentation should be carried out on the characterization and chemical composition of isolated ultraparticles and naked lipid cores of Pronase-treated LDL_p and LDL_g to help clarify the micelle structure.

REFERENCES

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, St. Paul, MN.

Burley, K.W. and Cook, W.H. 1961. Isolation and composition of avian egg yolk granules and their constituents α- and β-lipovitellins. Can. J. Biochem. Physiol. 39: 1295.

Chang, C.M. 1969. Studies on egg yolk. Ph.D. thesis, Univ. of Wisconsin, Madison, WI.

Chang, C.M., Powrie, W.D. and Fennema, O. 1977. Microstructure of yolk. J. Food Sci. 42: 1193.

Cook, W.H. 1968. Macromolecular components of egg yolk. In "Egg Quality, A Study of the Hen's Egg," Ed. Carter, T.C. Oliver and Boyd, Pub., Edinburgh, Scotland.

Garland, T. 1973. Studies on egg yolk myelin figures and granule low-density lipoprotein. Ph. D. thesis, Univ. of British Columbia, Vancouver, B.C.

Garland, T. and Powrie, W.D. 1978. Isolation of myelin figures and low-density lipoproteins from egg yolk granules. J. Food Sci. 43: 592.

Gornall, D.A. and A. Kuksis. 1971a. Resolution of egg yolk lipoproteins by chromatography on thin layers of hydroxylapatite. Can. J. Biochem. 49: 44.

Gornall, D.A. and A. Kuksis. 1971b. Molecular species of glycerophosphatides and triglycerides of egg yolk lipoproteins. Can. J. Biochem. 49: 51.

Johnston, P.V. 1971. Basic lipid methodology. Special Publ. 19, Univ. of Illinois, Pub., Urbana-Champaign.

Margolis, S. 1969. Structure of very low and low-density lipoproteins. In "Structural and Functional Aspects of Lipoproteins in Living Systems," Ed. Tria, E. and Scanu, A.M. Academic Press, New York, NY.

Martin, W.G., Augustyniak, J. and Cook, W.H. 1964. Fractionation and characterization of the low-density lipoproteins of hen's egg yolk. Biochem. Biophys. Acta. 84: 714.

Rhodes, D.N. and Lea, C.H. 1956. Phospholipids. 4. On the composition of hen's egg phospholipids. Biochem J. 65: 526.

Saari, A., Powrie, W.D. and Fennema, O. 1964. Isolation and characterization of low-density lipoproteins in native egg yolk plasma. J. Food Sci. 29: 307.

Saito, Z., Martin, W.G. and Cook, W.H. 1965. Changes in the major macromolecular fractions of egg yolk during embryogenesis. Can. J. Biochem. 43: 1755.

Steer, D.C., Martin, W.G. and Cook, W.H. 1968. Structural investigation of the low-density lipoprotein of hen's egg yolk using proteolysis. Biochemistry 7: 3309. Tattrie, N.H. and Cook, W.H. 1969. Lipoproteins and lipids of egg yolk. S.C.I. Monograph No. 32. p. 92.

Tattrie, N.H. and Cook, W.H. 1969. Lipoproteins and lipids of egg yolk. S.C.I. Monograph No. 32. p. 92.

Tu, C., Powrie, W.D. and Fennema, O. 1970. Steroids in egg yolk. J. Food Sci. 35: 601.

Waldi, D. 1965a. Steroids. In "Thin-Layer Chromatography, A Laboratory Handbook," Ed. Stahl, E. Academic Press, New York, NY.

Waldi, D. 1965b. "Spray Reagents for Thin-Layer chromatography, A Laboratory Handbook," Ed. Stahl, E. Academic Press, New York, NY.

White, A., Handler, P. and Smith, E.L. 1968. "Principles of Biochemistry," 4th ed, McGraw-Hill Book Co., New York, NY.

Zak, B., Luz, D.A. and Fisher, M. 1957. Determination of serum cholesterol. Am. J. Med. Technol. 23: 283.

Ms received 11/27/77; revised 2/10/78; accepted 2/15/78.

CHARACTERIZATION OF PLASTEIN REACTION PRODUCTS FORMED BY PEPSIN, α-CHYMOTRYPSIN, AND PAPAIN TREATMENT OF EGG ALBUMIN HYDROLYSATES

J. H. EDWARDS and W. F. SHIPE

ABSTRACT

Gelled plastein reaction products were prepared by treating concentrated peptic hydrolysates of egg albumin with pepsin, α-chymotrypsin, and papain. The relative firmness of these homogeneous gels was determined by comparing force-distance curves obtained from puncture tests made with the Instron Universal Testing Machine. The effects of the choice of enzyme and degree of substrate hydrolysis on the plastein yield (increase in 10% Trichloroacetic acid precipitable material) and water solubility were investigated. Electrophoresis of plastein products in the presence of sodium dodecyl sulfate (SDS) indicated that no high molecular weight, protein-like material was produced by the plastein reaction. It is suggested that plastein reactions lead to the formation of insoluble peptide aggregates. These aggregates are thought to be held together by noncovalent bonds rather than by covalent, peptide bonds, as has been reported previously.

INTRODUCTION

THE NEED FOR HIGH nutritional quality protein is increasing as a result of the expanding world population. A recognition of this need has prompted increased production of traditional proteins and the study and evaluation of unconventional protein sources. In many cases, these unconventional products are not acceptable due to poor nutritional, functional, and/or sensory qualities. The plastein reaction is a method of modifying proteins lacking quality attributes, thereby increasing the supply of acceptable protein products.

The plastein reaction reportedly involved the enzymatic hydrolysis of proteins into small molecular polypeptides, followed by enzymatic resynthesis into new protein-like substances (i.e., plastein). Little work has been done to determine what factors affect the properties of plastein reaction products.

Enzymes vary in their ability to synthesize plastein (Fujimaki et al., 1970; Yamashita et al., 1971). Tsai et al. (1972) has shown that the amount of plastein synthesized by a given enzyme is controlled by at least three factors: average molecular weight of substrate, substrate concentration, and reaction pH. Fujimaki et al. (1970) reported α-chymotrypsin to be more plastein productive than pepsin or papain at pH 7.0 with a peptic hydrolysate of soy protein. Yamashita et al. (1971) has reported a range of plastein yields obtained with various microbial proteases at their pH optimum. These workers also reported that maximum plastein production was obtained with pepsin, α-chymotrypsin, and papain at pH 5.0. Little work has been done to determine the effect of enzyme of the properties of plastein reaction products.

The degree of substrate hydrolysis also influences plastein yield. Cuthbertson and Tompsett (1931) observed that the longer a protein was allowed to digest during the hydrolysate step, the lower the yield of plastein. However, both Horowitz and Haurowitz (1959) and Tsai et al. (1972) have shown that small peptides provide the ideal substrate for plastein synthesis. Determann et al. (1963) suggested that a tetrapeptide represented the minimal peptide chain length for plastein reaction initiation. Presumably, over digestion produces small peptides and amino acids that do not enter into plastein formation. v. Hofsten and Lalasidis (1976) have also shown that the properties of plastein products are affected by the length of substrate hydrolysis time. Hydrolysates obtained by exhaustive digestion of a whey protein concentrate formed plasteins more slowly and gave softer gels than partial hydrolysates.

The exact mechanism of plastein formation has not been determined at present. Yamashita et al. (1973) demonstrated an actual peptidyl-enzyme intermediate stressing the enzymatic role in the reaction. These workers concluded the reaction was mainly a polycondensation reaction. If peptide bond formation is the only mechanism occurring, a decrease in free amino groups should occur. Several researchers have reported such a decrease (Wasteneys and Borsook, 1924; Collier, 1940; Virtanen and Kerkkonen, 1948) whereas other researchers have not (Horowitz and Haurowitz, 1959; v. Hofsten, 1974). These latter workers concluded that plastein is essentially a product of transpeptidation reactions.

Tauber (1951) reports obtaining plastein comprised of molecules with molecular weights of the order of 250,000 to 500,000 as determined by ultracentrifugal analysis. Yamashita et al. (1974) reports an increase from 2,600 to 21,600 during a plastein reaction initiated by chymotrypsin with a soybean globulin peptic hydrolysate as the reaction substrate. v. Hofsten and Lalasidis (1976) consider these molecular weight values as unreliable due to the poor solubility of plastein in water and ordinary buffers.

The present study was done to determine what effects the synthetic enzyme and the degree of substrate hydrolysis have on the properties of plastein reaction products. Electrophoresis in the presence of SDS was also performed to determine the extent of high molecular weight polypeptide formation during the plastein reactions studied.

MATERIALS & METHODS

Preparation of hydrolysates

A commercial egg albumin protein preparation (ICN Pharmaceuticals Inc., Cleveland, OH) containing approximately 70% protein (N × 6.25) was suspended in aqueous HCl (final pH, 1.6; final concentration, 1.0%) and incubated with pepsin (3 × crystallized, ICN Pharmaceuticals Inc.) with mild shaking. The enzyme to substrate ratio was set at 1:100. Lots were removed following allotted incubation times and the pH raised to 7.0 with 6N NaOH and held for one hour to inactivate the pepsin. The pH was then decreased to 5.0 with 6N HCl. Lots were freeze dried yielding powdered products which were used as substrates for the plastein reaction.

Preparation of plasteins

Freeze-dried reaction substrates and enzymes were dissolved in dilute HCl, pH 5.0, and incubated under the following conditions: enzyme-substrate ratio, 1/100 (w/w); substrate concentration, 40% (w/w); incubation temperature, 37°C; incubation time, 24 hr. In addition to the pepsin used during hydrolysis, the following enzymes were used to form plastein reaction products: α-chymotrypsin (3 × crystallized, ICN Pharmaceuticals Inc.) and papain (purified concentrate, ICN Pharmaceuticals Inc.). All papain reaction solutions contained L-cystein (free base) at a final concentration of 0.01M as a papain activator.

Degree of hydrolysis determination

Determinations of the degree of hydrolysis of the egg albumin protein preparation were based on a modified method Yamashita et al., 1970. Determinations were made in triplicate. The results were expressed in terms of percent hydrolysis. The following equation was used for making the calculations:

Authors Edwards and Shipe are with the Dept. of Food Science, Cornell University, Ithaca, NY 14853.

0022-1147/78/0004-1215$02.25/0
© 1978 Institute of Food Technologists

$$\frac{\text{10\% TCA insol N before hydrolysis} - \text{10\% TCA insol N after hydrolysis}}{\text{10\% TCA insol N before hydrolysis}} \times 100$$

Gel strength index determination

The Instron Universal Testing Maching Model #TM (Instron Corp., Canton, MA) was used to perform puncture tests on plastein gel surfaces. The force level required to penetrate the product (as indicated by an abrupt change in the slope of the force-distance curves) was taken as an index of plastein gel strength. Measurements were made with Compression Load Cell CB (Instron Corp.), ¼ in. drive pin punch (L.S. Starret Co., Anthos, MA), drive speed 2 in./min, chart speed 10 in./min, and a full scale load of 0–100g or 0–200g as required. Plastein gels were prepared in 100 ml beakers to yield a final volume of 15–20 ml.

Plastein yield determination

The method of Arai et al. (1975) was used to determine plastein yields. Determinations were made in triplicate. Plastein yields are calculated on the basis of the amount of 10% TCA insoluble N formed during the plastein step.

pH solubility determination

Solubility of 1% solutions of plastein reaction products and hydrolysates were determined at various pH values by a modified method of Lu and Kinsella, 1972. The nitrogen content of 3 ml aliquots before and after centrifugation (15,000 rpm, 15 min) were determined. Duplicate determinations were made for each sample at each pH valve.

$$\% \text{ N Solubility} = \frac{\% \text{ N supernatent}}{\% \text{ N total}} \times 100$$

Table 1—Degree of peptic hydrolysis of egg albumin protein preparation at varying incubation times[a]

Hydrolysis time (hr)	% Hydrolysis[b]
4	79.5
8	91.1
12	93.5
18	95.0
24	96.0

[a] Three replicates per determination

[b] $\frac{\text{10\% TCA insol N before hydrolysis} - \text{10\% TCA insol N after hydrolysis}}{\text{10\% TCA insol N before hydrolysis}} \times 100$

Table 2—Comparison of gel strength percent yield and percent plastein yield for plastein reaction products prepared with pepsin, α-chymotrypsin and papain

Sample designation		Gel strength yield point (g)	% Yield[a]	% Plastein yield[b]
Hydrolysis time (hr)	Enzyme			
4	pepsin	200	47	20
8	pepsin	125	45	25
18	pepsin	26	42	30
24	pepsin	11	40	28
4	α-chymotrypsin	103	43	16
8	α-chymotrypsin	81	38	18
18	α-chymotrypsin	21	35	23
24	α-chymotrypsin	11	32	20
4	papain	28	30	3
8	papain	14	26	6
18	papain	2	22	10
24	papain	1	20	8
4	control	0	27	0
8	control	0	20	0
18	control	0	12	0
24	control	0	12	0

[a] % yield [(10% TCA insol. N)/(Ttoal substrate N)] × 100.

[b] % plastein yield = % yield enzyme sample – % yield control.

Sodium dodecyl suflate (SDS) polyacrylamide gel electrophoresis

The method of Weber and Osborn (1969) was used with 12.5% acrylamide gels (N,N'-methylenebisacrylamide:acrylamide ratio = 1:25) to obtain resolution of lower molecular weight bands. The gels were stained with 0.25% Coomassie Blue, R-250 for 3 hr and destained in 7.5% acetic acid, 5.0% methanol solution until the background stain was removed. Samples were prepared by dissolving 0.10g sample in 10.0 ml 0.05N NaOH. Sample solutions were then mixed in a 1:1 ratio with tracking solution and boiled for 2 min (final concentration, 5μg/μl).

RESULTS & DISCUSSION

Peptic hydrolysis of egg albumin

Table 1 shows the degree of hydrolysis of egg albumin protein determined at various incubation times with pepsin. Increased hydrolysis was indicated by increased solubility of the hydrolysate in 10% TCA. Table 1 shows a leveling off of percent hydrolysis after 1 12-hr peptic incubation period. It is assumed that peptides soluble in 10% TCA may be further hydrolyzed at longer incubation times yielding products differing in component molecular weight distributions. Such further hydrolysis would not be reflected by TCA solubility measurements.

Plastein yield

Table 2 shows that the amount of plastein (increase in 10% TCA insoluble material) obtained from a particular hydrolysate is dependent on the enzyme used in its preparation. Pepsin exhibited the maximum synthetic activity on all hydrolysates. A reduced yield is obtained with α-chymotrypsin and papain is the least plastein productive. A fourfold increase in papain concentration did not result in a significant increase in plastein yield. This indicates that papain concentration was not the limiting factor in the original experiment.

Degree of substrate hydrolysis also influences plastein yield. Table 2 shows maximum plastein production was achieved with an 18 hr hydrolysate (degree of hydrolysis; 95.0%) with the three enzymes used.

Plastein gel strength

Typical puncture test force curves obtained with the Instron for plastein gels prepared from an 8 hr egg albumin hydrolysate (degree of hydrolysis; 91.1%) are shown in Figure 1. An initial steep slope is observed in the force-distance curves as the metal probe contacts the gel surface. An abrupt change in slope occurs when the probe penetrates the gel surface. The force at which this change occurs is the yield point according to the definition of Bourne (1976). In the present study this force level was interpreted as an indication of gel strength.

A yield point of 127g was obtained for the pepsin reaction product as is shown in Figure 1. Papain plastein reaction product prepared from the same hydrolysate had a much weaker gel structure (yield point; 14g). The fact that pepsin and α-chymotrypsin produces stronger gels is also shown in Figure 2. Regardless of the reaction mechanism, the strength of plastein gels is dependent on the enzyme used in their preparation.

Figure 2 shows that the gel strength of plastein products is dependent on the duration of the hydrolytic step. Since the molecular weight of the peptides decrease with prolonged hydrolysis it appears that weak gels may be produced if the starting peptides are too small. Larger peptides may interact to form larger particles lending firmness to the gel structure.

Figure 2 shows that the gel strength is more dependent of the specific enzyme used when the time of substrate hydrolysis is short. As hydrolysis time increases gel strength becomes less dependent on the enzyme and more dependent on the degree of substrate hydrolysis.

The present study shows that the physical gel strength of plastein gels is dependent on the enzyme used in their preparation and the degree of substrate hydrolysis. Tsai et al. (1972)

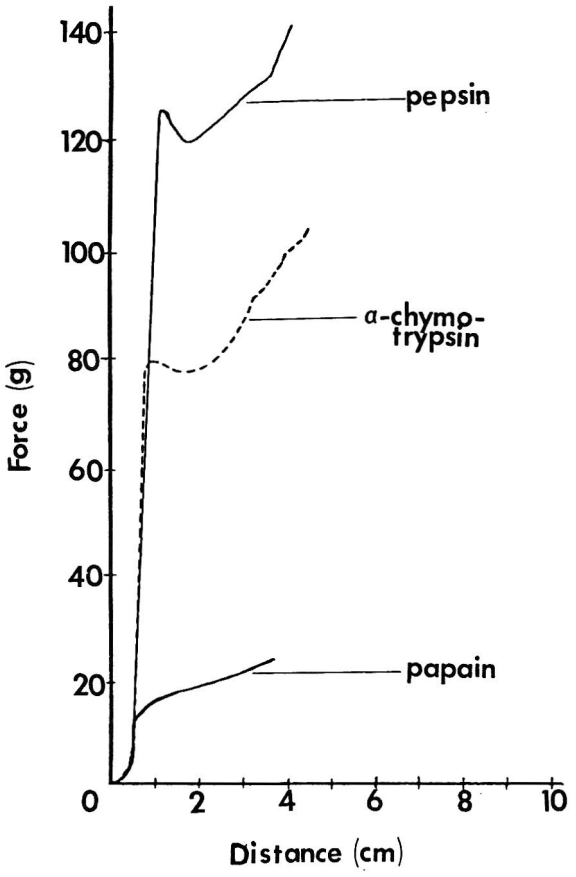


Fig. 1—Typical puncture test force-distance curves for plastein gels prepared with pepsin, α-chymotrypsin and papain from an 8 hr peptic hydrolysate of egg albumin.

using a curd tension meter has shown substrate concentration to be another factor governing gel strength. It is possible that plastein gels for utilization in food products could be engineered to the desired consistencies by controlling these parameters.

Plastein solubility

Figure 3 shows that the pH solubility profiles of plastein reaction products produced from 4 and 24 hr peptic hydrolysates is dependent on the enzymes used in their preparation. Pepsin produces the most insoluble products from both albumin substrates. The α-chymotrypsin plasteins are relatively insoluble between pH 3.0 and 7.0 but they approach the solubility of the hydrolysates at pH 8.0. Papain plastein solubility undergoes little change between pH 4.0 and 8.0 which is in contrast to all other plastein products. All reaction products and substrates approach complete solubility at the acidic and basic ends of the pH scale. All reaction products are more insoluble than the hydrolysates from which they are produced, which do not form gell products.

Figure 3 also shows that pH solubility profile is dependent on the degree of substrate hydrolysis. Plasteins produced from 4-hr hydrolysates were consistently more insoluble between pH 4.0 and 8.0 than corresponding plasteins produced from 24 hr hydrolysates.

Extent of molecular weight changes during the plastein reaction

Figure 4 shows that nearly all the protein fractions present in the egg albumin preparation had been hydrolyzed by a 4-hr peptic digestion. A high molecular band at 37,000 remains unhydrolyzed. A very faint band at 34,000 can be seen here and was completely visible at higher sample concentrations. The molecular weight range of the primary components of the

—Text continued on page 1218

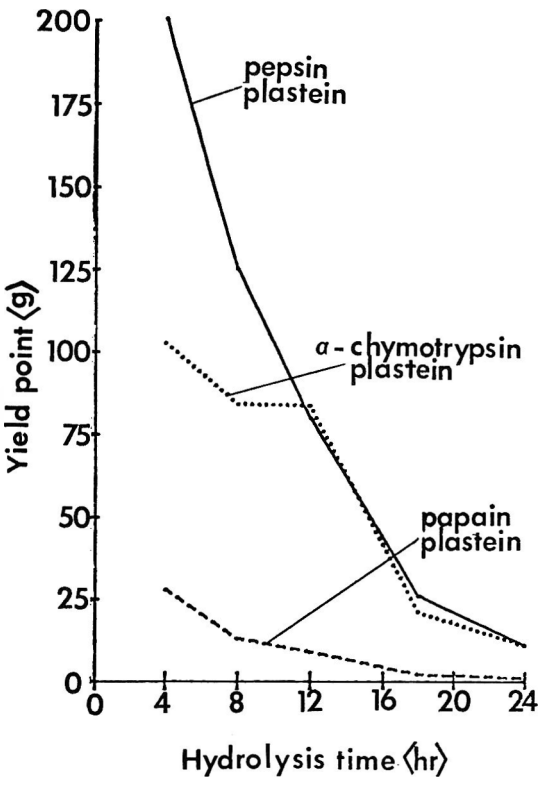


Fig. 2—Effect of substrate hydrolysis time and synthetic enzyme on the gel strength of plastein reaction products.

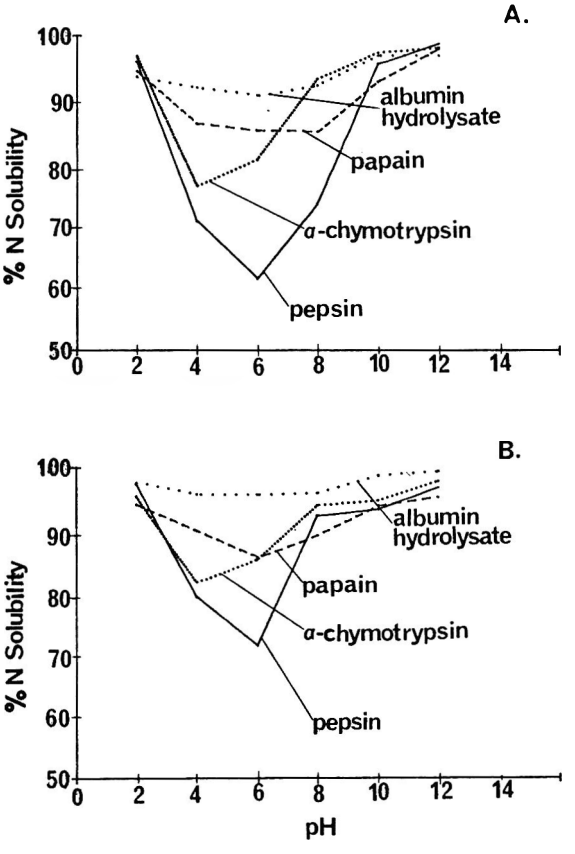


Fig. 3—pH solubility curves of albumin hydrolysates and corresponding pepsin, α-chymotrypsin, and papain plastein products: (A) 4-hr peptic hydrolysate; (B) 24-hr peptic hydrolysate.

hydrolysate lies between 6,500 and 10,500. The plastein reaction products produced with pepsin, α-chymotrypsin, and papain do not contain significant amounts of high molecular weight components approaching the complexity of the initial protein. The majority of material lies in the molecular weight range of the hydrolysate (6,500–10,500).

The pepsin plastein has a band corresponding to a molecular weight of 35,000 which is not present in the hydrolysate or other plastein samples. This band represents the pepsin added to synthesize the plastein product. α-chymotrypsin plastein shows a band at 25,000 which is not present in the hydrolysate or other plastein samples. This band was observed on SDS gels of the α-chymotrypsin enzyme preparation. The molecular weight of the two chains of α-chymotrypsin are 11,000 and 13,000 (Weber and Osborn, 1969). This band (25,000) may be an impurity or the two undissociated chains of α-chymotrypsin. Papain plastein shows no high molecular weight bands as the hydrolysate and other plastein samples do. This may indicate that further hydrolysis of this fraction is occurring simultaneously with plastein synthesis with papain.

All plastein samples have a diffuse protein band at 14,000 which is not present in the egg albumin hydrolysate. This may indicate that a small degree of higher molecular weight synthesis has occurred. However, it should be noted that diffuse staining on SDS gels in the low molecular weight region may indicate extensive association in occurring even in SDS (Weisel and Szent-Gyorgyi, 1975).

It is known that hydrophobic aggregations may be disrupted by SDS (Fukushima, 1969). When electrophoresis of plastein samples in 6 and 12% tris-glycine polyacrylamide gels at pH 8.5 and 9.5 was attempted, samples did not penetrate the gel but appeared as an insoluble mass on top of the gels. Such insolubility without SDS may indicate the role hydrophobic aggregation plays in the apparent high molecular weight formation determined by ultracentrifugation techniques.

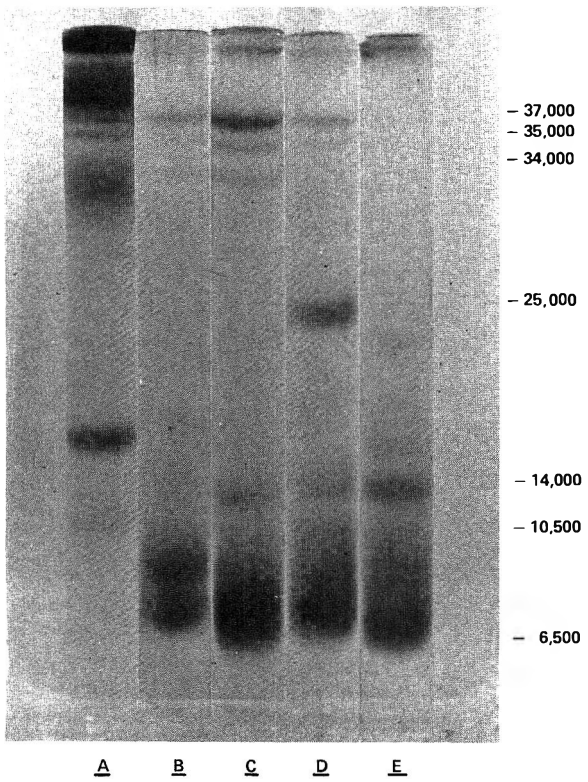


Fig. 4—12.5% SDS polyacrylamide gels of Coomassie Blue stained: (A) Egg albumin protein preparation; (B) 4-hr peptic hydrolysate; (C) Pepsin plastein; (D) α-chymotrypsin plastein; and (E) Papain plastein.

v. Hofsten and Lalasidis (1976) have estimated the molecular weight distributions of whey protein hydrolysate and plasteins produced with Esperase by chromatography on Sephadex G-50. They concluded the difference between these two materials lies mainly in their solubility properties and not in their molecular weights. The results of the present study are in agreement with this finding.

Plastein gels may be formed by a process whereby the dissolved compounds become insoluble. The inability of hydrolysate solutions to form gels in the absence of enzyme, indicates that this insolubility is enzymatically induced.

The insolubility of plastein has been attributed to the accumulation of nonpolar (hydrophobic) amino acids in a fraction of the total reaction product (Aso et al., 1973; Onoue and Riddle, 1973; Arai et al., 1975; v. Hofsten and Lalasidis, 1976). Plastein insolubility may be the result of hydrophobic amino acid incorporation into peptides by specific enzymatic reactions. These hydrophobic peptides may then aggregate as a consequence of their increased hydrophobicity. This aggregation process may be reponsible for the apparent increase in molecular weight which has been associated with the plastein reaction. Table 2 suggests that gel strength is not dependent solely on the amount of aggregation as reflected by the increase in TCA insoluble material. The ability of these aggregates to interact to form a continuous gel mesh network and/or the relative size and solubility of these aggregates may determine the strength of the resultant gel structure.

REFERENCES

Arai, S., Yamashita, M., Fujimaki, M. and Aso, K. 1975. A parameter related to the plastein formation. J. Food Sci. 40: 342.

Aso, K., Yamashita, M., Arai, S. and Fujimaki, M. 1973. General properties of a plastein synthesized from a soybean protein hydrolysate. Agr. Biol. Chem. 37: 2505.

Bourne, M.C. 1976. "Rheology and Texture in Food Quality." Ed. DeMan, J.M., Voisey, P.W., Rasper, V.F. and Stanley, D.W. Avi Publishing Co., Westport, CT.

Collier, H.B. 1940. The problem of plastein formation. I. The formation of a plastein by papain. Canadian J. of Research 18: 255.

Cuthbertson, D.P. and Tompsett, S.L. 1931. The enzymatic synthesis of proteins with special reference to the action of pepsin. Biochemistry 25: 2004.

Determann, H., Bomhard, K., Kohler, R. and Wieland, T. 1963. Unter suchgen uber die Plastein-Reaktion. Helv. Chim. Acta 46: 2498.

Fujimaki, M., Yamashita, M., Arai, S. and Kato, H. 1970. Enzymatic modification of proteins in foodstuffs. Part 1. Enzymatic proteolysis and plastein synthesis application for preparing bland protein-like substances. Agr. Biol. Chem. 34: 1325.

Fukushima, D. 1969. Denaturation of soybean proteins by organic solvents. Cereal Chem. 46: 156.

v. Hofsen, B. 1974. Enzymatisk hydrolys an naringsproteiner. STU report no. 733: 229. University of UPPsala, Sweden.

v. Hofsten, B. and Lasasidis, G. 1976. Protease-catalyzed formation of plastein products and some of their properties. J. Agric. Food Chem. 24: 460.

Horowitz, J. and Haurowitz, F. 1959. Mechanism of plastein formation. Biochimica et Biophysica Acta 33: 231.

Lu, P. and Kinsella, J. 1972. Extractability and properties of protein from alfalfa leaf meal. J. of Food Sci. 37: 94.

Onoue, Y. and Riddle, V.M. 1973. Use of plastein reaction in recovering protein from fish waste. J. Fish. Res. Board Can. 30: 1745.

Tauber, H. 1951. Synthesis of protein-like substances by chymotrypsin. J. Am. Chem. Soc. 73: 1288.

Tsai, S., Yamashita, M., Arai, S. and Fujimaki, M. 1972. Effect of substrate concentration on plastein productivity and some rheological properties of the products. Agr. Biol. Chem. 36: 1045.

Virtanen, A. and Kerkkonen, H. 1948. Structure of plasteins. Nature 161: 888.

Wasteneys, H. and Borsook, H. 1924—25. The synthesizing action of pepsin. J. Biol. Chem. 62: 15.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfatepolyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406.

Weisel, J.W. and Szent-Gyorgyi, A.G. 1975. The coiled-coil structure: identity of the two chains of mercanaria paramyosin. J. Mol. Biol. 98: 665.

Yamashita, M., Tsai, S., Arai, S., Kato, H. and Fujimaki, M. 1971. Plastein yields and their pH dependence. Agr. Biol. Chem. 35: 86.

Yamashita, M., Arai, S., Tanimoto, S., Fujimaki, M. 1973. Condensation reaction occurring during plastein formation by α-chymotrypsin. Agr. Biol. Chem. 37: 953.

Yamashita, M., Arai, S., Tanimoto, S. and Fujimaki, M. 1974. Mode of action of α-chymotrypsin in its reverse reaction at pH 5.3. Biochimica et Biophysica Acta 358: 105.

Ms received 10/5/77; revised 12/13/77; accepted 12/20/77.

INFLUENCE OF ELECTROLYTES ON THE SOLUBILITY OF RAPESEED PROTEIN ISOLATES

LARS GILLBERG

ABSTRACT

Rapeseed meal and protein isolates were analyzed for total nitrogen, total phosphorus, low molecular weight phosphorus, phytic acid, RNA, DNA, and phospholipids. The influence of electrolytes on the nitrogen and phosphorus solubility of rapeseed protein isolates was studied at different pH values. The solubility profiles were displaced differently by different salts. The displacements could be explained by ion binding to the proteins and to the other polymers present in the isolates. The higher binding strength anions had for proteins the more effective they were in decreasing the nitrogen solubility of the protein isolate at pH 0.7 and in increasing the nitrogen solubility at pH 5.4, the pH corresponding to the nitrogen solubility minimum in the absence of added salt.

INTRODUCTION

IN A PREVIOUS STUDY (Gillberg and Törnell, 1976a) it was shown that rapeseed meal proteins could be extracted without a simultaneous extraction of phytic acid, one of the antinutritive components of the meal. The extracted proteins were precipitated quantitatively by the addition of a polyacid and an acid (Lönnerdal et al., 1977). Also the polyacids in the extract were recovered in the protein isolates (Åman and Gillberg, 1977) and the protein isolates had excellent nutritive properties (Liedén et al., 1978).

The successful use of a protein isolate as a component in a food product depends on its functional properties, e.g. solubility, swelling, water-holding capacity, foam, gel, and emulsion properties. A protein isolate, as well as many other food components, contains polymers with fixed charges (polyelectrolytes), e.g. proteins, nucleic acids, and acidic carbohydrates. The functional properties of protein isolates therefore vary with the salt content. When salt is added to an aqueous dispersion of a protein isolate, the salt ions tend to screen the fixed charges and thereby weaken electrostatic interactions between the polyelectrolytes. The added ions may also bind strongly and even selectively to some sites and thereby change the charge of the polyelectrolytes involved. Depending on pH and composition of the protein isolate, this type of binding may result in the formation of new and in the dissolution of old complexes between polyelectrolytes. As the number of fixed sites to which ions can bind selectively or very strongly is often relatively small, the redistribution of complexes due to this type of binding is most extensive at low levels of salt addition. This is also true when ionic detergents are added, which can bind very strongly to a vast number of sites, as the number of fixed countercharges is restricted.

The aim of this study was to illustrate and attempt to explain the variation in solubility of rapeseed protein isolates at various pH's on the addition of small amounts of salts. To get a complete and detailed picture of the formation and dissolution of complexes between the molecules of the protein isolates, the composition and concentration of all complexes would have had to be examined. For the sake of simplicity and feasibility, only the nitrogen and phosphorus solubility was measured.

Author Gillberg formerly with the Dept. of Chemical Technology, The Lund Institute of Technology, P.O.B. 740, S-220 07 Lund 7, Sweden, is now affiliated with Pharmacia Diagnostics, P.O. Box 17, S–751 03 Uppsala 1, Sweden.

0022-1147/78/0004-1219$02.25/0
© 1978 Institute of Food Technologists

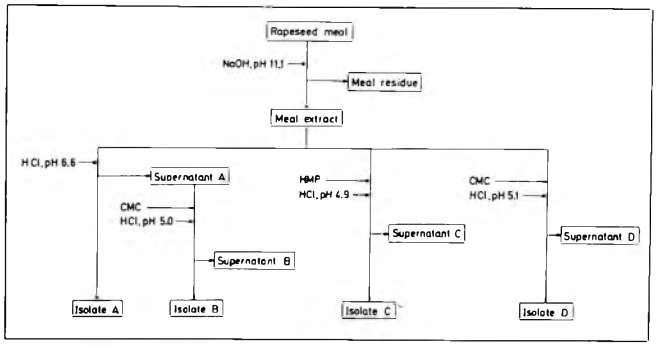


Fig. 1—Scheme for the preparation of the isolates.

Of studies previously published on the effects of addition of small amounts of salts on the solubility of complex proteinaceous systems, reference is given to Smith and Circle (1938), who reported that the nitrogen solubility of soybean meal was strongly affected by small amounts of salts, and to van Megen (1974), who described how the nitrogen solubility of concentrated dispersions of a soybean protein isolate varied at various pH's on the addition of small amounts of NaCl and $CaCl_2$.

MATERIALS & METHODS

Preparation of the protein isolates

About 70 kg of an industrially heat treated, prepressed and defatted rapeseed meal (kindly supplied by AB Karlshamns Oljefabriker, Sweden), with a nitrogen content of 6.5%, was dispersed in deionized water at ambient temperature. The pH of the slurry was increased to 11.1 by the addition of aqueous sodium hydroxide. The extraction was allowed to proceed at a constant pH level and continuous agitation for 45 min. This required successive additions of sodium hydroxide. The final solid to liquid ratio was approximately 1/10 (w/v). Insoluble material was removed by centrifuging the dispersion in a continuous centrifuge. The nitrogen content of the extract corresponded to a dissolution of approximately 70% of the nitrogen content of the meal. Four protein isolates A, B, C and D were prepared from the alkaline meal extract as shown schematically in Figure 1. The isolates were separated by continuous centrifugation, washed two times with deionized water, neutralized to pH 7 and spray dried. Isolate A was prepared by adding 0.5M HCl to the alkaline rapeseed meal extract until a pH of 6.6 was reached; isolate B by adding 27.7L (liters) of a 1% aqueous solution of CMC (Carboxymethyl cellulose, Cellugel 3000 Special, kindly supplied by SCA, Sweden) to 100L of supernatant A and adjusting the pH of the mixture to 5.0; isolate C by adding 11.7L of a fresh 2% solution of HMP (sodium hexamethaphosphate, BDH Chemicals Ltd., Poole, U.K.) to 100L of the alkaline meal extract and adjusting the pH to 4.9; isolate D by adding 31.3L of the 1% aqueous CMC solution to 100L of the alkaline meal extract and adjusting the pH to 5.1. The amounts of HMP and CMC were chosen to give high nitrogen recoveries.

Analysis of the products

Phospholipid phosphorus was determined by the amount of phosphorus extracted by chloroform/methanol (3/1, v/v) for 2 hr at room temperature. Phytic acid was determined by extracting the products for 60 min at 0°C by 0.4M $HClO_4$ and then determining the phytic acid

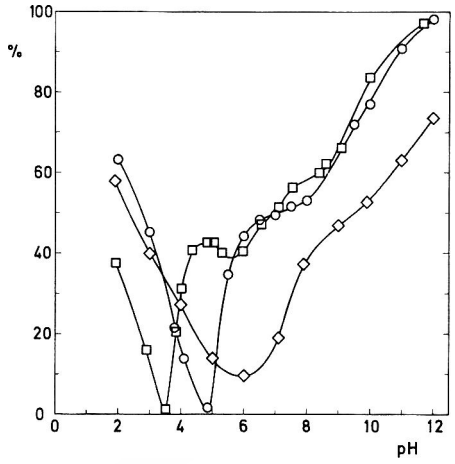


Fig. 2—Influence of pH on the nitrogen solubility of isolate D in the absence of added salt (○), in the presence of 0.004M NaLS (□) and in the presence of 0.01M $CaCl_2$ (◇).

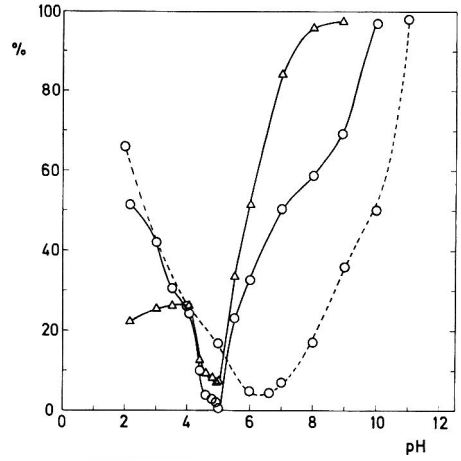


Fig. 3—Influence of pH on the dissolution of nitrogen (○) and phosphorus (△) of isolate C (solid curves) and isolate A (broken curve) in the absence of added salt.

content in the extract by the procedure of Wheeler and Ferrel (1971) except that 0.4M $HClO_4$ was used throughout the analytical procedure instead of trichloroacetic acid (TCA). Nucleic acids were analyzed using a modified Schmidt-Thannhauser technique (1945). Low molecular weight compounds were first extracted with 3 portions of 0.4M $HClO_4$ for 15 min each at 0°C. These extracts, which contained the phytic acid and other low molecular weight compounds, were mixed and the phosphorus content was reported as low molecular weight phosphorus. Low molecular weight nonphytic phosphorus was calculated from the difference between low molecular weight phosphorus and phytic phosphorus. The residue was extracted at room temperature, twice with 95% ethanol, twice with chloroform/methanol (3/1, v/v) and twice with 95% ethanol and then hydrolyzed in 0.3M KOH at 37°C for 1 hr. The hydrolyzate was cooled and an equal volume of 0.8M $HClO_4$ was added at 0°C. The mixture was centrifuged and the sediment extracted twice at 0°C with 0.4M $HClO_4$. The centrifugate and the two $HClO_4$-washings were filtered and mixed. Its adsorption at 260 nm was used for the RNA-determinations using RNA from yeast (Nutritional Biochemical Corp., Cleveland, Ohio) as standard. The sediment was extracted by three portions of 0.2M KOH at 37°C. The DNA content was calculated from the phosphorus contained in these extracts (assuming that the four DNA bases were present in equal amounts) as being 10.5 times the phosphorus content.

Dissolution experiments

Rapeseed protein isolate (0.4000 g) was dispersed in deionized water at room temperature. A salt solution was added in some experiments and the pH was adjusted to the predetermined value by the addition of aqueous sodium hydroxide or hydrochloric acid. The dissolution was allowed to proceed at a constant pH level and continuous agitation for 30 min. In some experiments this required small successive additions of sodium hydroxide or hydrochloric acid. The final isolate/liquid ratio was 1/100 (w/v). Insoluble material was removed by centrifugation for 5 min at 3,100 × G (Wifug X1 with swing-out rotor). The solution phase was analyzed for nitrogen with the Kjeldahl technique and for phosphorus using the method of Chen et al. (1956). The precision of the nitrogen determinations, which were run in duplicate, was 1% and that of the phosphorus determinations was 4%.

RESULTS

THE RESULTS from the analysis of the protein isolates and of a rapeseed meal (*Brassica napus*, winter variety Panter) prepared as described by Gillberg and Törnell (1976b) are summarized in Table 1. In the rapeseed meal 63% of the total amount of phosphorus was analyzed as phytic-, 22% as low molecular weight nonphytic-, 7% as RNA- (assuming that the RNA contained 10.4% phosphorus), 4% as phospholipid- and 1% as DNA-phosphorus.

In the preparation of the extract of the rapeseed meal from which the protein isolates were prepared, the extraction conditions were chosen in such a way that only little phytic acid

Table 1—Composition in weight % of the protein isolates and a rapeseed meal (RM) on an oven dry basis

	RM	A	B	C	D
Total nitrogen	6.9	15.2	14.3	14.5	14.1
Total phosphorus	1.22	0.78	0.23	1.14	0.43
Low mol wt phosphorus	1.03	0.20	0.17	0.82	0.22
Phytic phosphorus	0.764	0.004	0.006	0.011	0.007
Phospholipid phosphorus	0.044	0.052	0.003	0.016	0.012
RNA	0.85	3.24	0.36	1.59	1.21
DNA	0.15	0.44	0.05	0.36	0.19

was dissolved (Gillberg and Törnell, 1976a). This explains the low content of phytic acid in the protein isolates.

During the alkaline extraction carried out when preparing the protein isolates, some hydrolysis of the RNA most likely occurred. The RNA was, however, not broken down completely. Of the protein isolates, isolate A, in which all high molecular weight proteins of the meal extract were recovered (Lönnerdal et al., 1977), had the highest (3.68%) and isolate B the lowest (0.41%) content of nucleic acids. Although isolates C and D were prepared from the same meal extract, isolate C had a somewhat higher RNA content than isolate D. This fact indicates that presence of the CMC decreased the tendency of the RNA to coprecipitate with the proteins.

In addition to what can be seen from Table 1, isolate C contained 2% hexametaphosphoric acid and isolates B and D about 12% and 5% respectively of CMC. All of the protein isolates also contained acidic carbohydrates originating from the rapeseed meal (Åman and Gillberg, 1977).

From Figure 2 it can be seen that the presence of a small amount of $CaCl_2$ or sodium lauryl sulphate (NaLS) had a large effect of the pH of the nitrogen solubility minimum of isolate D. Presence of NaLS displaced the minimum towards a lower and presence of $CaCl_2$ towards a higher pH than the one in the absence of added salt.

From Figure 3 it can be seen that isolate A had a wider solubility minimum than isolate C. A comparison of Figures 2 and 3 shows that isolates C and D had similar nitrogen solubility profiles. Separate experiments showed that also the solubility profile of isolate B was similar to that of isolates C and D.

Figure 4 shows the phosphorus and nitrogen solubility of isolate C at various concentrations of NaCl and $CaCl_2$ at pH 2.0, 4.0, 5.0, 6.0 and 8.0. At pH 2.0 the phosphorus solubility increased and the nitrogen solubility decreased on the addition of salt. At both pH 2.0 and 4.0 the phosphorus and nitrogen solubility was higher in the presence of $CaCl_2$ than in the

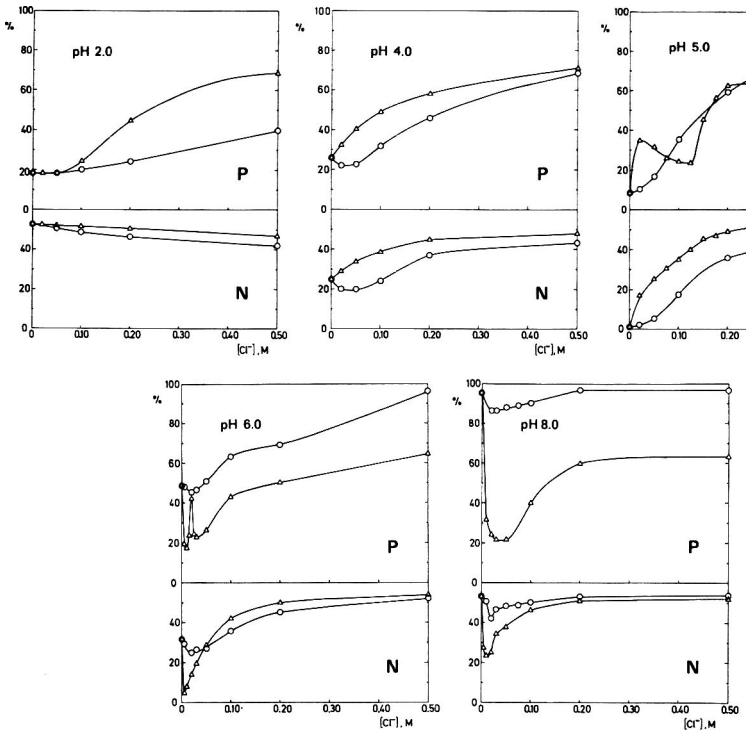


Fig. 4—Dissolution of phosphorus and nitrogen on extraction of isolate C at pH 2.0, 4.0, 5.0, 6.0 and 8.0 as a function of added NaCl (○) and $CaCl_2$ (△).

presence of NaCl. At pH 6.0 and 8.0 the reverse situation was true for the phosphorus solubility and at pH 8.0 also for the nitrogen solubility. At pH 6.0 and 8.0, which both are pH-values above the precipitation pH used when the isolates were prepared, both the phosphorus and nitrogen solubilities went through minima on increasing concentrations of salt.

Separate experiments showed that the effect of NaCl and $CaCl_2$ on the nitrogen solubility of isolates B and D at pH 2.0, 4.0, 5.0, 6.0 and 8.0 were similar to those of isolate C.

The effect of various concentrations of NaCl and $CaCl_2$ at pH 3.0, 5.0, 6.3 and 8.0 on the phosphorus and nitrogen solubility of isolate A is illustrated in Figure 5. Isolate A and C have quite different compositions. Isolate A is especially rich in acidic proteins (Ip 4–6) whereas isolate C contains proteins with a wide variety of isoelectric points (4.5–11) (Lönnerdal et al., 1977). Isolate C contained HMP and of the total phosphorus content of isolate C 72% was analyzed as low molecular weight phosphorus and 13% as nucleic acids. The corresponding figures for isolate A were 26% and 45% respectively. In spite of the different compositions of isolate A and C the effect of NaCl and $CaCl_2$ on the solubility of isolate A was similar to that of isolate C, as can be seen when comparing Figures 4 and 5. The main difference was that isolate A had a lower phosphorus solubility than isolate C.

The solubility of the protein isolates changed on storage (room temperature). Such an effect has also been reported for soybean meal (Smith and Circle, 1938). Experiments showed that the minimum in nitrogen solubility of isolate C occurred at pH 5.4 in 1976 and at pH 5.0 in 1974. The protein isolates were prepared in January 1974. The experiments underlying Figures 6 and 7 and the experiments with NaLS in Figure 2 were carried out in October 1976–January 1977. All other solubility experiments were carried out in April–October 1974.

In Figure 6 is shown a logarithmical diagram of the nitrogen and phosphorus solubility of isolate C at pH 5.4 on increasing concentrations of different sodium salts. From the diagram it can be seen that the nitrogen solubility increased on increasing salt concentrations and that the different univalent anions affected the nitrogen solubility to significant different extents. The phosphorus solubility was also affected. Of the various

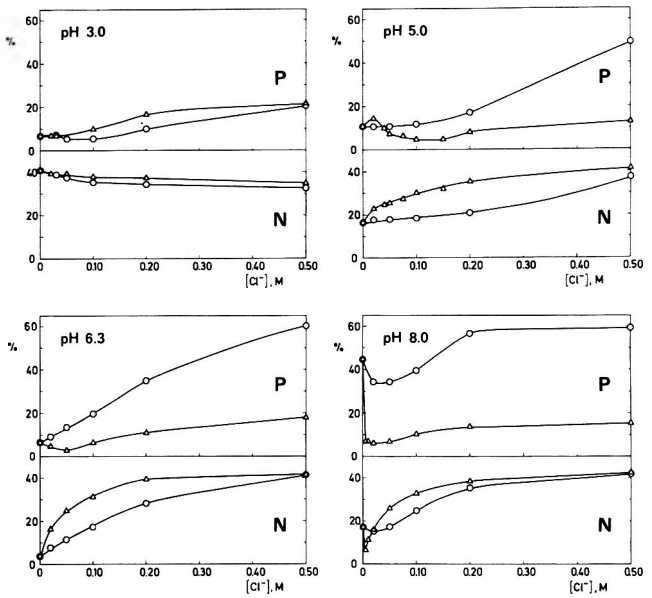


Fig. 5—Dissolution of phosphorus and nitrogen on extraction of isolate A at pH 3.0, 5.0, 6.3 and 8.0 as a function of added NaCl (○) and $CaCl_2$ (△).

salts NaLS had the largest effect on both the phosphorus and nitrogen solubility.

Figure 7 gives the nitrogen and phosphorus solubility of isolate C at pH 0.7 at various concentrations of monovalent sodium salts. On increasing the concentration of NaLS the nitrogen solubility curve went through a minimum. For the other salts the nitrogen solubility decreased on increasing the concentration of salt. Except for NaLS the phosphorus solubility went through a minimum on increasing salt concentrations.

DISCUSSION

THE RAPESEED PROTEIN ISOLATES studied have a very complex composition. A large proportion of the rapeseed pro-

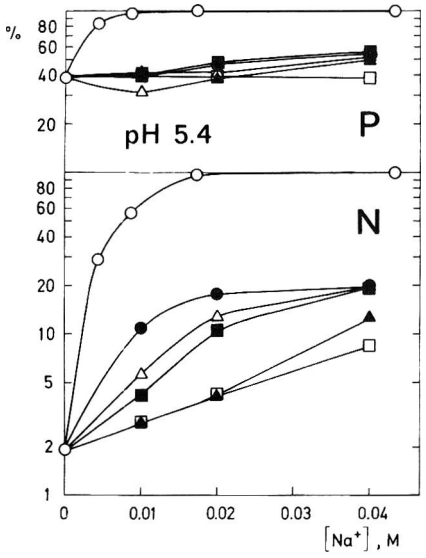


Fig. 6—Dissolution of phosphorus and nitrogen on extraction of isolate C at pH 5.4 as a function of added NaLS (○), $NaClO_4$ (●), NaTCA (△), NaI (■), NaBr (▲), and NaCl (□). N.B. The diagram is drawn in a logarithmical scale.

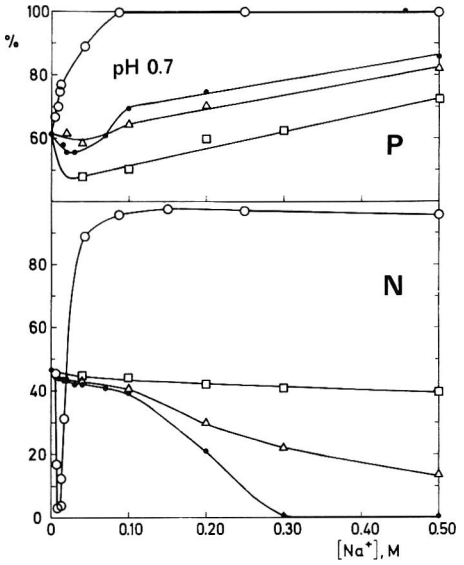


Fig. 7—Dissolution of phosphorus and nitrogen on extraction of isolate C at pH 0.7 as a function of added NaLS (○), NaCl (□), NaTCA (△), and $NaClO_4$ (●).

teins, 20–40%, has an isoelectric point close to 11 and a molecular weight of about 13,000 (Lönnerdal and Janson, 1972). The other rapeseed proteins have isoelectric points spread out in the interval from 4 to 8 and can with respect to their molecular weights be divided into three groups with molecular weights of 320,000, 150,000, and 50–75,000 (Lönnerdal, 1976). The rapeseed protein isolates consist to the major part of various polyelectrolytes (proteins, CMC, hexametaphosphate, nucleic acids, and acidic carbohydrates deriving from the rapeseed meal). These polyelectrolytes, which contain ionogenic groups with widely different pk_a-values, tend to form complexes with each other, soluble as well as insoluble.

When a salt is added to a solution of a polyelectrolyte the salt ions tend to screen the electric field of the fixed, charged groups of the polyelectrolyte. The counter-ion concentration will be much higher within the volume occupied by the polyelectrolyte than at longer distances. Interactions between many fixed charges will be weakened and this affects the conformation of the polyelectrolyte. The addition of salt may also affect the degree of dissociation of the ionogenic groups of the polyelectrolyte in the same way as salts affect the protolysis of weak, low molecular weight electrolytes (Tanford, 1967). Beside these nonspecific or general salt effects, which can be correlated with the change in ionic strength, ions may bind selectively or very strongly to the polyelectrolyte. This type of binding affects the charge of the polyelectrolyte and may also affect its solvatization. When salt is added to a system as complex as a rapeseed protein isolate all of these mechanisms would be effective. The addition of salt may result in a dissociation of existing complexes and in the formation of new ones. As the number of fixed sites to which ions can bind selectively or very strongly is often relatively small, the redistribution of complexes due to this type of binding is most extensive at low levels of salt addition. This is also true when ionic detergents are added, which can bind very strongly to a vast number of sites, as the number of fixed countercharges is restricted. Finally, it should be noted that the water activity varies with the type and amount of salt present. However, when salt is added in a small amount, and the concentration of other molecules is low, the change in water activity is so small that it has no effect on the complex formation.

The observed variation in solubility illustrates the net effect of the formation and dissolution of insoluble complexes. Since the redistribution of the individual components between the solid phase and the liquid phase is unknown, the variation in solubility is difficult to explain in detail.

From Figure 2 and 3 it can be seen that in the absence of added salt isolates C and D had a sharp minimum in nitrogen solubility. This means that the nitrogen solubility was very sensitive to a change in the charge of the molecules constituting the protein isolates. The displacement of the nitrogen solubility induced by NaLS and $CaCl_2$ (Fig. 2) can be explained by selective ion binding. It is well known that LS^- has a very high affinity for proteins (Karush and Sonenberg, 1949). Binding of LS^- will increase the number of negative charges on the proteins. This increase can be compensated for by lowering the pH so that carboxyl groups on glutamic and aspartic acid residues become protonized. In this way the charge of the proteins can be restored, so that the formation of insoluble complexes are favored. The displacement of the solubility minimum with $CaCl_2$ may be a result of binding of Ca^{2+} to the phosphate groups of the nucleic acids and to carboxyl groups on proteins, CMC, and other acidic carbohydrates. This will change the charge of these polyelectrolytes. By increasing the pH the charges of the proteins and possibly nucleic acids and acidic polycarbohydrates are changed so that the formation of insoluble complexes is favored. The Ca^{2+} will partly neutralize and may even totally neutralize some polyacids which otherwise would form insoluble complexes with the proteins. Such an effect may explain why the nitrogen solubility could not be suppressed to as low a value with $CaCl_2$ as with NaCl, but this could also be due to the fact that the ionic strength was higher in the experiments with $CaCl_2$ than NaLS.

It is possible, in principle at least, to explain the solubility curves of isolates A and C (Fig. 4 and 5) in terms of general salt effects, ion binding, and by considering the fact that the charge of the polyelectrolytes varied with the pH. At pH 2 and 3 proteins have a net positive charge. At these pH's the nitrogen solubility decreased on increasing salt concentrations. This probably was due to binding of Cl^- to proteins, which to some extent neutralized some of the positive charges of the proteins and thereby made it possible for an increasing amount of proteins to form insoluble complexes with the polyacids. This effect evidently outweighed the general salt effect. The fact that the nitrogen solubility at these pH's was higher in the

presence of $CaCl_2$ than in an equivalent amount of NaCl might be due to the higher ionic strength induced by the $CaCl_2$ but also to the higher tendency of Ca^{2+} than Na^+ to bind to HMP and nucleic acids and thereby decrease the negative charge of these compounds and thereby decrease the tendency of these compounds to form insoluble complexes with proteins. As the phosphorus solubility increased on the addition of salt soluble complexes might have been formed between the phosphorus containing molecules and the cations.

At pH 8 a large proportion of the rapeseed proteins are negatively charged. At this pH (c.f. Fig. 4 and 5) the phosphorus and nitrogen solubilities went through minima on the addition of salt. In spite of the fact that the ionic strength was larger in the presence of $CaCl_2$ than in an equivalent Cl^- concentration of NaCl, $CaCl_2$ gave more pronounced minima. This indicates that the minima were due to cation binding. Binding of cations decreases the negative charge of the polyacids and increases the number of positive charges on the proteins. At a certain concentration of cations, the charge of the molecules was such that the formation of insoluble complexes was favored the most.

Kawade and Kitamura (1957) have reported that nucleic acids precipitate in the presence of low concentrations of $MgCl_2$ and that low concentrations of NaCl do not have this effect. In view of this fact it seems likely that nucleic acids were involved in the formation of insoluble complexes with the added Ca^{2+} at weakly acidic to alkaline pH's. This may explain why isolate A, in which 45% of the total phosphorus content was analyzed as nucleic acids, had a lower phosphorus solubility than isolate C, in which 13% of the total phosphorus content was nucleic acids.

In Figure 4 it can be seen that the nitrogen solubility minima at pH 6 and 8 occurred at a somewhat lower $CaCl_2$ concentration than the phosphorus solubility minima. It can also be seen that $CaCl_2$ affected the relative phosphorus solubility in a higher extent than the relative nitrogen solubility. These facts show that insoluble complexes between proteins, Ca^{2+}, phosphorus containing molecules and probably also other polyacids were formed at the very low $CaCl_2$ concentrations. At higher $CaCl_2$ concentrations complexes between Ca^{2+} and phosphorus containing molecules probably also were formed. Such effects may explain the presence of the two minima at pH 6 and the minimum at pH 5 of the phosphorus solubility.

van Megen (1974) has studied the nitrogen solubility profiles of concentrated dispersions of a soybean protein isolate at various concentrations of NaCl at pH 4, 5, 6 and 7, and various concentrations of $CaCl_2$ at pH 4 and 7. He measured both the nitrogen and salt content in the sediment and in the supernatant. At pH 7 he found that the nitrogen solubility had a minimum when the overall $CaCl_2$ concentration was 0.02–0.08M. At this salt concentration he found a pronounced accumulation of Ca^{2+} in the sediment. This observation supports the conclusion in the present work. It is also interesting to note that the nitrogen solubility profiles of the soybean protein isolate reported by van Megen agrees with those of the rapeseed protein isolates reported in the present study.

The experiment underlying Figure 6 were carried out at a pH where the nitrogen solubility of the protein isolate (in the absence of added salt) started to increase with an increase in pH. At this pH, binding of anions would more or less have the same effect as an increase in pH. The stronger the anions bind, the stronger the effect will be. From Figure 6 it can be seen that the anions according to the molar concentrations of salt needed to increase the nitrogen solubility were ordered as:

$$LS^- < ClO_4^- < TCA^- < I^- < Br^- < Cl^-$$

This series, as expected, runs parallel with the order of decreasing binding strength of anions to protein (Norne et al., 1975).

Dispersion forces, which are independent of pH changes, govern the relative binding strength of anions to proteins (c.f. e.g. Norne et al., 1975). Anions would therefore be sequenced in the same order according to their relative binding strength to a protein at all pH's where the protein has the same positive charge. The experiments underlying Figure 7 were carried out at a pH of 0.7. At this pH all anionogenic groups of the protein are protonized, and as compared to pH 5.4 no new kind of positive charged groups are introduced to the proteins. From Figure 7 it can be seen that according to the molar concentration needed to decrease the nitrogen solubility the anions, as expected, were also sequenced as:

$$I.S^- < ClO_4^- < TCA^- < Cl^-$$

The nitrogen solubility minimum obtained on increasing the concentration of NaLS reflects the very strong binding of LS^- to proteins. At low concentrations ($\leqslant$ 0.01M) binding of LS^- neutralized the positively charged proteins and thereby faciliated the formation of insoluble protein-LS complexes. At higher concentrations, binding of LS^- probably induced a charge reversal of the proteins. It is possible that LS^- bound to the nucleic acids similarily as to proteins.

Cl^- is more hydrated than ClO_4^- (Hertz, 1973). The results of Figure 7 imply that the effect of specific anion binding outweighs both the general salt effect of the added salts, which act for an increased nitrogen solubility, and the effect the added salts had on the water activity. The results also imply that the binding strength of ClO_4^- to proteins is not sufficient to induce a charge reversal. As the phosphorus solubility increased on salt concentrations higher than 0.1M it seems probable that binding of phosphorus to proteins to some extent was replaced by binding of the added salt ions.

TCA concentrations of 3–10%, which correspond to a pH of 1.0–0.6, are often used for the precipitation of proteins. The results in Figure 7 indicate that TCA is not effective for the precipitation of rapeseed proteins. Separate experiments have shown that it is the low molecular weight rapeseed proteins (Mw 13,000) with an isoelectric point around 11 which are soluble in TCA (Lönnerdal, 1976). The results of Figure 7 indicate that $HClO_4$ is better than TCA for the precipitation of nitrogen compounds in rapeseed protein isolates. This agrees with the results of Bhatty and Finlayson (1973) who found that TCA extracted more nitrogen than $HClO_4$ from rapeseed, sunflower, and soybean meal.

In many analytical procedures TCA is used for the extraction of low molecular weight compounds, which are not to be contaminated by proteins and nucleic acids. The results of Figure 7 together with those of Bhatty and Finlayson (1973) show that it is better to use $HClO_4$ for this purpose. For this reason $HClO_4$ was used in the analytical procedures for the determination of phytic acid and nucleic acids in the present study.

In this study it has been exemplified how small amounts of salts can affect the solubility of rapeseed protein isolates. As the variation in interactions between the molecules of a food system govern the variation in any functional property, and as ions bind to molecules with different strengths and thereby affect the interactions between charged molecules to different extents, it seems most likely that functional properties other than solubility are also varied with the concentration of small amounts of salts.

REFERENCES

Åman, P. and Gillberg, L. 1977. Preparation of rapeseed protein isolates. A study of the distribution of carbohydrates in the preparation of rapeseed protein isolates. J. Food Chem. 42: 1114.

–Continued on page 1228

AN ELECTROPHORETIC INVESTIGATION OF A PEPTIC HYDROLYSATE OF COTTONSEED STORAGE PROTEIN

S. D. CUNNINGHAM, C. M. CATER and K. F. MATTIL

ABSTRACT

Cottonseed storage protein that had been hydrolyzed with pepsin in an ultrafiltration cell and in a batch system was subjected to electrophoresis. Bands of protein occur in the peptic hydrolysates but these bands are not as sharp as known protein standards. The peptides were not present in native (unhydrolyzed) SP and are considered to be peptic resistant peptides. Molecular weights of these peptides are estimated.

INTRODUCTION

ENZYMATIC MODIFICATION of cottonseed proteins may offer a viable alternative to the use of functionally limited native proteins. One approach to enzymatic modification is to include the modifying enzyme within an ultrafiltration cell. The containment of an enzyme within a semipermeable membrane device is one form of immobilized enzyme technology. The membrane in such a device should retain the enzyme but at the same time be permeable enough to allow passage of the products of hydrolysis.

Cottonseed protein products have not been as well characterized as protein products from other plant sources such as soybean and peanuts. Martinez and co-workers (1970) investigated the composition and functionality of selected cottonseed protein products including storage protein but a complete characterization of the protein or the protein components of cottonseed storage protein does not appear likely in the near future. This paper will present data on the characteristics of polypeptides in the storage protein (SP) fraction of glandless cottonseed that has been hydrolyzed with pepsin.

In another paper (Cunningham et al., 1978) cottonseed storage protein was hydrolyzed within an ultrafiltration device. Storage protein is soluble in acid conditions (pH 2) and these conditions were used to meet the demands for substrate solubility within the membrane reactor. It was noted that pepsin is capable of hydrolyzing cottonseed storage protein and peptides were noted in the peptic hydrolysate.

MATERIALS & METHODS

STORAGE PROTEIN (SP) was prepared from glandless cottonseed by the two-stage selective precipitation method of Berardi et al. (1969). Acrylamide, 2-mercaptoethanol (2-ME) and cyanogen bromide were purchased from Eastman Chemicals, Inc. Sodium dodecyl sulfate (SDS) was purchased from Pierce Chemicals, Inc.

Electrophoresis

Two methods of electrophoresis were used. One procedure was the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) procedure of Weber et al. (1972) except that isobutyl alcohol was used to layer the gel surface rather than water. Gel tubes (0.5 cm × 10.0 cm) were filled with a 10% acrylamide monomer solution. Gels were run at 6 ma/tube for 5 hr. Molecular weights were calculated from the marker proteins; Bovine Serum Albumin (BSA) (68,000), Ovalbumin (45,000), Pepsin (35,000) Chymotrypsin A (25,000) and Ribonuclease (13,700). Relative mobility (Rm) was calculated by the formula, Rm = Pm/Dm, where the distance of protein migration (Pm) was divided by the distance of dye front migration (Dm).

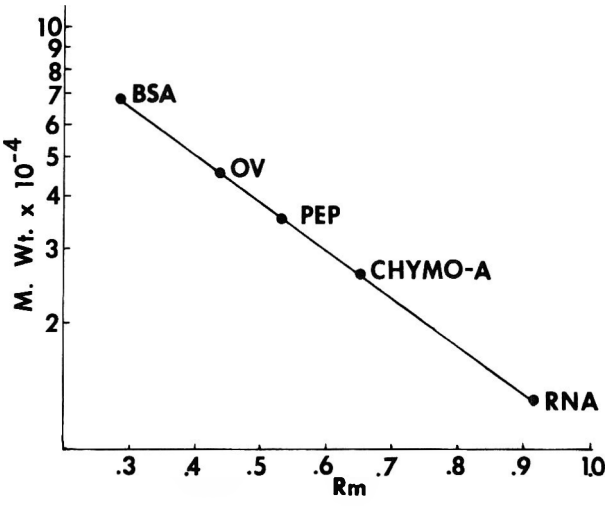


Fig. 1—Standard curve for molecular weight determination on 10% SDS-PAGE gels.

Molecular weights of peptides lower than 12,000 were determined by using highly crosslinked SDS-Urea gels as demonstrated by Swank and Munkres (1971). The acrylamide concentration of the gel was 12.5% and the ratio of acrylamide to methylenebisacrylamide (Bis) was 10:1. Acrylamide gels contained 0.1% SDS (w/v), 0.075% TEMED (v/v), 0.07% ammonium persulfate (w/v), 0.1M phosphate, 8M Urea and enough Tris (hydroxymethylaminomethane) to adjust the final pH to pH 6.8. Gel tubes were 0.5 × 10 cm. Tank buffer was 0.1% SDS and 0.1M phosphate, adjusted to pH 6.8 with Tris.

Protein samples were prepared by dissolving the protein in 1% SDS, 8M urea, 1% 2-ME and 0.01M phosphate buffer adjusted to pH 6.8 with Tris (sample buffer). After heating the solution to 90°C for 5 min it was cooled to ambient temperature and 5–50 μl were applied to a small section of parafilm. Five sucrose crystals and 5 μl of bromophenol blue (0.02% w/v) were added to the sample and all samples were brought to equal volume with tank buffer. Samples were applied to the surface of the gel with a 100 μl syringe and gels were run (ambient temp) at 2 ma/tube for about 6 hr. Gels were stained with Coomassie brilliant blue (R-250). Destaining was accomplished by electrophoresis or by diffusion.

Cyanogen bromide (CNBr) was used to cleave myoglobin and cytochrome c to provide necessary marker proteins for a standard curve. Myoglobin was dissolved in 0.1N HCl and a 30-fold molar excess CNBr was added (Edmunson, 1963). Cytochrome c was also dissolved in 0.1N HCl and incubated at 40°C in 250-fold molar excess CNBr (Chu and Yasunobu, 1964). After 24 hr, the solutions were lyophilized and prepared as protein samples for electrophoresis.

RESULTS & DISCUSSION

IN THE INVESTIGATION of the effects of pepsin on cottonseed storage protein (SP), two different methods of electrophoresis were used. Figure 1 represents the standard curve constructed from data on the relative mobilities (Rm) of 5

At the time the research reported here was done, all authors were with the Food Protein R&D Center, Texas A&M University, College Station, TX 77843. Authors Cater and Mattil are now deceased. Author Cunningham is now affiliated with the Food Technology & Science Department, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901.

0022-1147/78/0004-1224$02.25/0
© 1978 Institute of Food Technologists

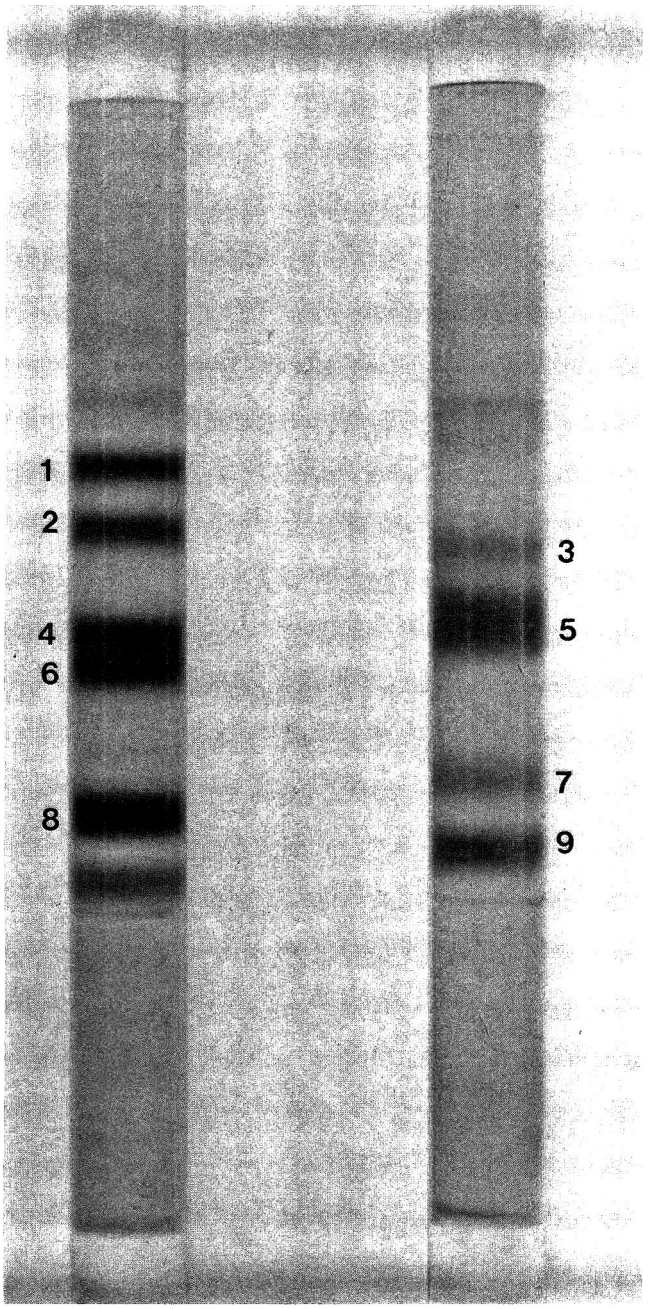


Fig. 2—SDS-urea gels (12.5% acrylamide) loaded with cyanogen bromide (CNBr) cleavage peptides from (A) myoglobin and (B) cytochrome c. Numbers assigned to peptide bands above correspond to CNBr peptides listed in Table 1.

standard proteins. The linear regression coefficient was greater than 0.99 for the standard curve on 10% SDS-acrylamide gels. Figure 2 contains a photograph for SDS-urea gels loaded with CNBr cleavage peptides of myoglobin and cytochrome c. The molecular weights of the CNBr peptides are listed in Table 1. Figure 3 contains the standard curve for calculation of lower molecular weights (MWt).

Peptic hydrolysates

In another paper (Cunningham et al., 1978) SP was placed in an enzyme reactor and samples taken at various times of hydrolysis. Figure 4 shows that SDS-PAGE was accomplished with SP from the enzyme reactor at 0 min (4a), 10 min (4b) and 60 min (4c) of hydrolysis with pepsin. Native storage protein is present in gel 4a and after 10 min (4b) substantial

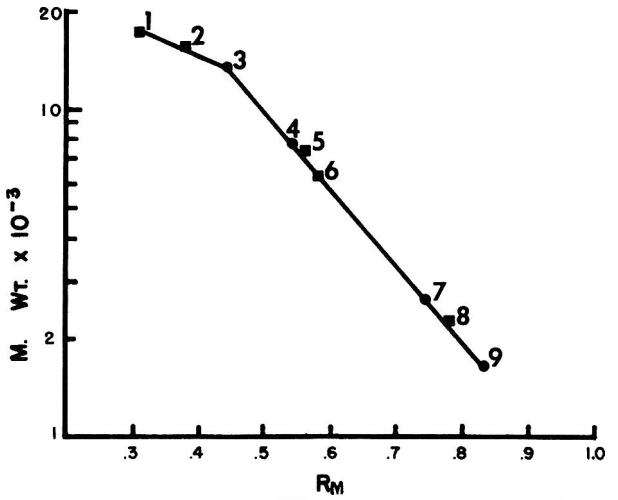


Fig. 3—Standard curve for SDS-urea gel electrophoresis. Numbers correspond to cyanogen bromide cleavage peptides identified in Table 1.

Table 1—Peptides resulting from cyanogen bromide cleavage of myoglobin and cytochrome c[a]

Label	Peptide	
1	Myoglobin	17,200
2	Myoglobin I + II	14,900
3	Cytochrome c	12,300
4	Myoglobin I	8,270
5	Cytochrome c I	7,760
6	Myoglobin II	6,420
7	Cytochrome II	2,780
8	Myoglobin III	2,550
9	Cytochrome c III	1,810

[a] From Swank and Munkres (1971)

protein hydrolysis occurred. In gel 4c two, wide polypeptide bands occur near the dye front. The lower molecular weight peptide (higher Rm) is very wide and diffuse and has a molecular weight of 12,000–13,000. The higher mol wt band (lower Rm) has an estimated mol wt of 17,500–18,400.

Figure 5 is a photograph of SDS-urea gels which were loaded with lyophilized ultrafiltrates. When the ultrafiltrate from UM-30 membranes and hollow-fibers were applied to SDS-urea gels, protein stained zones were detected. These bands were generally wider and more diffuse than generally observed for shown proteins (i.e. standard marker proteins). Although the bands may not be produced from a single polypeptide chain, they are at least produced by polypeptide chains of very similar molecular weights. These gels in Figure 5 are slightly streaked from the transverse migration of very low molecular weight peptides through the gel during electrophoretic destaining of these gels.

Samples of ultrafiltrate (UM-30 membrane) were subjected to electrophoresis both with (5b) and without (5c) the addition of 2-ME to the sample buffer. In both cases, the major portion of the protein had a high Rm and was estimated to be smaller than 4,000 daltons. The ultrafiltrate in the presence of 2-ME indicated the presence of 3 peptide bands. When 2-ME was withheld from the sample buffer, 5 peptide bands were seen in the ultrafiltrate samples (Fig. 5). None of these bands had the same Rm as the bands present in gels containing 2-ME. These 5 peptides (Fig. 5) could be composed of smaller peptides linked by disulfide bonds and the reduction of those disulfide bonds could result in the 3 peptides that are present in the gel (5b) containing 2-ME. However, the largest peptide with the lowest Rm in any of these hydrolysates appears in

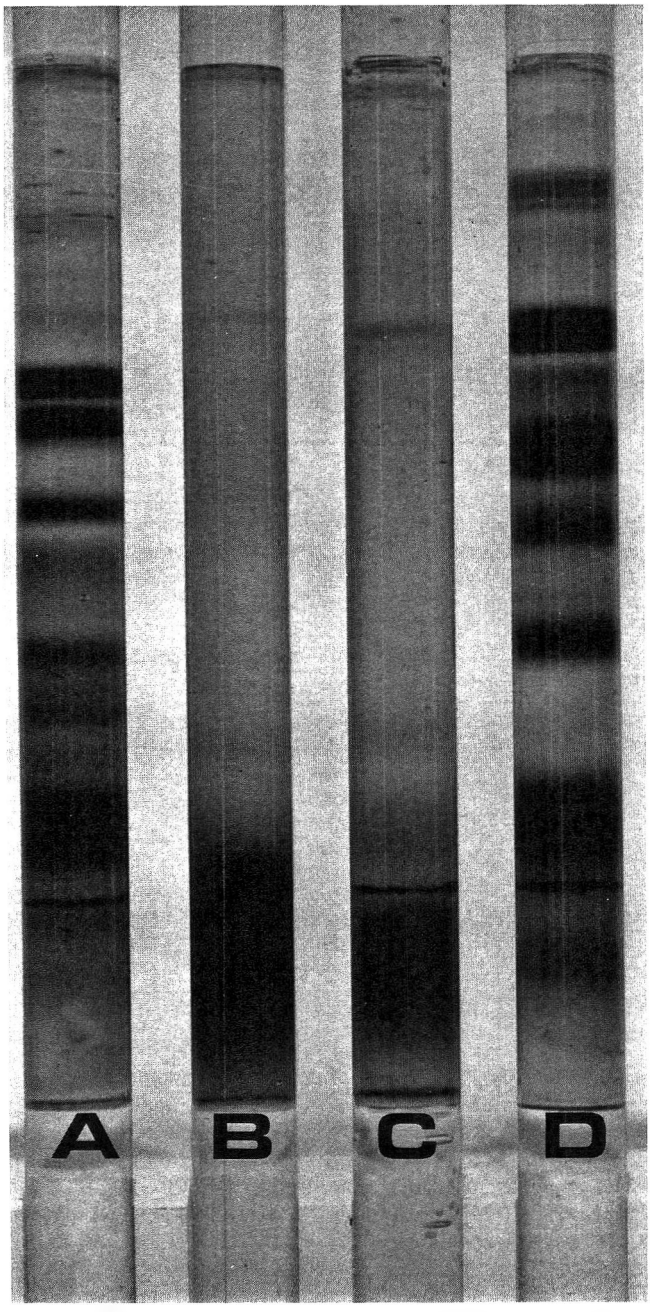


Fig. 4—SDS-PAGE gels (10% acrylamide) of (A) SP prior to addition of pepsin (B) 10 min after pepsin addition, (C) 1 hr after enzyme addition and (D) the standard marker proteins.

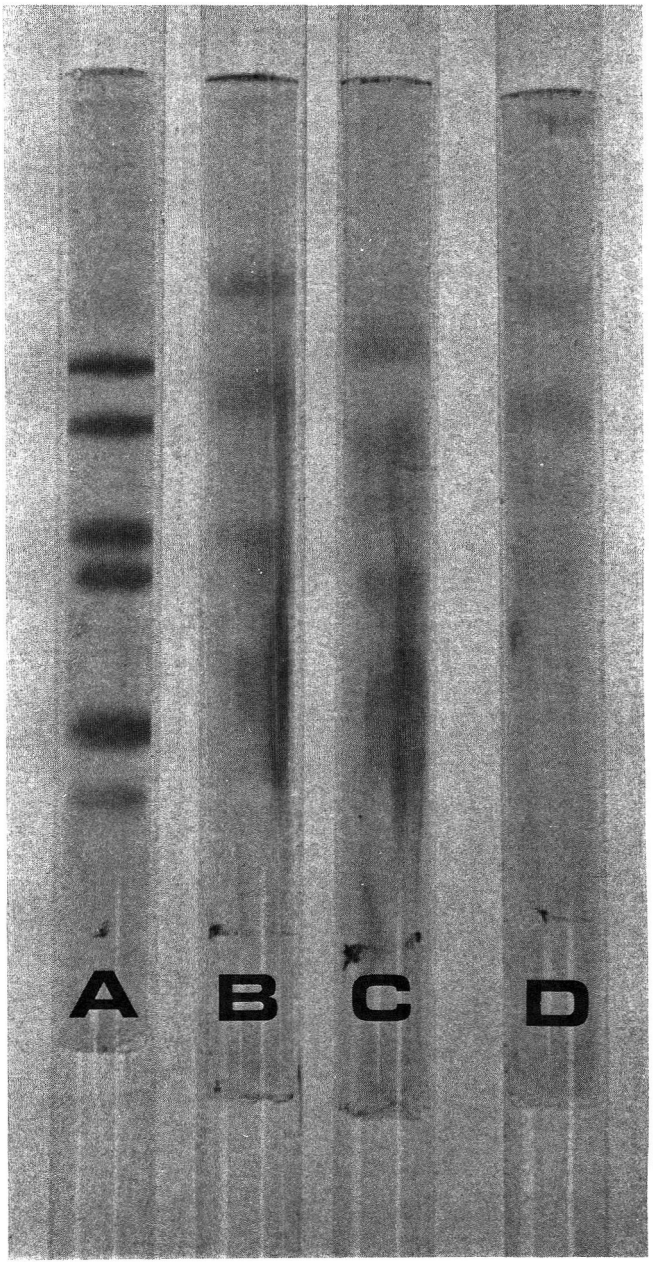


Fig. 5—SDS-urea gels (12.5% acrylamide). (A) CNBr peptides of myoglobin. Ultrafiltrate protein (B) with and (C) without 2-mercaptoethanol in sample buffer. (D) Ultrafiltrate through hollow fibers.

gels containing the disulfide reducing agent. The peptides present in gels without reducing agent have a calculated molecular weight different from the peptides in gels with reducing agent. This is true of the ultrafiltrates as well as the batch hydrolysates. It may follow that the addition of 2-ME changes the acrylamide gel pore size which ultimately affects the Rm of the peptides.

Peptides in the ultrafiltrates could have originated from low molecular weight subunits present in native storage protein. These peptides could have also resulted from the peptic cleavage of storage protein subunits and may represent proteins derived from native SP that may not be further degraded by pepsin. In order to determine if the low molecular weight peptides present in native SP could be the same as the protein bands present in the ultrafiltrates from enzyme reactors, SDS-urea gels were loaded with samples of each. Figure 6 contains the results. Low molecular weight bands from native SP (6c) do not have the same mobility as peptides present in the ultrafiltrates (6b). Also, the ultrafiltrate bands are not as sharp and well defined as the native SP bands.

In order to determine if the above peptides produced in a UF cell would be present in a batch system, a separate experiment was conducted using batch hydrolysis. Storage protein was hydrolyzed by 1% (w/w) pepsin for 24 hr and the resulting solution was lyophilized and loaded onto SDS-urea gels. Figure 7 is a photograph of these gels. Gels were prepared in the same manner as the ultrafiltrate gels (Fig. 5) except destained by diffusion. The presence of a low Rm (high molecular weight) peptide in gel 7b (2-ME present) closely resembles the peptide in the gel (5b) from the ultrafiltrates. Again 5 peptides (3 major zones) were present in gels without 2-ME. Estimated molecular weights of the three major pep-

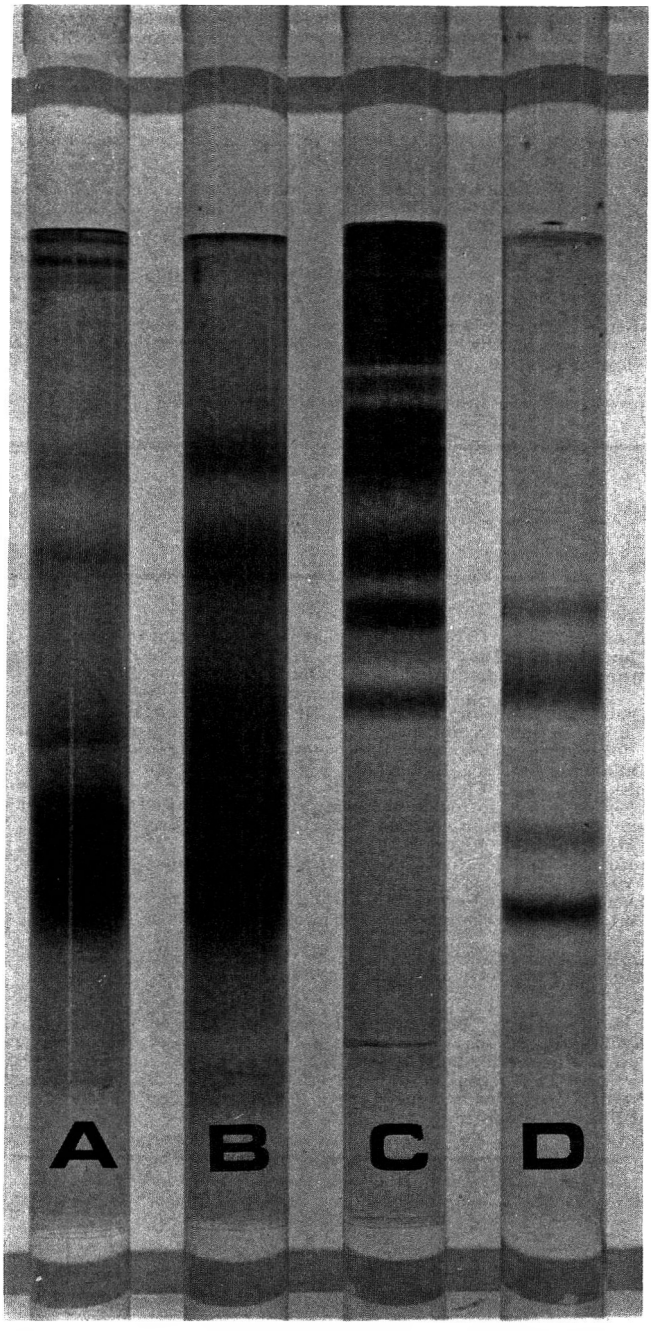


Fig. 6—SDS-urea gels (12.5% acrylamide). Peptides in (A) and (B) were ultrafiltrates through a PM-30 membrane. Gel (C) is native SP and (D) is cytochrome c CNBr peptides for standard curve.

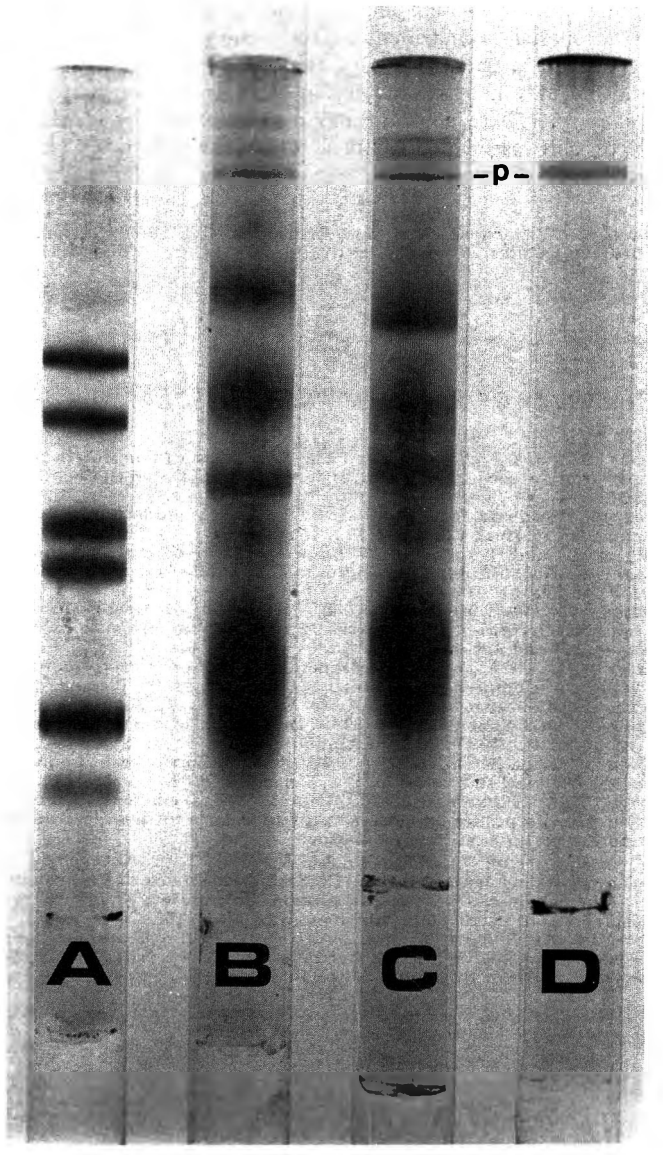


Fig. 7—SDS-urea gels (12.5% acrylamide). Gels are (A) myoblobin CNBr peptides, SP hydrolyzed in batch with 1% (w/w) pepsin; (B) with 2-ME in sample buffer, 25 µl; (C) without 2-ME in sample buffer, 25 µl; and (D) 10 µl pepsin.

tides are presented in Table 2. Molecular weights of the peptides from ultrafiltration and batch hydrolysis were similar.

Gels 7b and 7c were run under the same conditions but 7b contained 2-ME while 7c did not. The upper band (p) in these gels is pepsin. Comparison of the separation of the two bands immediately above pepsin indicates that these gels had different peptide separation characteristics. The presence of 2-ME in the sample buffer seems to influence peptide separation to the extent that differences in the major peptides other than Rm cannot be observed.

Table 2—Molecular weight estimates of pepsin resistant peptides present in storage protein hydrolysates

	SDS-Urea		SDS-PAGE
Band	UF (daltons)	Batch (daltons)	UF (daltons)
1	23,000	20,500	17,000
2	16,200	15,800	12,000–13,000
3	8,300	7,800	–

These results indicate that pepsin does not completely hydrolyze cottonseed storage protein and that several relatively large peptides remain. These results also indicate that the peptides are not native SP subunits but instead a result of hydrolysis of SP with pepsin.

Pepsin is known to have a rather broad range of action. Pepsin cleaves proteins at the point where tryosine, phenylalanine, methionine, tryptophan or leucine contribute the car-

boxyl group of a peptide bond. The peptides which result from peptic action on native SP subunits represent partially hydrolyzed SP that appears to be resistant to further attack by pepsin. These peptic resistant subunits may be long polypeptide chains that are devoid of the pepsin specific sites or it may be that these peptides represent the internal portion of a globular storage protein and sites of peptic attack are buried by the spatial arrangement of the molecule. These peptides may provide a useful tool for the study of subunit arrangement and may help in elucidation of the primary structure of SP subunits. These peptides may also be useful for identification of cottonseed protein products in the presence of other foods.

REFERENCES

Berardi, L.C., Martinez, W.H. and Fernandez, C.J. 1969. Cottonseed protein isolates: Two-step extraction procedure. Food Technol. 23: 75.

Chu, R.C.L. and Yasunobu, K.T. 1964. The reaction of cyanogen bromide and N-bromosuccinamide with some cytochrome c. Bicchim. Biophys. Acta 89: 148.

Cunningham, S.D., Cater, C.M. and Mattil, K.F. 1978. Cottonseed protein modification in an ultrafiltration cell. J. Food Sci. In Review.

Edmundson, A.B. 1963. Cleavage of sperm whale myoglobin with cyanogen bromide. Nature 198: 354.

Martinez, W.H., Berardi, L.C. and Galdblatt, L.A. 1970. Cottonseed protein products-composition and functionality. J. Agr. Food Chem. 18(6): 961.

Swank, R.T. and Munkres, K.D. 1971. Molecular weight analysis of oligo-peptides by electrophoresis in poly-acrylamide gel with sodium dodecyl sulfate. Anal. Biochem. 39: 462.

Weber, K., Pringle, J.R. and Osborn, M. 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. Methods Enzymol. 26: 3.

Ms received 7/15/77; revised 2/4/78; accepted 2/8/78.

SOLUBILITY OF RAPESEED PROTEIN ISOLATES . . . *From page 1223*

Bhatty, R.S. and Finlayson, A.J. 1973. Extraction of nonprotein nitrogen from oilseed meals with different solvents. Cereal Chem. 50: 329.

Chen, P.S., Toribara, T.Y. and Warner, H. 1956. Microdetermination of phosphorus. Anal. Chem. 28(11): 1756.

Gillberg, L. and Törnell, B. 1976a. Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. J. Food Sci. 41: 1063.

Gillberg, L. and Törnell, B. 1976b. Preparation of rapeseed protein isolates. Precipitation of rapeseed proteins in the presence of polyacids. J. Food Sci. 41: 1070.

Hertz, H.G. 1973. "Nuclear Magnetic Relaxation Spectroscopy in Water: A Comprehensive Treatise," Ed. Franks, F. Plenum Press, New York — London, 111: 301.

Karush, F. and Sonenberg, M. 1949. Interaction of homologous alkyl sulphates with Bovine Serum Albumin. J. Am. Chem. Soc. 71: 1369.

Kawade, Y. and Kitamura, T. 1957. Precipitation of pentosenucleic acid by neutral salt. J. Chem. Soc. Japan 78: 1801.

Liedén, S.-Å., Gillberg, L., Hambreus, L. and Lönnerdal, B. 1978. Nutritive properties of rapeseed protein isolates for the reproducing and the growing rat. To be published.

Lönnerdal, B. 1976. Unpublished data.

Lönnerdal, B. and Janson, J.-C. 1972. Studies on Brassica seed proteins. 1. The low molecular weight proteins in rapeseed. Isolation and characterization. Biochim. Biophys. Acta 278: 175.

Lönnerdal, B., Gillberg, L. and Törnell, B. 1977. Preparation of rapeseed protein isolates. A study of rapeseed protein isolates by molecular sieve chromatography. J. Food Sci. 42: 75.

van Megen, W.H. 1974. Solubility behavior of soybean globulins as a function of pH and ionic strength. Agr. Food Chem. 22(1): 126.

Norne, J.-E., Hjalmarsson, S.-G., Lindman, B. and Zeppezauer, M. 1975. Anion binding properties of human serum albumin from halide ion quadropole relaxation. Biochem. 14(15): 3401.

Schmidt, G. and Thannhauser, S.J. 1945. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoprotein in animal tissues. J. Biol. Chem. 161: 83.

Smith, A.K. and Circle, S.J. 1938. Peptization of soybean proteins. Extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts. Ind. Eng. Chem. 30(12): 1414.

Tanford, C. 1967. "Physical Chemistry of Macromolecules," Ch 8. John Wiley & Sons, Inc.

Wheeler, E.L. and Ferrel, R.E. 1971. A method for phytic acid determination in wheat and wheat fraction. Cereal Chem. 48: 312.

It is a pleasure to thank Professor Bertil Törnell for support and valuable suggestions with respect to the writing of this article; Mr. Sigvard Winsnes for help in the preparation of the protein isolates; Mr. Per-Christian Persson, Mrs. Eva Tjerneld and Mrs. Jana Polackova for technical assistance and the Swedish Board for Technical Development for financial support.

USE OF CLUSTER ANALYSIS TO EVALUATE SENSORY-OBJECTIVE RELATIONS OF PROCESSED GREEN BEANS

DAVID R. GODWIN, ROLF E. BARGMANN and JOHN J. POWERS

ABSTRACT

Cluster analysis was applied to the acceptability scores of panelists to partition the panel into groups showing a preference for either canned or frozen green beans. Cluster analysis was then applied to 20 sensory and 28 objective attributes to ascertain if the correlations changed according to whether differences had been judged by panelists preferring the canned or frozen product. The order of inclusion of terms and the correlations were affected. Flavor was the most important determinant of acceptability and mouthfeel was next most important. The utilization of particular descriptors to detect differences and sensory-objective relations varied according to whether the panelist scored canned or frozen green beans higher in acceptability.

INTRODUCTION

IN A PRIOR PAPER (Powers et al., 1977) relationships among various sensory attributes and several objective measurements made on canned and frozen green beans were examined by factor and by cluster analysis. Reported here are findings relative to relations between sensory tests for difference and the objective measurements when the panelists were first grouped into two classes according to their preference for the canned or frozen product. Recently, von Sydow (1976) commented that a question which should be answered is whether a "possible relation between degree of liking (hedonic response) and taste discriminatory ability" exists. This paper describes observations which touch in part upon that question.

Bargmann et al. (1976), Wu et al. (1977) and Powers et al. (1977) respectively used component analysis, factor analysis, and factor and cluster analysis to examine the applicability of specific descriptive terms to product differences. Various descriptive profiling methods have been used (Cairncross and Sjöström, 1950; Brandt et al., 1963; Clapperton, 1973; Stone et al., 1974; Williams, 1975). These methods require that a descriptor list be formulated and a consensus as to meaning be arrived at by the panelists. This may necessitate comparison with reference chemicals or other substances (Szczesniak et al., 1963) illustrative of the particular attribute sought to be described. Development of a common language may thereby be facilitated. Greater refinement of descriptors can be accomplished if statistical methods are applied to seek out underlying order among the descriptors (Harper, 1956) and to assess their utility (Harries, 1973).

Palmer (1974) reported that panelists used different descriptors depending upon whether they were differentiating among specimens of tea or describing them. Lehrer (1975) examined, by factor analysis, descriptors for assessment of the quality of wine. She observed that the qualities panelists liked in wine did not always coincide with the qualities they said they liked. In a later study, Lehrer (1978) used cluster analysis to relate descriptors to sensory attributes of wines. The methods above and other multivariate ones have been used to correlate descriptors with food quality (Andersson et al., 1973; Henry et al., 1971; Frijters, 1976; Dravnieks, 1976; Bargmann et al., 1976; Qvist et al., 1976; Wu et al., 1977), to particular chemicals (Schiffman and Dackis, 1975; Duewer and Kowalski, 1975; Schiffman et al., 1977), or odors "sniffed" at the effluent port of a gas chromatograph to particular peaks (Dravnieks et al., 1973, 1974; Kendall et al., 1974; Clark and Nursten, 1976; Jobbágy and Holló, 1976; Dravnieks et al., 1977).

Authors Godwin and Powers are with the Dept. of Food Science, University of Georgia, Athens, GA 30602. Author Bargmann is with the Dept. of Statistics & Computer Science, University of Georgia, Athens, GA 30602.

0022-1147/78/0004-1229$02.25/0
© 1978 Institute of Food Technologists

EXPERIMENTAL

ONLY A BRIEF DESCRIPTION of the experimental procedure will be given here because the details have been described before (Powers et al., 1977).

Sensory procedure

In 1975, a panel of 21 judges was asked to evaluate three canned and three frozen brands of green beans of standard-to-fancy quality. Fresh beans were also evaluated. One of the canned products of intermediate grade was used both as a reference and as a coded sample. The panelists evaluated the seven coded samples and the reference beans for acceptability, appearance, color, flavor, and mouthfeel by hedonic descriptions which were later transposed to a 9-point scale. The panelists also scored the seven coded samples against the reference beans for each of 27 specific descriptors on a scale of −4 to +4, which scale was later coded from 1 to 9. The procedure was an adaptation of that used by Vuataz et al. (1974) (See also Wu et al., 1977). Before the sensory trials started, four products from among the seven were randomly assigned to each session. Each of the seven products was eventually evaluated four times by each of the 21 panelists.

One-way analysis of variance (ANOV) was used to examine each judge's scores to determine whether he/she was consistent and could discriminate among the samples. The same thing was done for the descriptors, to determine whether the between F value was significantly greater than the within error. Seven terms were dropped for being nonsignificant.

Sensory-objective trial

A second trial was carried on in 1976 to permit correlating of chemical-physical and sensory variables. Samples for sensory and chemical-physical analyses were drawn from the same cooking vessel at the same time. Within the limitations of sampling from a batch of particulates, the sensory and objective samples were probably as identical as is possible. For the 1976 trial, four brands of canned beans, three of frozen beans, and fresh beans were the test samples, the processed products again representing beans in the standard-to-fancy quality range. The same sensory procedure was used for the 1976 trial except the seven descriptors which had proved to be ineffective in 1975 were dropped and 27 judges instead of 21 were used. The 20 specific descriptors retained are listed in the first column of Table 1.

For the objective tests, samples were extracted with an acetone-chloroform-hexane solvent and the resulting solution analyzed spectroscopically over the visible range. Absorbancy was later used to calculate ratios at 467/525, 525/610 and 525/665 nm. An aliquot of the spectral absorbancy sample was also used for measurement of the non-volatile components of the beans by high-pressure, liquid-solid chromatography. The volatile compounds were extracted by the combined steam distillation, ether-extraction method of Likens and Nickerson (1964) and condensed for gas-liquid chromatographic (GLC) analysis by the procedures described by Milutinović et al. (1970) and Young et al. (1970). Tensile strength, shear force, and coefficients of friction for the beans were measured on an universal testing instrument (Instron). Details as to each of the methods above have been described by Powers et al. (1977).

—Text continued on page 1230

Statistical

No judge from either the 1975 or 1976 panel had to be dropped for being statistically nondiscriminatory. Only three of the panelists who had served on the 1975 panel also served on the 1976 one.

Table 1—List of descriptors and correlation of each term with centroid for all descriptors, with the panelists partitioned into subsets preferring canned or frozen green beans

Variable	Abbreviation	Correlation of descriptor with centroid: Judges preferring Canned green beans	Correlation of descriptor with centroid: Judges preferring Frozen green beans
Acceptability	ACP	0.949	0.722
Appearance	APR	0.785	0.857
Flavor	FLA	0.956	0.617
Mouthfeel	MOU	0.906	0.597
Color	COL	0.618	0.913
Sweet	SWET	0.925	0.496
Buttery taste	BUTY	0.950	−0.358
Green vegetable taste	GVTA	−0.923	0.694
Hay-like flavor	HAY	−0.923	0.332
Bland	BLAN	−0.621	0.064
Crisp	CRIP	−0.879	0.715
Processed flavor	PROC	−0.385	−0.830
Off-flavor	OFFL	−0.894	−0.582
Persistent aftertaste	PERS	−0.626	−0.474
Pleasant aftertaste	PLES	0.895	0.454
Coarse	COAR	−0.894	0.800
Juicy	JUIC	0.895	−0.516
Slimy	SLIM	0.646	−0.717
Soggy	SOGY	0.531	−0.674
Fibrous	FIBR	−0.911	0.843
Tender	TEND	0.950	−0.739
Pale color	PALE	0.227	−0.819
Color off-shade	COSH	−0.120	−0.874
Bright color	BRIT	−0.878	0.896
Uniform appearance	UNIF	0.499	−0.317
GLC peaks			
Peak No. 9	GC09	0.370	−0.424
Peak No. 10	GC10	0.329	−0.347
Peak No. 11	GC11	0.470	−0.466
Peak No. 12	GC12	0.228	−0.309
Peak No. 13	GC13	−0.420	0.428
Peak No. 20	GC20	−0.635	0.522
Peak No. 24	GC24	0.617	−0.649
Peak No. 26	GC26	−0.070	−0.102
Peak No. 32	GC32	0.547	−0.528
Peak No. 33	GC33	−0.423	0.304
Peak No. 34	GC34	0.221	−0.161
Peak No. 36	GC36	0.496	−0.515
Peak No. 37	GC37	−0.474	0.570
Peak No. 39	GC39	0.051	−0.173
Peak No. 40	GC40	−0.224	0.253
Peak No. 41	GC41	−0.366	0.315
Peak No. 47	GC47	−0.402	0.510
Absorbancy ratio			
Ratio @ 467/525 nm	CR01	−0.332	0.382
Ratio @ 525/610 nm	CR02	0.840	−0.844
Ratio @ 525/665 nm	CR03	0.531	−0.530
LC peaks			
Peak No. 1	LC01	0.352	−0.300
Peak No. 2	LC02	0.404	−0.379
Peak No. 3	LC03	−0.215	0.269
Peak No. 9	LC09	−0.754	0.757
Mechanical forces			
Tensile strength	TENS	−0.590	0.572
Shear force	SHER	−0.763	0.744
Coefficient of friction			
Parallel to axis	FPAR	0.359	−0.397
Perpendicular to axis	FPER	0.459	−0.491

The 25 scores per replicate for each judge were weighted as described by Powers et al. (1977) so as to get a mean sensory value to insert in each cell of a 53 × 53 correlation matrix consisting of the 5 general acceptability descriptors, 20 specific descriptors, 17 GLC measurements, 3 spectral ratios, 4 LC measurements and 4 mechanical measurements. There were thus 32 experimental units (8 products × 4 replications) for each of the sensory and chemical-physical measurements. Means for the objective measurements were obtained by simple averaging.

RESULTS & DISCUSSION

INSPECTION of the sensory scores suggested the existence of at least two distinct groups of judges. Cluster analysis verified this by identifying three groups, based on the acceptability scores of the judges. A few of the judges showed no preference for canned or frozen beans. The other two groups consisted of panelists who liked canned green beans more than frozen beans and of panelists who liked frozen beans more than canned beans. The procedure of Trivedi (1971) was used to form the clusters. Table 2 shows the acceptability scores assigned to the beans by judges who preferred canned beans and those who preferred frozen beans. The results for the 1975 and 1976 trials could not be pooled because different panelists and different commercial brands had been used, but the two trials corroborated each other. Judges who scored canned beans higher in acceptability than frozen beans tended to score appearance, color, flavor, and mouthfeel in a different order than did those who scored frozen beans higher in acceptability. Judges who considered canned beans more acceptable generally rated the beans high in flavor and mouthfeel and somewhat lower in appearance and color while the judges who found fresh and frozen beans more acceptable apparently considered color very important. If the product was bright green, then appearance was also rated high; appearance flaws, such as split pods or separated seeds, apparently had little weight.

Table 3 lists the simple and multiple correlation coefficients for the two sets of judges. As may be seen, judges liking the frozen product better than the canned product were influenced more by color-appearance than were the judges preferring the canned product. For both groups of judges, flavor and mouthfeel were the greatest determinants of acceptability.

In previous studies (Powers and Quinlan, 1974; Powers, 1976), we examined for heterogeneity in preference among panelists by visually scanning the correlation matrix for the scores of each judge versus all other judges. Negative correlations between judges indicate heterogeneity. By classifying the judges into sub-groups so that the scores of all judges within a sub-group are positively correlated, homogeneous sub-groups can be established. In this study, only two judges were placed in different sets by cluster analysis than had been done by visual inspection, but they were borderline cases anyway. Unless one is already doing cluster analysis, its use to group judges into homogeneous sets is statistical "overkill" when a small number of judges is involved, but for a large number of judges, cluster analysis is not only more objective than visual inspection, it is also less time consuming.

Reasons for analysis

The primary reason for arranging judges into sub-groups was to learn whether the two subsets of panelists who had distinct preferences for canned or frozen green beans had used the specific descriptors in the same way. Furthermore, we wished to know whether relations between specific descriptors and the objective tests were different when the two subsets were considered separately. Table 1 shows that the correlation coefficients and the signs did indeed differ by subset.

Formation of clusters

Before discussing the results further, the clustering procedure used needs to be explained. One concept is old. Pearson (1901) pointed out that correlation coefficients can be ex-

Table 2—Mean acceptability scores for canned and frozen green beans upon the panels being partitioned into subsets of judges whose overall acceptability scores were higher for the canned or the frozen product

	Judges preferring canned green beans					Judges preferring frozen green beans				
Form of product	Accept.	App.	Color	Flavor	Mouthfeel	Accept.	App.	Color	Flavor	Mouthfeel
					1975 trial					
Canned	5.7	5.3	5.1	5.6	5.6	5.8	5.5	5.4	5.8	5.5
Frozen	4.0	4.7	4.6	3.6	4.1	6.9	7.0	7.2	6.7	6.5
					1976 trial					
Canned	6.4	5.9	5.6	6.7	6.4	4.5	4.4	4.3	4.4	4.9
Frozen	4.0	5.1	5.0	3.9	4.1	6.0	6.6	6.8	5.6	5.8

Table 3—Simple and multiple correlations for acceptability factors, panelists partitioned into those scoring canned green beans higher in overall acceptibility and those scoring frozen green beans higher

Judges preferring canned product					Judges preferring frozen product				
	App.	Color	Flavor	Mouthfeel		App.	Color	Flavor	Mouthfeel
Acceptability	0.584	0.532	0.905	0.833	Acceptability	0.721	0.713	0.858	0.724
Appearance		0.751	0.546	0.526	Appearance		0.862	0.673	0.525
Color			0.457	0.468	Color			0.610	0.576
Flavor				0.807	Flavor				0.551
Accept. vs ACFM[a] = 0.927					Accept. vs ACFM = 0.920				
Accept. vs AFM = 0.925					Accept. vs AFM = 0.918				
Accept. vs FM = 0.922					Accept. vs FM = 0.909				
Accept. vs AC = 0.601					Accept. vs AC = 0.743				

pressed as vectors. If r = 0.79, its inverse cosine is 37.8°; if r = 0.97, the angle is 14.1°. Relations between attributes can thus be expressed either as correlation coefficients or as vectors.

The particular form of clustering used is known as "virtual" clustering (Bargmann and Graney, 1969) because the number of dimensions of the cluster is at least one dimension less than the actual number. To illustrate, the stars making up the constellation, the Pleiades, are not clustered in actuality (three dimensions) because the distances from their center point vary widely. However, projected in two dimensions against the celestial vault, the stars appear to form a cluster.

Clustering is begun by selecting the pair of descriptors which has the highest correlation, i.e., smallest angular separation. The third member of the cluster is chosen as follows. The center of the imaginary galaxy or sphere encompassing the two descriptors is calculated, and the correlations of the remaining descriptors with the center are obtained. The attribute with the largest correlation to this centroid is added. The fourth member is added in the same way. The procedure is programmed to go on until the first sharp drop occurs in the correlation. The sharp drop terminates the "cluster core." Additional descriptors are added until a second sharp drop occurs. This is the "cluster extension." Descriptors in the cluster extension are considered in forming the second cluster, but descriptors assigned to the cluster core are not. The two-stage procedure of a cluster core and a cluster extension was adopted to permit some degree of overlap between clusters. The reason for this provision is illustrated below.

Suppose that one has made the following measurements on solutions of acids and monosaccharides: pH, total acidity, buffering capacity, sourness, sweetness, mutarotation equilibrium, reducing capacity, and the presence or absence of a ring structure. One might conclude that pH, total acidity, buffering capacity, and sourness are attributes of acids whereas mutarotation of light, reducing capacity, a ring structure, and sweetness are attributes of monosaccharides. However, some consideration has to be given to the fact that some acids are sweet (picric), some are reductants (ascorbic acid), some rotate light (lactic acid), and the phenolic acids possess a ring structure. Carrying on the clustering procedure through the cluster extension stage before closing out the cluster core permits attributes which are common to different classes to be evaluated as to the cluster to which they should be assigned.

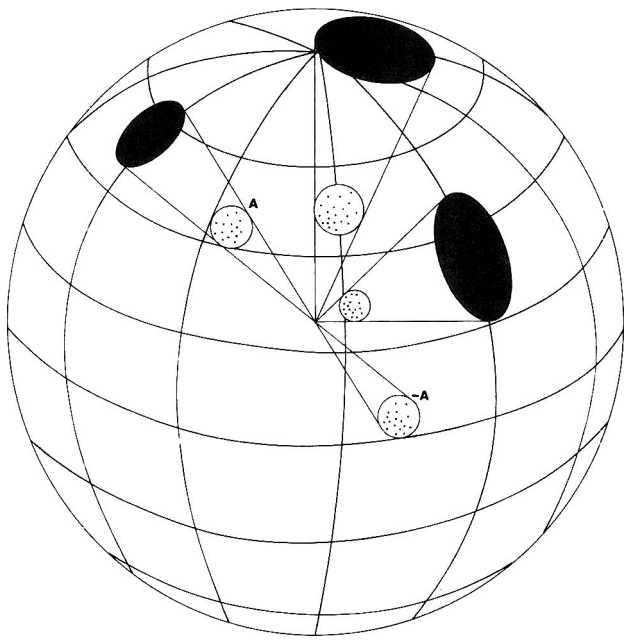


Fig. 1—Simplified concept of descriptors in a three-dimension space, showing the smallest angle subtending the descriptors, the cone of which projected onto the surface of a hypersphere causes the variables to cluster in two-dimension space. The spheroid, —A, illustrates reflected variables corresponding to those in the spheroid, A. Similar reflected variables might exist for the other two spheroids.

Another facet of the clustering procedure which needs to be explained is the way of handling positive and negative correlations. If a correlation is negative instead of positive (being 190° instead of 10°, for example), the attribute is said to be "reflected." In the illustration above, hydroxyl ions are the reflected attribute of hydrogen ions. In our study, since sweetness had originally been scaled in the positive direction, its reflected attribute was "lack of sweetness." Figure 1 shows

projections of clusters onto the surface of a hypersphere. Cluster −A could be made to coincide with Cluster A by reversing the direction of scoring of the attributes in −A or by using the antonyms of the descriptors.

Difference–preference relations

To return to Table 1 and the results, the correlation coefficients given are for each descriptor versus the centroid for all descriptors. The values are listed by descriptor rather than by their order in the clusters so as to permit the values and signs to be compared.

One is not really interested in master clusters but rather in the relations of specific descriptors to the main acceptability factors. To find out which specific descriptors were correlated with the individual acceptability factors, clustering was not done by letting the pair of attributes with the highest correlation serve as the starting point for the cluster but rather by designating particular terms. Appearance-color, flavor, and mouthfeel were thus successively designated as the origin of the cluster. When appearance-color was to be the origin, the other acceptability factors (overall acceptability, flavor, and mouthfeel) were deleted. The results are shown in Table 4. Detailed comment does not need to be made about the relation of descriptors included in a cluster and the panelists' preferences for canned or frozen beans because one would conclude the same as from the other analyses: the panelists' preferences affect the order and correlation within a cluster. The important thing to be noted here is that the panelists' preferences influenced the relation of the specific sensory descriptors to the acceptability factors although scoring of the specific descriptors was a *difference* test. This study thus throws some light on the question raised by von Sydow (1976). All of our panelists were statistically significant as judges for the acceptability phase of the study and they were significantly discriminatory as to most of the difference criteria. However, the fact that the correlation coefficients and even the signs for the various difference attributes varied according to whether they preferred canned or frozen green beans indicates that judgment is affected by the liking of the panelist for that product. Some of the difference in performance can be accounted for by the fact that one cannot expect every judge to be equally sensitive to each form of difference. Since the judges in our study were statistically significant for most of the difference criteria, the relation to acceptability seems to be that they put more weight on one difference factor than another. Whether they are insensitive to a particular difference attribute or they sence it but weight it differently has an influence on their acceptability judgment because subconsciously they are assessing all these factors when they make acceptability judgments, whether or not they are being asked about a particular difference attribute.

Table 4—Clusters[a] formed when acceptability of appearance-color, flavor, and mouthfeel were successively designated as the origin for the cluster, and the various descriptors were returned to the pool of all terms for formation of the next acceptability cluster.

Appearance-color cluster		Flavor cluster		Mouthfeel cluster	
Panelists preferring canned beans					
SWET	−	−FLA	−	−COAR	−
PLES	0.942	−HAY	0.971	JUIC	0.983
−BRIT	0.846	−GVTA	0.955	TEND	0.964
CR02	0.801	SWET	0.907	−FIBR	0.953
APR	0.722	−OFFL	0.883	BUTY	0.943
−LC09	0.644	PLES	0.879	−CRIP	0.935
−SHER	0.598	−BRIT	0.741	−GVTA	0.919
−GC20	0.593	CR02	0.742	SWET	0.907
−PERS	0.533			−HAY	0.896
COL	0.545			MOU	0.883
				OFFL	0.810
				PLES	0.815
				CR02	0.738
				−BRIT	0.711
Panelists preferring frozen beans					
APR	−	−OFFL	−	COAR	−
COL	0.982	PLES	0.939	FIBY	0.953
−COSH	0.879	SWET	0.751	BRIT	0.842
BRIT	0.848	FLA	0.752	−PALE	0.788
−PALE	0.828	−PROC	0.697	SHER	0.756
−PROC	0.797	−SOGGY	0.697	−CR02	0.692
−CR02	0.752	−COSH	0.662	−COSH	0.708
−SOGY	0.699	−PALE	0.676	−PROC	0.709
−SLIM	0.668	−SLIM	0.678	CRIP	0.642
GVTA	0.654	BRIT	0.668	−TEND	0.628
		GVTA	0.597	GVTA	0.590
		−CR02	0.606	−SOGY	0.594
				−SLIM	0.612

[a] A minus sign in front of the abbreviation for the term indicates that the antonym of the word listed is the word which actually belongs in the cluster, or that the direction of the scale for that particular descriptor should be reversed, i.e., "stronger" in the attribute should be interpreted as "weaker." See Table 1 for abbreviations.

Terms appearing together

The clusters shown in Table 4 reveal some relations which might not be expected. In the appearance-color cluster for judges preferring frozen beans, it seems quite logical that the term, bright color should be positive with reference to color acceptability, for almost invariably one looks upon bright color as being desirable. Brightness, however, was a negative factor in the appearance-color cluster for the judges liking canned beans. Both sets of judges evaluated the brightness of both canned and frozen beans, but brightness apparently denotes different things to them. The judges preferring frozen green beans rated the chlorophyll-green color of the frozen beans high on the positive side of the scale while not downgrading the brightness of the canned beans too severely. (The reader should remember the panelists were judging beans cooked for serving. Although the frozen beans lost some of their bright, chlorophyll-like green color upon being cooked, they were not quite as olive-green as the canned beans). Judges preferring canned beans apparently placed more emphasis on the "brightness" part regardless of whether the color was chlorophyll-like or pheophytin-like. They also tended not to depart as widely from the neutral point of the scale.

One thing which is quite evident from Table 4 is that words we associate with one of the senses often appears in two or three of the clusters. In fact, bright color appears in all six clusters listed. The descriptors, "sweet" and "pleasant aftertaste," were the first variables picked up in the appearance-color cluster for the judges liking canned beans while "green vegetable taste" was a term in the appearance-color cluster for the judges liking frozen beans. There may be common elements to which the responses are related such as color compounds having taste qualities.

Figures 2 and 3 show the general relations of the clusters, each to the other. They represent 31-dimension centroids projected onto a two dimensional surface. The figures are as if clusters within an enveloping sphere were projected onto the surface of the sphere and then one looks at the sphere much like a polar projection. The abbreviations with a negative sign are for terms which had to be reversed (reflected) and thus they should be read as the antonyms of the specific descriptors. The actual clusters may not overlap as extensively as shown in Figures 2 and 3 because one centroid could be well toward the center of the hypersphere while another is near the outer surface, but upon being projected onto the surface, they overlap. The 31-dimension centroids probably do encompass portions of each other as judged by the fact that the same words appear in different clusters.

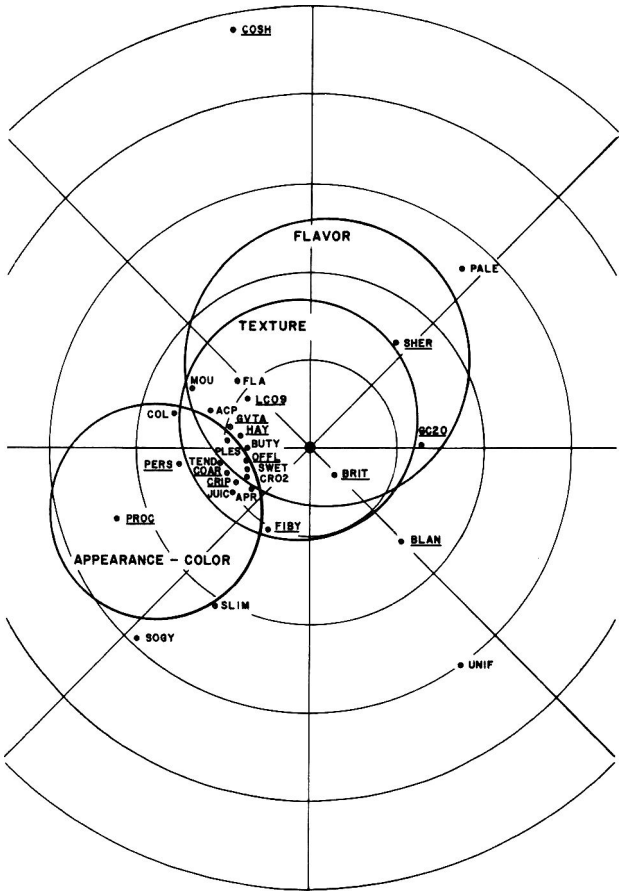


Fig. 2—Appearance-color, flavor and mouthfeel clusters formed by successively designating one acceptability factor and then searching for specific descriptors most closely correlated with that acceptability factor. All terms were returned to the pool of descriptors for formation of the next cluster. Clusters are for judges preferring canned green beans.

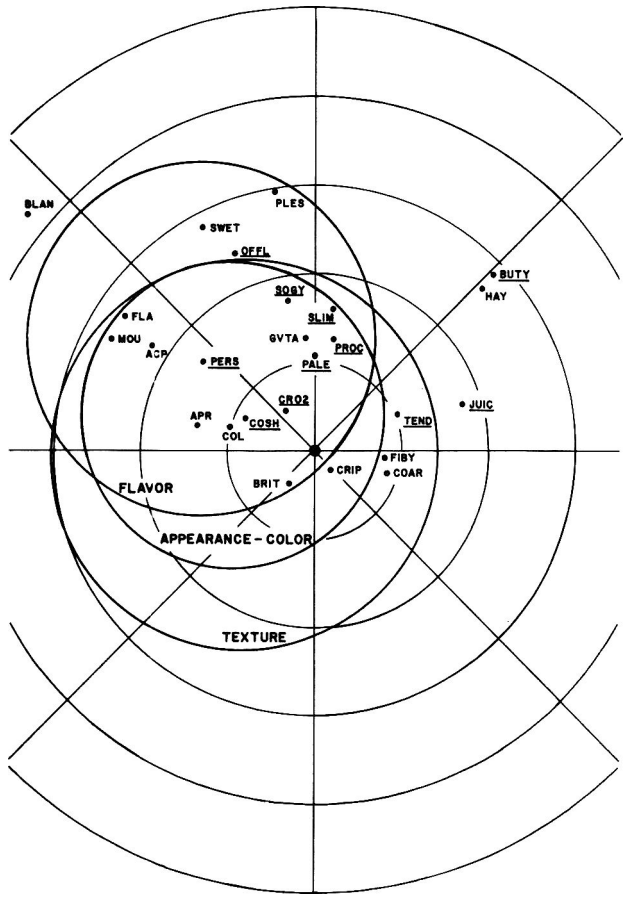


Fig. 3—Appearance-color, flavor, and mouthfeel clusters formed by successively designating one acceptability factor and then searching for specific descriptors most closely correlated with that acceptability factor. All terms were returned to the pool of descriptors for formation of the next cluster. Clusters are for judges preferring frozen green beans.

Sensory-objective relations

In the study reported by Powers et al. (1977) , none of the objective terms occurred in the first three clusters formed. This was understandable. The specific sensory descriptors were originated in the first place on the assumption they were components of general descriptors such as flavor or color; if they are components, they should be highly correlated with the general descriptors. In this study where opposing sensory judgments have been eliminated, there was a greater tendency for the sensory and objective terms to be correlated and thus to appear in the same cluster.

Just as partitioning of the panelists into homogeneous sets is desirable to obtain maximum insight as to preference:difference relations, so too is partitioning necessary to establish relations between particular chemical or physical properties and sensory responses. Note in Table 1 the objective term, CR02 (the spectral absorbancy ratio of 523/603 nm). For those liking the canned beans, the relation between it and color is positive; for those liking frozen beans, the relation is negative. The color coordinates x, y, z and L, a, b were calculated for the range of 400–700 nanometers at 10 nanometers intervals. The correlations of the x, y, z coordinates with color-related variables are listed in Table 5. None of the descriptors were significantly correlated with the z coordinate. The L, a, b coordinates provided no information that the x, y, z ones had not. As commented upon above, for those panelists preferring canned beans, frequently a given correlation was positive while it was negative for those preferring frozen beans. This is a second reason panelists should be partitioned into homogeneous sets. If one were to try to establish a relation for the panel as a whole versus the absorbancy ratios, one could be seriously misled. Either one would find no relation at all, or, if a particular relation appeared to exist, it would only be because the number of panelists in one group was greater.

Table 5—Correlations of color and color related descriptors with x, y, and z color coordinates

	Panelists preferring canned green beans			Panelists preferring frozen green beans		
Descriptor	x	y	z	x	y	z
APR	–0.026	–0.408	0.225	–0.128	0.484	–0.171
COL	0.314	–0.113	–0.136	–0.158	0.514	–0.167
SWET	0.143	–0.475	0.154	–0.191	0.236	–0.005
GVTA	–0.051	0.562	–0.255	–0.073	0.560	–0.249
PROC	–0.425	0.137	0.333	0.297	–0.338	–0.007
PERS	–0.117	0.266	–0.276	–0.085	–0.111	0.113
PLES	0.268	–0.374	0.265	–0.157	0.104	0.043
SLIM	–0.069	–0.494	0.295	–0.034	–0.442	0.254
SOGY	–0.060	–0.382	0.235	–0.028	–0.496	0.276
PALE	–0.604	–0.437	0.599	–0.133	–0.538	0.361
COSH	–0.509	–0.219	0.425	0.304	–0.290	–0.375
BRIT	–0.155	0.488	–0.137	–0.093	–0.539	–0.222

–Text continued on page 1234

There are numerous problems in trying to ascertain causative relations between chemical or physical measurements and sensory response. This study demonstrates in a concrete way that a problem which whould be taken into account is that relationships between sensory and objective attributes vary widely according to the individual judge. One cannot, of course, carry partitioning of a panel so far as to get down just to a few judges. That would be *reductio ad absurbum* and would be self-defeating in terms of ascertaining practical, working relations between objective measurements and sensory response. However, as long as we have to rely on descriptions of sense response to determine what the sensory stimulus is, there should be some clustering of panelists, the responses of whom are similar, into sets so that specific objective attributes can be matched more closely with the various sensory responses of the panelists.

REFERENCES

Anderson, Y., Brake, B., Granquist, A., Halldin, L., Johansson, B., Pangborn, R.M., and Akesson, C. 1973. Fracture force, hardness and brittleness in crisp bread, with a generalized regression analysis approach to instrumental-sensory comparisons. J. Texture Studies 4: 119.

Bargmann, R.E. and Graney, R.W. 1969. An algorithm for identifying and testing virtual clusters. Themis Report No. 1, Dept. of Statistics and Computer Science, University of Georgia, Athens, GA 30602.

Bargmann, R.E., Wu, L.S. and Powers, J.J. 1976. Search for the determiners of food quality ratings—description of methodology with applications to blueberries. In "Correlating Sensory Objective Measurements—New Methods for Answering Old Problems," Ed. Powers, J.J. and Moskowitz, H.R., Publ. STP 594, Am. Soc. for Testing and Materials, Philadelphia, PA, p. 56.

Brandt, M.A., Skinner, E.Z. and Coleman, J.A. 1963. Texture profile method. J. Food Sci. 28: 404.

Cairncross, S.E. and Sjöström, L.B. 1950. Flavor profile—A new approach to flavor problems. Food Technol. 4: 308.

Clapperton, J.F. 1973. Derivation of a profile method for sensory analysis of beer flavor. J. Inst. Brew. 79: 495.

Clark, R.G. and Nursten, H.E. 1976. Volatile flavour components of Brazil nuts, Bertholletia excelsa (Humb. and Bonpl.). J. Sci. Food Agr. 27: 713.

Dvavnieks, A. 1976. Approaches to subjective/objective correlations in flavor. In "Correlating Sensory Objective Measurements—New Methods for Answering Old Problems," Ed. Powers, J.J. and Moskowitz, H.R., Publ. STP 594, p. 5. Am. Soc. Testing and Materials, Philadelphia, PA.

Dravnieks, A., Keith, L., Krotoszynski, B.K. and Shan, J. 1974. Vaginal odors: GLC assay method for evaluating odor changes. J. Pharmaceutical Sci. 63: 36.

Dravnieks, A., McDaniel, H.C. and Powers, J.J. 1977. Comparison by 12 laboratories of the odor qualities of nine chemicals sniffed from the bottle and as GLC effluents. Submitted to J. Agr. Food Chem.

Dravnieks, A., Reilich, H.G., Whitfield, J. and Watson, C.A. 1973. Classification of corn odor by statistical analysis of gas chromatographic patterns of headspace volatiles. J. Food Sci. 38: 34.

Duewer, D.L. and Kowalski, B.R. 1975. Source identification of oil spills by pattern recognition analysis of natural elemental composition. Anal. Chem. 47: 1573.

Frijters, J.E.R. 1976. Evaluation of a texture profile for cooked chicken breast meat by principal component analysis. Poultry Sci. 55: 229.

Harper, R. 1956. Factor analysis as a technique for examining complex data on foodstuffs. Applied Statistics 5: 32.

Harries, J.M. 1973. Complex sensory assessment. J. Sci. Fd. Agr. 24: 1571.

Henry, W.F., Katz, M.H., Pilgrim, F.J. and May, A.T. 1971. Texture of semi-solid foods: sensory and physical correlates. J. Food Sci. 36: 155.

Jobbágy, A., and Holló, J. 1976. Über die gaschromatographische duftqualifizierung von weinen 2. Mitt. entwicklung einer masszahl zur qualifizierung des duftes durch bestimmung des zusammenhanges mit den peakflächen und anwendungsbedingungen. Nahrung 20: 295.

Kendall, D.A., Levins, P.L. and Leonardes, G. 1974. Diesel exhaust odor analysis by sensory techniques. Soc. Auto. Eng. Congress, Detroit, MI.

Lehrer, A. 1975. Talking about wine. Language 51: 901.

Lehrer, A. 1978. We drank wine, we talked about it. Semiotica (In press).

Likens, S.T. and Nickerson, G.B. 1964. Detection of certain hop oil constituents in brewing products. Am. Soc. Brewing Chemists Proc., Annual Meeting, 1964, 5—13.

Milutinovic, L., Bargmann, R.E., Chang, K-Y, Chastain, M. and Powers, J.J. 1970. Comparison of flavor and volatiles of tomato products and of peanuts. J. Food Sci. 35: 224.

Palmer, D.H. 1974. Multivariate analysis of flavour terms used by experts and non-experts for describing teas. J. Sci. Fd. Agric. 25: 153.

Pearson, K. 1901. On lines and planes of closest fit to systems of points in space. Philosophical Magazine 2: 559.

Powers, J.J. 1976. Experiments with subjective/objective correlations. In "Correlating Sensory Objective Measurements—New Methods for Answering Old Problems," Ed. Powers, J.J. and Moskowitz, H.R., Publ. STP 594, p. 111. Am. Soc. for Testing and Material, Philadelphia, PA.

Powers, J.J., Bargmann, R.E. and Godwin, D.R. 1977. Relations between sensory and objective measurements for quality evaluating of green beans. In "Flavor Quality: Objective Measurement," Ed. Scahlan, R.A., Symposium Series 51, Am. Chem. Soc., p. 51. Washington, D.C.

Powers, J.J. and Quinlan, M.C. 1974. Refining of methods for subjective-objective evaluation of flavor. J. Agr. Fd. Chem. 22: 774.

Qvist, I.H., von Sydow, E.C.F. and Akesson, C.A. 1976. Unconventional proteins as aroma precursors: instrumental and sensory analysis of the volatile compounds in a canned meat product containing soy or rapeseed protein. Lebensmittel-Wissenschaft u. Technologie 9: 311.

Schiffman, S.S. and Dackis, C. 1975. Taste of nutrients: amino acids, vitamins, and fatty acids. Perception & Psychophysics 17: 140.

Schiffman, S., Robinson, D.E. and Erickson, R.P. 1977. Multidimensional scaling of odorants: examination of psychological and physicochemical dimensions. Chemical Senses & Flavor 3: 375.

Stone, H., Sidel, J., Oliver, S., Woolsey, A. and Singleton, R.C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 29: 24.

Szczesniak, A.S., Brandt, M.A. and Friedman, H.H. 1963. Development of standard rating scales for mechanical parameters of texture and correlation between the objective and the sensory methods of texture evaluation. J. Food Sci. 28: 397.

Trivedi, S.J. 1971. Configuration and classification of clusters in n-dimensions. Themis Report No. 17, Technical Report No. 75, Dept. of Statistics and Computer Sci., Univ. of Georgia, Athens, GA 30602.

von Sydow, E. 1976. Annual Report 75/76, SIK, The Swedish Food Institute, Goteborg.

Vuataz, L., Sotek, J. and Rahim, H.M. 1974. Profile analysis and classification. Proceedings IV International Congress of Food Sci. and Technology, Madrid, Spain. Vol 1a: 25.

Williams, A.A. 1975. The development of a vocabulary and profile assessment method for evaluating the flavour contribution of cider and perry aroma constituents. J. Sci. Fd. Agric. 26: 567.

Wu, L.S., Bargmann, R.E. and Powers, J.J. 1977. Factor analysis applied to wine descriptors. J. Food Sci. 42: 944.

Young, L.L., Bargmann, R.E. and Powers, J.J. 1970. Correlation between gas-liquid chromatographic patterns and flavor evaluation of chemical mixtures and of cola beverages. J. Food Sci. 35: 219.

Ms received 8/30/77; revised 12/23/77; accepted 12/30/77.

The authors acknowledge the aid of Messrs. Tommy Mundy and David Staser, NSF high-school summer participants, who conducted the 1975 sensory trials and carried on preliminary statistical analysis. Appreciation is also expressed to Mrs. Louise Wu and Messrs. Jose Novó, Randall Evans, and Ronald Cox whose efforts permitted the sensory and objective tests of the 1976 trial to be carried on simultaneously.

TANNIN HAZES IN DEPROTEINIZED APPLE JUICE

J.P. VAN BUREN and R. D. WAY

ABSTRACT

Haze development in deproteinized juices was followed during incubation at 50°C. Haze increased 15 times faster at pH 2.5 and 5 times faster at 3.0 than at pH 3.5. Salts enhanced haze formation, with their molar effectiveness in the order $CaCl_2$ > $MgCl_2$ > KCl > NaCl > Na_2SO_4 > $NaNO_3$. The rate of haze production in 0.7M NaCl was 16 times that found with no salt addition. The materials, presumably flavonoids, most readily precipitating with gelatin are the quickest to form hazes in deproteinized systems. Juices from different cultivars varied in their tendency to form haze. Pectin and gum arabic (0.1%) retarded haze development.

INTRODUCTION

THE DEVELOPMENT OF HAZES and associated sediments in bottled apple juices creates a consumer acceptance problem for the juice processor. There seems to be general agreement that such hazes in apple juices consist largely of phenolic materials or flavonoid tannins mixed with varying amounts of protein-like materials (Johnson et al., 1968). The amount of protein in apple juices is quite low, normally ranging from 50–180 mg/L (Travernier and Jacquin, 1946), considerably less than the 300–1500 mg/L of tannin material usually present (Beech, 1972). Because of the direct relation of tannins and sediment (Marshall, 1951), the usual excess of phenolic or tannin materials and the known tendency of these types of compounds to condense with each other (Weinges et al., 1969), it seemed possible that some apple juice hazes might be primarily tannin condensation products. The idea was explored in the present investigation by studying the conditions affecting the formation of hazes in deproteinized apple juices. In such juices the complications of protein-tannin hazes are reduced (Van Buren and Robinson, 1969).

EXPERIMENTAL

Juice preparation

Juice preparation was carried out by pulping apples in a hammer mill to pass a ¼-inch screen, followed by pressing in a hydraulic press. The juice was then heated to 50°C, mixed with Klerzyme 100 (Wallerstein Co.) at the rate of 0.2 g/L, allowed to stand for 2 hr, followed by heating to 70°C. This depectinized juice was cooled overnight in a 1.5°C cold room. The supernatant juice was decanted, heated to 80°C and bottled hot into sterilized bottles. The bottled juices were stored in a 1.5°C cold room. Before use in the test incubations, the juices were decanted from sediment that might have formed and deproteinized by mixing well with bentonite (Kain, 1967) at the rate of 2 g/L. This was followed by centrifugation and filtration of the supernatant through a 0.65 micron filter (Millipore Corp.). The filtrates showed no indications of protein when tested with tannic acid (Mejbaum-Katzenellenbogen, 1955), or dinitrosalicylic acid, or sulfosalycilic acid (Sumner and Somers, 1949).

Measurements

Juice haze was measured in nephlos units using a Model 9 Coleman Nephlo-Colorimeter calibrated with the nephlos standards supplied with the instrument. Nephlos units are proportional to the amount of light scattered, by a solution or suspension, at a 90° angle from the incident beam. Nephlos values of 20–30 are common for nonhazy, clear, commercial apple juices. Haze would be readily detected by eye in juices having nephlos values over 100. Incubations of juices and mixtures were carried out in nephlometer cuvettes sealed with soft paraffin wax. Experimental mixtures were deaerated in vacuum prior to being placed in the cuvettes. All incubations were in a 50°C air oven.

Total phenolic materials were measured with the Folin-Ciocalteu reagent according to the procedure developed for wine analyses (Singleton and Rossi, 1965). Nonflavonoid phenolic material was determined on the soluble material remaining after precipitation of the flavonoids with formaldehyde (Kramling and Singleton, 1969). Subtraction of the nonflavonoid values from the total phenolics yielded values for the flavonoids in the juice or mixture. A measure of the concentration of materials having a non-substituted meta di-hydroxybenzene nucleus, the A ring of catechin, was obtained using the vanillin-sulfuric acid method of Swain and Hillis, 1959. All the above values are expressed in terms of an equivalent weight of catechin.

Other materials

Polyclar AT (PCAT), supplied by General Aniline and Film Corp., is an insoluble polyvinyl pyrrolidone powder used for removal of polyphenols from beverages. The Eastman Kodak calfskin gelatin used had a Bloom strength of 330. The pectin was a high methoxyl rapid set citrus pectin from California Fruit Growers Exchange. Other chemicals used were reagent grade.

RESULTS & DISCUSSION

Haze development

The development of haze in a deproteinized McIntosh apple juice is shown in Figure 1. When the same juice had been treated with PCAT, 2 g/100 ml, to remove the tannins and give a flavonoid free juice, there was no haze formed under the same incubation conditions.

pH Effect

Flavonoids comprise a large part of the tannins of apple juice. The tendency of flavonoids to undergo acid catalyzed condensation reactions (Weinges et al., 1969) suggested that one could expect an effect of pH on the rate of haze formation in apple juice. Juices were adjusted to pH 3.5, 3.0 and 2.5, using 1N NaOH or H_2SO_4, and incubated. The results, Figure 2, given for several incubation periods, show that hazes are promoted under the lower pH conditions and that the pH sensitive range for haze formations extends into the pH 3.0–3.5 region found for many apple juices (Bowen et al., 1959).

The haze in samples incubated at pH 2.5 and 3.0 was not decreased when the pH was adjusted to 3.5 with NaOH.

Pectin and gum arabic

One of the difficulties that arose in these experiments was that the measurement of haze formation over a long incubation time was hampered by the development of precipitates often accompanied by a clearing of the supernatant liquid. In their extensive work on sediment formation in apple juices, Neubert and Veldhuis (1944) reported that pectin could prevent sediment formation. Mejbaum-Katzenellenbogen (1955) used gum arabic to stabilize haze. These observations led to the testing here of both pectin and gum arabic with the results shown in Table 1. Both of these polysaccharides delayed precipitation during formation of tannin haze. They also decreased the rate of haze formation.

Salt effects

Juices containing added NaCl become turbid rapidly (Van Buren et al., 1976). Incubation of deproteinized juice containing various concentrations of NaCl showed that the rate of

Author Van Buren is with the Dept. of Food Science and Technology, Cornell University, Geneva, NY 14456. Author Way is with the Dept. of Pomology and Viticulture, Cornell University, Geneva, NY 14456.

0022-1147/78/0004-1235$02.25/0
© 1978 Institute of Food Technologists

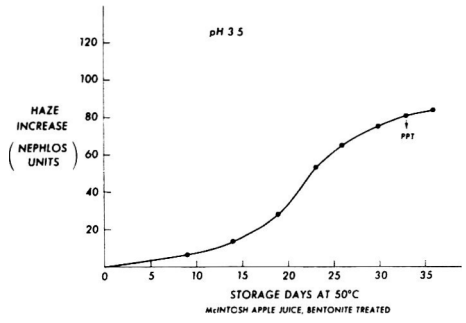


Fig. 1—Haze development in a deproteinized McIntosh apple juice.

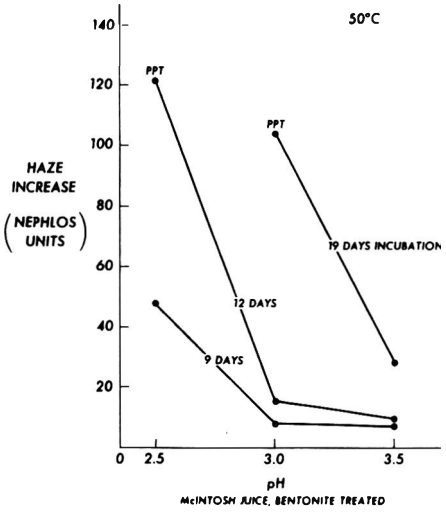


Fig. 2—Influence of pH on haze formation in a deproteinized McIntosh apple juice; ppt indicates a precipitate was present.

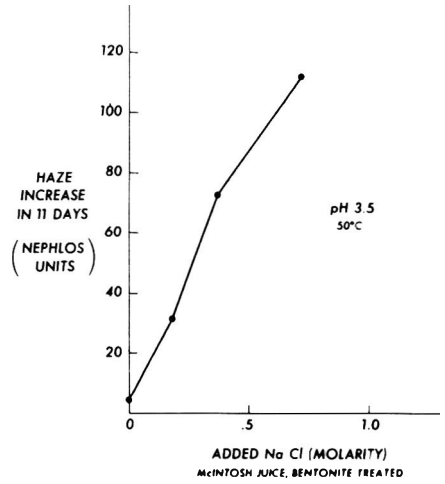


Fig. 3—Influence of NaCl concentration on haze formation in a deproteinized McIntosh apple juice.

haze formation was directly related to the NaCl concentration, Figure 3. Of some interest is the effectiveness of the NaCl at low concentrations, too low to be causing a salting out of the tannins (Glories, 1974).

A possibility was that NaCl acted to promote flocculation by altering the electric double layer (van Olphen, 1963). If this were the case, one could expect divalent ions to be 20 to 100 times as effective as monovalent ions in promoting haze formation. This possibility was tested with various salts. For each salt a set of data was obtained by incubation of juice with a range of salt concentrations giving different rates of haze formation from which one could calculate the concentration of the different salts that produced the same haze at given incubation times, Table 2. The kinds of anions and cations had an effect on the efficacy of the salts. Calcium was the most active cation while chloride was the most active anion. The relative equivalent concentrations of the salts and ions do not follow the Schulze-Hardy rule (van Olphen, 1963) for colloidal flocculation by modification of the electric double layer. The effectiveness of both anions and cations seems to rule out an action connected exclusively to charges on colloidal particles. The results suggest that salts play a role in haze formation aside from, or in addition to, promoting flocculation, perhaps by accelerating the chemical condensation of the flavonoids.

Table 1—Influence of pectin and gum arabic on haze and sediment formation at 50° C

Juice additive final conc[a]	Haze at 17 days (Nephlos units)	Days to produce sediment
none	95	17
0.1% gum arabic	38	35
0.1% pectin	41	>40

[a] Bentonite treated McIntosh juice at pH 3.0

Table 2—Effectiveness of different salts in promoting haze formation[a]

Salt	Salt molarity to get a haze increase of 75 nephlos units in 20 days
$CaCl_2$	0.019
$MgCl_2$	0.038
KCl	0.071
NaCl	0.111
Na_2SO_4	0.187
$NaNO_3$	0.259

[a] Bentonite treated McIntosh juice, pH 3.5, 50° C incubation, 0.04% gum arabic. With no added salt nephlos was 33 after 20 day's incubation.

Flavonoid effects

With an important involvement of flavonoids in haze formation one would expect that selective removal of some flavonoids would result in changes in the haze forming ability of a given amount of remaining flavonoid. The flavonoid mixtures in apple juices have a wide range of molecular weights (Lea and Timberlake, 1974) and the ability of flavonoids to precipitate with proteins increases as the flavonoid molecular weight goes up (Bate-Smith, 1973). This allowed the selective removal of flavonoids based roughly on their molecular weight through the use of small amounts of added gelatin and removal of the precipitate by centrifugation. Excess gelatin was removed from the gelatin-treated supernatants by a two times repeated bentonite (2 g/L) treatment. A number of different Nehou apple juices were prepared in this manner having different amounts of residual flavonoid (Table 3).

The gelatin treatment had little effect on the relative amounts of flavonoids to vanillin-sulfuric acid reactive material remaining in the juice. This was somewhat surprising since more highly polymerized flavonoids have often been found to have a higher ratio of flavonoid to vanillin-sulfuric acid reactive material (Goldstein and Swain, 1963). However, in similar work involving apple juice, Poffet (1971) also found that gelatin treatment did not alter the ratio of flavonoids to vanillin-sulfuric acid reactive material remaining in solution.

For a comparison of haze forming tendency of the different juices resulting from these gelatin treatments, mixtures were prepared by dilution of A, B and C treatment juices with flavonoid free "treatment E" juice so that the final incubation mixtures had similar amounts of flavonoids, Table 3.

The results of the incubations show that the greater the degree of flavonoid removal, as in treatment D, the less active the remaining flavonoid was in producing haze. Flavonoids removed by gelatin were of a nature to produce haze more readily than flavonoids not removed by gelatin. Analysis of hazy juice showed moderate decreases in the concentration of flavonoids and more marked decreases in the vanillin-sulfuric acid reactive material. These decreases in flavonoids and vanillin-sulfuric acid reactive materials on incubation are similar to those found by List (1969) and by Weiss (1973) during storage of apple juice. This is what might be expected if an important chemical reaction taking place among the flavonoids was sim-

Table 3—Effect of partial removal of flavonoids from Nehou apple juice on the ability of remaining flavonoids to form haze

	Juice[a] treatment (mg gelatin/100 ml)	Flavonoid removal (%)	Incubation mixture[b] analysis, mg/100 ml				Haze formed, nephlos units		
			Flavonoids		Vanillin-H_2SO_4 material				
			0 days	46 days	0 days	46 days	28 days	35 days	46 days
A	0	0	93	63	52	15	212	ppt	ppt
B	25	15	93	67	53	16	170	ppt	ppt
C	100	43	101	69	59	19	49	192	ppt
D	150	58	106	89	64	21	19	86	479
E	150 + 2 g/100 ml PCAT	99							

[a] After gelatin treatment juices were made protein free by a double bentonite treatment.
[b] Mixture of indicated juices A, B, or C, with juice E. Treatment D juice was used in its incubation mixture with no addition of juice from the E treatment. Mixtures contained 0.05% gum arabic and 0.1M NaCl, pH 3.5, 50°C incubation temperature.

lar to the acid-catalyzed condensations described by Weinges et al. (1969). Such condensations would have little effect on measured flavonoid levels but would decrease the amount of vanillin-sulfuric acid reactive material by one molecular unit for each individual condensation.

The formation of hazes in flavonoid free apple juices augmented with catechin (1 mg/ml) was extremely slow during incubation at 50°C, with little change over a 40-day period in nephlos, flavonoid content or vanillin-sulfuric acid reactive material.

Cultivar effects

The haze forming ability of juices from different apple cultivars showed considerable differences when compared either per mg of juice flavonoid or per ml of juice, Table 4. These values were obtained from sets of incubations having ranges of juice concentrations that allowed calculation of the amounts of juices giving equivalent haze formation.

The difference in the amount of juice needed to attain a haze level of 100 nephlos can be ascribed to the nature of the flavonoid of the various juices, since removal of the flavonoids eliminates haze formation. It may be of no significance that there was variation between these cultivars, indeed the variations due to year and location are not known. What may be more important, as indicated by this experiment and the prior one dealing with the partial removal of flavonoids with gelatin, is that the ability to form hazes in these deproteinized systems is related to the nature of the flavonoids as well as their amount.

Table 4—Flavonoid amounts and haze formation. Comparison of different apple cultivars.

Cultivar	Flavonoid in juice mg/100 ml	Amount per 100 ml of mixture[a] to reach 100 nephlos in 30 days	
		mg flavonoid	ml of juice
McIntosh	41	32	77
Marechal	248	42	17
Large Yellow Siberian	233	56	24
Hyslop	271	58	21
Yarlington Mill	170	92	54
Nehou	192	136	71

[a] Mixture of indicated juice with flavan-free deproteinized McIntosh juice; pH 3.5, 0.05% gum arabic, 0.2M NaCl, 50°C. McIntosh is a dessert apple; Marechal, Yarlington Mill and Nehou are bittersweet apples; Large Yellow Siberian and Hyslop are bitter-sharp apples.

SUMMARY

TWO ASPECTS of tannin haze formation in deproteinized juices have been studied. The effect of juice conditions has shown the importance of salts and pH. The importance of the flavonoids, both the amount and type, has been illustrated by the results with juices from different cultivars and with different degrees of flavonoid removal.

The effect of pH and salts are likely due to an effect on chemical condensation of the flavonoids. There is no evidence that there is any difference in the type of condensations involved. The salt may influence the degree of aggregation producing particles large enought to cause appreciable haze, but such an effect would be in addition to an earlier chemical condensation.

The haze-forming ability of the juice flavonoids, as indicated by the flavonoid removal experiment, is probably closely related to their molecular weight or degree of polymerization.

REFERENCES

Bate-Smith, E.C. 1973. Haemanalysis of tannins: the concept of relative astringency. Phytochem. 12: 907.
Beech, F.W. 1972. English cidermaking. Progress in Ind. Microbiol. 2: 135.
Bowen, J.F., MacGregor, D.R. and Atkinson, F.E. 1959. Effect of fruit variety and maturity on quality of apple wine. Food Technol. 13: 676.
Glories, Y. 1974. Recherches sur la structure et les proprietés des composés phénoliques polymérisés des vins rouge. Conn. Vigne Vin 8: 375.
Goldstein, J.L. and Swain, T. 1963. Methods for determining the degree of polymerization of flavans. Nature 198: 587.
Johnson, G., Donelly, B.J. and Johnson, D.K. 1968. The chemical nature and precursors of clarified apple juice sediment. J. Food Sci. 33: 254.
Kain, W. 1967. Untersuchung und Beurteilung von Bentoniten zur Weinbehandlung. Mitteil. Klosterneuburg 17: 201.
Kramling, T.E. and Singleton, V.L. 1969. An estimate of the nonflavonoid phenols in wines. Am. J. Enol. Vitic. 20: 86.
Lea, A.G.H. and Timberlake, C.F. 1974. The phenolics of ciders. 1. Procyanidins. J. Sci. Food Agric. 25: 1537.
List, D. 1969. Veränderungen der Inhaltsstoffe von Apfelsäften unter dem Einfluss der Warmebehandlung. Ind. Obst-u. Gemüseverw. 54: 489.
Marshall, C.R. 1951. Oxidation in apple juice. 3. Oxidation turbidities. J. Sci. Food Agric. 2: 342.
Mejbaum-Katzenellenbogen, W. 1955. Eine neue turbidimetrische Mikromethode zur Eiweissbestimmung. Bull. de l' Academie Polanaise des Sciences 3: 171.
Neubert, A.M. and Veldhuis, M.K. 1944. Clouding and sedimentation in clarified apple juice. Fruit Prod. J. 23: 324.
Poffet, J.R. 1971. Unpublished data. Eidg. Technischen Hochschule, Zürich, Switz.
Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144.
Sumner, J.B. and Somers, G.F. 1949. "Laboratory Experiments in Biological Chemistry," p. 64. Academic Press, New York.
Swain, T. and Hillis, W.E. 1959. The phenolic constituents of Prunus Domestica. 1. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63.
Travernier, J. and Jacquin, P. 1946. Influence de l'élimination partielle des matières azotés des moûts de pommes sur la fermentation des cidres. Chemie et Industrie 56: 104.
Van Buren, J., de Vos, L. and Pilnik, W. 1976. Polyphenols in Golden Delicious apple juice in relation to method of preparation. J. Agric. Food Chem. 24: 448.
Van Buren, J.P. and Robinson, W.B. 1969. Formation of complexes between protein and tannic acid. Agr. Food Chem. 17: 772.
van Olphen, H. 1963. "Clay Colloid Chemistry." Interscience Publishers, New York.
Weinges, K., Bähr, W., Ebert, W., Göritz, K. and Marx, H.D. 1969. Konstitution, Entstehung und Bedeutung der Flavonoid-Gerbstoffe. Fortschr. Chem. Org. Naturst. 27: 158.
Weiss, J. 1973. Zeit-und lagertemperature-abhängig Veränderungen sensorischer und nichtsensorischer Qualitätsmerkmale von Apfelsaftkonzentraten. Mitteil. Klosterneuburg 23: 367.
Ms received 6/21/77; revised 2/6/78; accepted 2/15/78.

FOLATE LEVELS IN FOOD—A COMPARISON OF MICROBIOLOGICAL ASSAY AND RADIOASSAY METHODS FOR MEASURING FOLATE

J. E. RUDDICK, J. VANDERSTOEP and J. F. RICHARDS

ABSTRACT

Concentrations of N^5-methyltetrahydrofolic acid (N^5MTHFA) were determined by the radioassay and the *L. casei* microbiological assay methods. The correlation coefficient for samples analyzed by both methods was 0.93. The radioassay method was found to be faster, simpler and more accurate than the microbiological method. The milk folate binder and *L. casei* were found to react differently to pteroylglutamic acid compared to N^5-MTHFA acid. The two methods are suitable only for measuring the form of folate used to construct the standard curve for the assay. Therefore, neither method can be used to accurately quantitate folate levels in foods.

INTRODUCTION

THE TERM "folic acid" is presently accepted to have two meanings (Malin, 1975). Generically, it covers a broad range of closely related pteroic acid compounds while specifically, it is used to describe the compound, pteroylglutamic acid (PGA). Thus, much confusion has arisen in the literature as to the meaning of the term "folic acid." It has been proposed by the IUPAC-IUB commission (Stokstad and Thenen, 1972) that the names "folic acid" and "folate" be used in the general sense for any member of the family of compounds, or a mixture of these, with the basic structure shown in Figure 1, either free or conjugated with two or more glutamic acid residues.

Throughout this paper, therefore, the term "folate" will be used only in the general sense to denote compounds with this structure. Where reference is made to specific forms of folate, appropriate nomemclature will be used e.g. pteroylglutamic acid (PGA).

Until recently, microbiological assays have been the most frequently used method for quantitatively measuring folate content of food, serum and other materials. The reported presence of a folate-binding fraction in milk (Ghitis, 1967) and the later association of this folate-binding activity with the lactoglobulin fraction of milk proteins (Ford et al., 1969) has been shown to provide the basis for a rapid and sensitive in vitro test for folates (Waxman et al., 1971; Archibald et al., 1972; Rothenburg et al., 1972; Tajuddin and Gardyna, 1973). Because of the increasing use of the radioassay method for the quantitative measurement of folate and the frequently contradictory reports on the correlation of results obtained by this method with those obtained by microbiological assays (Waxman et al., 1971; Rothenburg et al., 1972; Mincey et al., 1973; Dunn and Foster, 1973; Tajuddin and Gardyna, 1973; Shaw et al., 1974; Kamen and Caston, 1974; Waddell et al., 1976; Rudzki et al., 1976) it was of interest to determine both the reproducibility of standard curves and the correlation of results obtained by the two methods using buffered solutions of N^5-methyletrahydrofolic acid (N^5MTHFA) as the standard and as the unknown. The structure of N^5MTHFA is shown in Figure 2. In an attempt to partially explain both the variation

Authors are with the Dept. of Food Science, University of British Columbia, Vancouver, B.C.

0022-1147/78/0004-1238$02.25/0
© 1978 Institute of Food Technologists

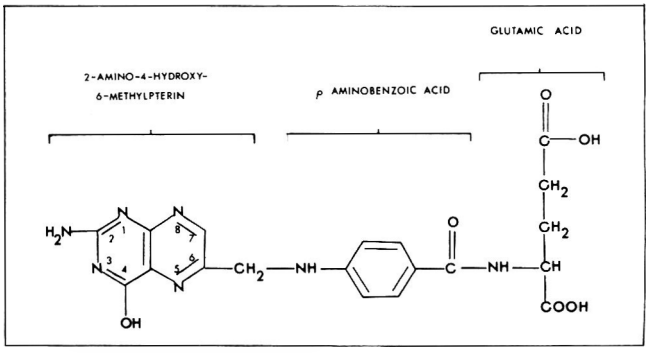


Fig. 1—Basic structure of folate compounds.

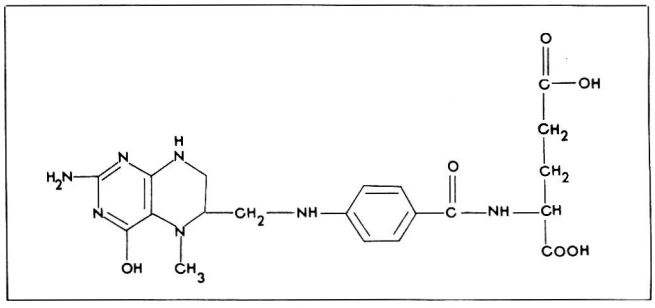


Fig. 2—N^5-methyltetrahydrofolic acid.

in previously reported results and the disagreement between folate levels of baby food analyzed by the radioassay method and *L. casei* microbiological method in this laboratory (Ruddick, 1977), standard curves obtained using PGA (Fig. 1) were then compared with those obtained using N^5MTHFA.

Microbiological methods for the determination of folate have been well documented (Teply and Elvehjem, 1945; Herbert, 1966; Cooperman, 1967; Herbert and Bertino, 1967). *Lactobacillus casei* was utilized in these experiments since this organism exhibits a growth response to N^5MTHFA. The radioassay technique used was a modification of that described by Rothenburg et al., 1972, and utilizes a two step sequential addition technique.

MATERIALS & METHODS

Preparation of N^5MTHFA standards

Samples of N^5MTHFA (Clinical Assays, Inc., Massachusetts) were diluted with 0.1M phosphate buffer (pH 7.3) containing 0.3% mercaptoethanol to give an initial N^5MTHFA concentration of 50.0 ng/ml. This stock solution was then serially diluted to give the concentrations shown in Table 1. Additional dilutions to give concentrations of 5.0, 7.5, 10.0, 15.0 and 20.0 ng/ml N^5MTHFA were later prepared in order to increase the accuracy of the microbiological assay standard curve. N^5MTHFA is sensitive to air oxidation and to the action of ultra violet light. Standard dilutions and assay tubes were therefore kept in diffuse light and a fresh standard vial, reconstituted immediately prior to use, was utilized for the preparation of each set of standard curves.

Preparation of unknown concentrations of N^5MTHFA

A series of unknown concentrations of N^5MTHFA in phosphate

buffer (0.3% mercaptoethanol) was made up such that all samples were within the concentration range of the standard curves.

Preparation of pteroylglutamic acid standards

Samples of PGA (Nutritional Biochemical Corp., Ohio) were diluted in 0.1M phosphate buffer (pH 7.3) containing 0.3% mercaptoethanol to give concentrations of 50.00, 16.67, 5.56, 1.85 and 0.62 ng/ml PGA.

Maintenance of stock culture and preparation of inoculum

Lactobacillus casei ATCC 7469 was maintained as a stab culture in tubes of prepared Bacto-Lactobacilli Agar. Cultures were incubated at 35–47°C for 18–24 hr and then stored in a refrigerator at 4°C. Transfers were made at monthly intervals. Inoculum for the assay was made by subculturing from the stock culture into a tube containing 10 ml prepared Bacto-Lactobacilli Broth. After incubation for 16–18 hr at 37°C the tubes were centrifuged, the supernatant discarded and cells resuspended in 10 ml 0.85% sterile saline. The culture was recentrifuged and suspended in fresh sterile saline twice more. An aliquot was diluted 100-fold in a sterile container with sterile saline and one drop of this was used to inoculate the assay tubes.

Microbiological assay procedure

5 ml of rehydrated Bacto-Folic Acid Casei Medium (Difco) were dispensed into test tubes. 0.1 ml of the N^5MTHFA dilution (or PGA dilution) was added to each tube and the volume made up to 10 ml with 0.1M phosphate buffer (1% ascorbate). All tubes were autoclaved for 5 min at 15 psi (121°C) and then cooled to room temperature. One drop of the diluted *L. casei* cell suspension was added to each tube. Tubes were incubated for 18 hr at 37°C. After thoroughly mixing absorbance was read at a wavelength of 660 nm.

Radioassay procedure

The procedure for radiometric assay was that outlined in the Clinical Assays ^{3}H Folic Acid Radioassay Kit (Clinical Assays, Massachusetts, 1975). All solutions used in the radioassay were made up and the assay procedure followed exactly as described in the kit with the exception that 0.1M phosphate buffer pH 7.3 was used in place of tris-NaCl buffer. Duplicate samples of N^5MTHFA or PGA were incubated with milk folate binder for 20 min at room temperature. After addition of ^{3}HPGA there was a further 20 min, room temperature incubation. The protein-bound and the free ^{3}HPGA were then separated by adsorption of the latter on dextran coated charcoal. Supernatant and charcoal were separated by centrifugation in a refrigerated centrifuge (Sorvall Model RC2-B) at 4°C, 2,000 × G for 20 min. The supernatant was decanted into vials and 12 ml scintillation fluid (Scintiverse – Fisher Universal LSC Cocktail) were added. After cooling, sample radioactivity was counted for 10 min in a Nuclear Chicago Isocap 300 liquid scintillation counter. Correction for counting efficiency (quench correction) was carried out at each assay using the channels ratio technique. A maximum binding control was run in duplicate, with every assay to determine the amount of radioactive material which was able to compete with unlabeled N^5MTHFA for milk protein binding sites. This counteracted the effect of any impurities which may have been present in the ^{3}HPGA. In addition, a "blank" sample containing buffer and ^{3}HPGA was run in duplicate with each assay to determine levels of radioactivity not removed by dextran coated charcoal.

Experimental procedure

Microbiological and radioassays were carried out concurrently using duplicate samples of the freshly prepared N^5MTHFA dilutions. This ensured that any variation in the standard curves due to differing concentrations of N^5MTHFA between batches was taken into account by both methods. Ten N^5MTHFA standard curves were prepared by each method.

Twenty-five unknown dilutions of N^5MTHFA were assayed by both methods. Concentrations were determined from N^5MTHFA standard curves prepared at the same time and under identical conditions as the unknown samples.

Five PGA standard curves were prepared by each method using duplicate samples of each dilution. PGA standard curves were prepared at the same time as an additional five N^5MTHFA standard curves in order to ensure that conditions of assay remained as identical as possible for each standard used.

RESULTS

N^5MTHFA standard curves–Microbiological assay

Regression analysis was carried out on the data from the 10 standard curves obtained by the microbiological assay method. This is shown in Figure 3. The correlation coefficient (r) of 0.61 illustrates that there is a significant ($p \leq 0.001$) relationship between concentration of N^5MTHFA and growth response of *L. casei* as measured by absorbance at 660 nm. The standard error of estimate was found to be 11.40.

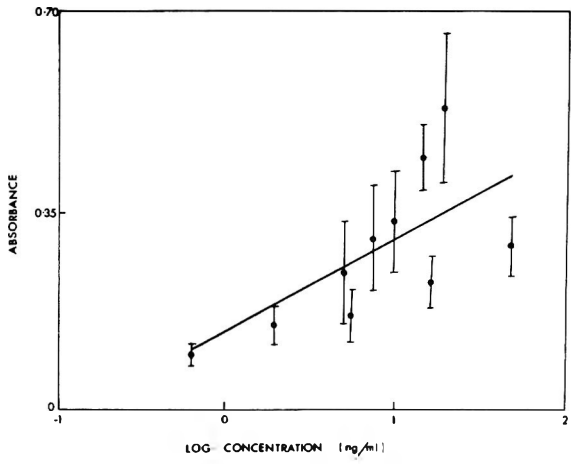


Fig. 3–Regression analysis performed on N^5MTHFA standard curves obtained by L. casei microbiological assay method.

Table 1–N^5MTHFA concentrations used in preparation of standard curves

Dilution	N^5MTHFA in 100 µl (ng)	Sample equivalent (ng/ml)
1/1	5.000	50.00
1/3	1.667	16.67
1/9	0.556	5.56
1/27	0.185	1.85
1/81	0.062	0.62

Radioassay

Standard curves were obtained by plotting the percent tracer bound for each amount of standard on semi log paper. The percent of tracer bound for each level of standard was calculated from the normalized (quench corrected) counts per minute, thus:–

$$\%\ ^3\text{HPGA bound} = \frac{\left(\begin{array}{c}\text{cpm in supernatant}\\ \text{of standard}\end{array} - \begin{array}{c}\text{cpm in supernatant}\\ \text{of blank}\end{array}\right)}{\left(\begin{array}{c}\text{cpm in supernatant}\\ \text{of maximum binding}\\ \text{control}\end{array} - \begin{array}{c}\text{cpm in supernatant}\\ \text{of blank}\end{array}\right)} \times 100$$

Regression analysis performed on the standard curves obtained from the 10 sets of data is shown in Figure 4. The correlation coefficient (r) of 0.99 illustrates that there is a highly significant ($p \leq 0.001$) relationship between concentration of N^5MTHFA and percent binding of tracer. The standard error of estimate was found to be 4.01.

Comparison of folate concentrations obtained using both methods

Folate concentrations for the 25 unknown N^5MTHFA samples were calculated by reference to standard curves obtained by both methods. Results obtained by the radioassay and microbiological methods are shown in Figure 5. Radioassay values ranged from 0.62–35 ng/ml while microbiological assay values ranged from 0.75–38 ng/ml. The correlation coefficient (r) for values obtained by both methods was 0.93 and the regression line was found to have a slope of 0.76 and a y intercept of 3.10. The standard error of estimate was 3.65.

–Text continued on page 1240

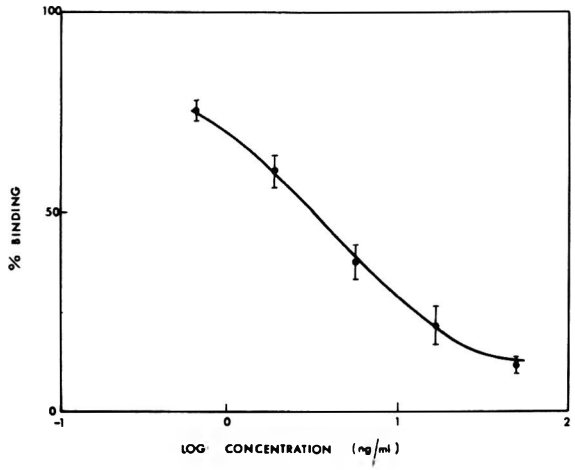


Fig. 4—Regression analysis performed on N^5MTHFA standard curves obtained by radioassay method.

Table 2—Paired comparison Student t-test values of PGA and N^5MTHFA standard curves obtained by microbiological and radioassay techniques

	Concentration of folate (ng/ml)				
	50.00	16.70	5.57	1.85	0.62
Microbiological assay					
t value	8.10	6.27	9.07	4.06	12.06
degrees of freedom	4	4	4	4	4
P	0.01	0.01	0.001	0.02	0.001
Radioassay					
t value	11.51	7.08	4.74	2.50	3.74
degrees of freedom	4	4	4	4	4
P	0.001	0.01	0.02	0.10	0.05

Comparison of N^5MTHFA and PGA standard curves

Regression analysis was performed on the five sets of standard curves produced by both methods of assay using the two forms of folate. Results are presented in Figures 6 and 7. Paired comparison Student t-tests were carried out on the data. These results are presented in Table 2. A significant difference in the two standard curves is evident. This is particularly the case at higher concentrations of folates when the radioassay method is used. At concentrations lower than 6.0 ng/ml the difference in the two standard curves is less evident in the radioassay method where similar percent binding values were frequently obtained for both standards. The difference in the growth response of *L. casei* with PGA and N^5MTHFA is significant at all concentrations of folate. This is particularly the case at lower concentrations of folate although the difference does not appear to be evident from the regression analysis.

It would appear from the limited data presented that the milk protein shows a greater affinity for binding to PGA than to N^5MTHFA at pH 7.3 and that PGA causes a greater growth response of *L. casei* than N^5MTHFA.

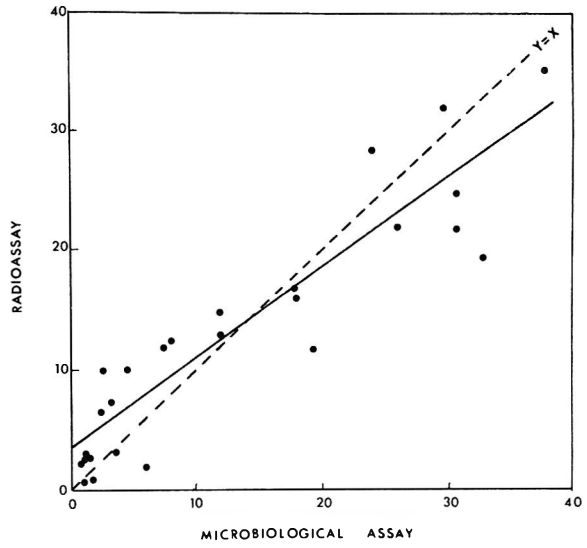


Fig. 5—Regression analysis of radioassay on microbiological assay method of measuring N^5MTHFA concentration.

DISCUSSION

UNTIL RECENTLY, microbiological assays have been the most frequently used method for measuring folate levels. However, such assays are extremely time consuming, requiring both maintenance of the stock culture and an overnight incubation period. *L. casei* has been the most frequently used microorganism for folate assay. However, it has been found to be affected by the presence of absence of several unidentified growth factors (Stokstad and Hutchings, 1947), it is capable of converting various other growth factors to folate in periods of stress (Baker et al., 1971), and it can also be subject to growth changes such that folate is no longer required. These factors, together with the varying effects on growth of different commercial media, temperature and time of autoclaving, tempera-

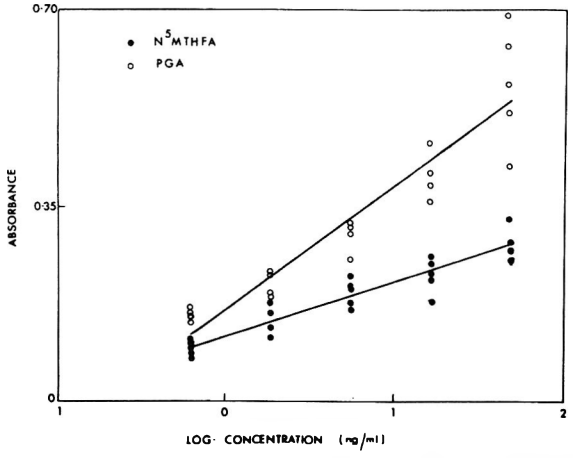


Fig. 6—Regression analysis performed on PGA and N^5MTHFA standard curves obtained by microbiological assay method.

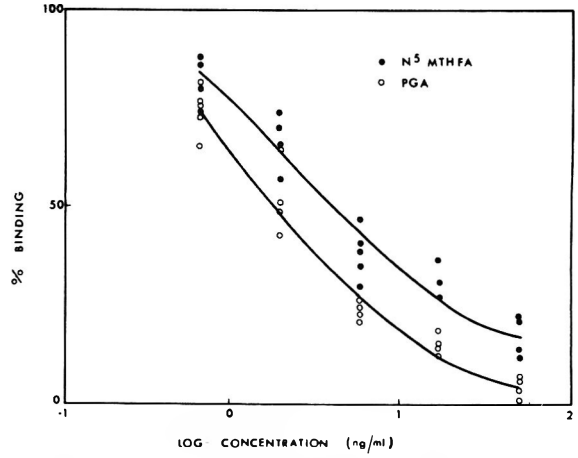


Fig. 7—Regression analysis performed on PGA and N^5MTHFA standard curves obtained by radioassay method.

ture and time of incubation etc., make the use of this method unsuitable for accurate determination of folate levels.

The radioassay method, by comparison, is fast and can be carried out with simple laboratory equipment over a period of 3–4 hr. Although milk folate binding is influenced by such factors as temperature and time of incubation, pH and charcoal preparation (Givas and Gutcho, 1975; Zettner and Duly, 1974, 1975) the effects of these factors are much less significant than in the microbiological method. Thus, as shown by our results, a much more reproducible standard curve can be obtained using the radioassay method than by the microbiological assay.

It should be noted that when using commercially prepared radioassay kits, there may be a difference in the folate binding activity of the milk protein from one kit to another causing differences in standard curves to occur.

Previously published material pertaining to the comparison of folate levels obtained using the radioassay and microbiological methods, vary considerably. Serum folate levels were compared in every case. Archibald et al. (1972), Waxman et al. (1971) and Tajuddin and Gardyna, (1973) showed good agreement between the two methods. Mincey et al. (1973) and Waddell et al. (1976) found that mean serum folate levels as determined by the radioassay method were slightly lower than those obtained by the microbiological method but still offered the same degree of diagnostic information. Rothenburg et al. (1972) found that the radioassay folate concentrations were approximately half those obtained by the microbiological assay and Rudzki et al. (1976) concluded that the microbiological assay gave the more accurate indication of folate levels in serum. In all cases, N^5MTHFA was used as standard for the radioassay method but PGA or an unidentified standard was used for preparation of the microbiological standard curve.

It can be seen from the results obtained in this experiment that the growth of *L casei* and the milk-protein binding ability are dependent, in part, on the type of folate present in the system. It is to be expected, therefore, that comparisons of the two methods of assay using PGA as standard for the microbiological assay and N^5MTHFA as standard for the radioassay will yield different results.

Since PGA and N^5MTHFA react differently in each system of assay, it may also be expected that results read from a standard curve prepared from a singular pure form of folate will not give an accurate estimate of the total folate contained in a multiple system. It has been well documented that N^5MTHFA is the predominant form of folate found in serum (Herbert et al., 1962). However, forms of folate other than N^5MTHFA may also be present which are able to show a different affinity for the milk protein or cause a dissimilar growth response with *L. casei*. This may partially explain why Shaw et al. (1974) obtained a poor correlation coefficient for serum folate levels even though N^5MTHFA was used as standard for both assays. It may also be a reason why, when total and free folate levels in baby food were assessed using both methods of assay and N^5MTHFA as standard, results obtained by the radioassay method differed from those obtained by the microbiological assay (Ruddick, 1977). Also, values did not compare well with levels determined by Hoppner (1971). Hoppner used *L. casei* microbiological assay for determining folate levels and PGA as the standard curve.

CONCLUSIONS

THESE EXPERIMENTS demonstrate that the radioassay method herein described is a faster, simpler and more accurate method for measuring levels of N^5MTHFA than the *L. casei* microbiological method. When N^5MTHFA standard curves are used, concentrations of buffered N^5MTHFA as determined by the radioassay method compare well with concentrations obtained by the *L. casei* microbiological assay. However, when the radioassay method of folate assay is carried out at pH 7.3 there is a marked difference in the ability of the milk protein to bind PGA and N^5MTHFA. Similarly *L. casei* also exhibits a difference in its growth response to the two forms of folate. The two assay systems therefore, when carried out by the methods herein described, are suitable only for measuring singular forms of folate using standard curves constructed from data obtained using the same folate.

N^5MTHFA and PGA are just two of the many forms of folate that have been identified. Other forms may show greater or lesser binding affinities and may exhibit further differences in the growth response of microorganisms. In addition, the presence of twc or more folate types together in any material may also cause variations. It becomes obvious therefore that the folate levels of foodstuffs which are known to contain many forms of folate cannot be accurately estimated by either of the two methods described in this paper until more information pertaining to the number, types and distribution of folate in foodstuffs and the interaction of the various forms with each other and with biological materials is obtained.

REFERENCES

Archibald, E.L., Mincey, E.K. and Morrison, R.T. 1972. Estimation of serum folate levels by competitive protein binding assay. Clin. Biochem. 5: 232.

Baker, H., Frank, O. and Hunter, S.H. 1971. Simplified Lactobacillus casei assay for folates. In "Methods in Enzymology," Vol 18, Part B.

Cooperman, J.M. 1967. Microbiological assay of serum and whole blood folic acid. Am. J. Clin. Nat. 20: 1015.

Dunn, R.T. and Foster, L.B. 1973. Radioassay of serum folate. Clin. Chem. 19: 1101.

Ford, J.E., Salter, D.N. and Scott, K.J. 1969. The folate binding protein in milk. J. Dairy Res. 36: 435.

Ghitis, J. 1967. The folate binding in milk. Amer. J. Clin. Nutr. 20: 1.

Givas, J.K. and Gutcho, S. 1975. pH dependence of the binding of folates to milk binder in radioassay of folates. Clin. Chem. 21: 427.

Herbert, V. 1966. Aseptic addition method for Lactobacillus casei assay of folate activity in human serum. J. Clin. Pathol. 19: 12.

Herbert, V. and Bertino, J. 1967. Folic acid, In "The Vitamins: Chemistry, Physiology, Pathology, Methods," Vol. 7 p. 243. Academic Press, New York.

Herbert, V., Larrabee, A.R. and Buchanan, J.M. 1962. Studies on the identification of a folate compound of human serum. J. Clin. Invest. 41: 1134.

Hoppner, K. 1971. Free and total folate activity in strained baby foods. Can. Inst. Food Technol. J. 4: 51.

Kamen, B.A. and Caston, J.D. 1974. Direct radiochemical assay of serum folate. J. Lab. Clin. Med. 83: 164.

Malin, J.D. 1975. Folic acid. In "World Review of Nutrition and Dietetics," Vol. 21, p. 198. Karger, Basel.

Mincey, E.K., Wilcox, E. and Morrison, R.T. 1973. Estimation of serum and red cell folate by a simple radiometric technique. Clin. Biochem. 6: 274.

Rothenburg, S.P., DaCosta, M. and Rosenberg, Z. 1972. A radioassay for serum folate. Use of a two-phase sequential incubation, ligand binding system. New Eng. J. Med. 286: 1335.

Ruddick, J.E. 1977. Unpublished data. The University of British Columbia, Vancouver, B.C.

Rudzki, Z., Nazaruk, M. and Kimber, R.J. 1976. The clinical value of the radioassay of serum folate. J. Lab. Clin. Med. 87: 859.

Shaw, W., Slade, B.A., Harrison, J.W. and Nino, H.V. 1974. Assay of serum folate: Difference in serum folate values obtained by L. casei bioassay and competitive protein-binding assay. Clin. Biochem. 7: 165.

Stokstad, E.L.R. and Hutchings, B.L. 1947. The microbiological assay of Lactobacillus casei factor (vitamin B_c folic acid). Biological Symposia 12: 353.

Stokstad, E.L.R. and Thenen, S.W. 1972. Chemical and biochemical reactions of folic acid. In "Analytical Microbiology." Academic Press. New York.

Tajuddin, M. and Gardyna, H.A. 1973. Radioassay of serum folate, with use of a serum blank and nondialyzed milk as folate binder. Clin. Chem. 19: 125.

Tepley, L.J. and Elvehjem, C.A. 1945. The titrimetric determination of Lactobacillus casei factor and folic acid. J. Biol. Chem. 157: 303.

Waddell, C.C., Domstad, P.A., Pricher, F.J., Lerner, S.R., Brown, J.A., Lawhorn, B.K. 1976. Serum folate levels: Comparison of microbiologic assay and radioisotope kit methods. Am. J. Clin. Path. 66: 746.

Waxman, S., Schreiber, C. and Herbert, V. 1971. Radioisotopic assay for measurement of serum folate levels. Blood 38: 219.

Zettner, A. and Duly, P.E. 1974. Principles of competitive binding assays (saturation analyses) 2. Sequential saturation. Clin. Chem. 20: 5.

Zettner, A. and Duly, P.E. 1975. Relative efficacy of separation of "free" and "bound" [3'5'-^{3}H] pteroylglutamate by charcoal coated with various materials. Clin. Chem. 21: 1927.

Ms received 9/6/77; revised 1/16/78; accepted 1/30/78.

LIPID AUTOXIDATION AND AMINO ACID CHANGES IN PROTEIN–ENRICHED SWEET POTATO FLAKES

W. M. WALTER JR., A. E. PURCELL, M.W. HOOVER and A. G. WHITE

ABSTRACT

Three sweet potato flake formulations, containing (1) soy flour plus methionine, (2) casein and (3) no supplementary protein, were prepared and stored at room temperature (21–23°C) in air for 16 months. The formulations were analyzed periodically to ascertain changes in carotene content, amino acid levels and water-binding capacity. On the basis of carotene degradation as a measure of lipid autoxidation, it was found that after an induction period of 19 days required by the flakes supplemented with soy-methionine, all formulations were oxidized in an identical manner. Among the amino acids, only glutamic acid was lost from all three formulations, and isoleucine levels decreased in the control formulation only. Lysine concentration in the fortified flakes decreased while histidine decreased in the soy-methionine formulation. Storage had no effect on the water-binding capacity of any of the three formulations.

INTRODUCTION

SWEET POTATO FLAKES stored in air undergo autoxidation in which carotene and fatty acids are destroyed (Walter and Purcell, 1974). The autoxidation of such lipids in foods can mediate destruction of other compounds present, some with high nutritional value. Research with both model systems and dehydrated foods has shown that protein interacts with lipid oxidation products thus undergoing both denaturation and loss of biological value (Labuza, 1972).

No information is available concerning the effect of autoxidizing lipids on the amino acid composition of dehydrated sweet potato flakes. A previous study has shown that dehydrated sweet potato flakes and flakes fortified with soy flour, cottonseed flour, and wheat gluten, retained essential amino acids when stored at 23°C and 40°C under nitrogen (Walter et al., 1978). However, water-binding capacity of flakes stored at 40°C decreased. The purpose of the present study was to investigate the relationship between lipid autoxidation and changes in amino acid content of sweet potato flakes, flakes fortified with casein, and flakes containing soy flour plus methionine. In addition, it was desired to determine if autoxidation modified other food components in such a way as to change water-binding capacity.

MATERIALS & METHODS

JEWEL SWEET POTATOES, which had been cured for 1 wk and then stored for 1 month (Purcell et al., 1976), were washed, hand-peeled and cut into 2-inch cubes. Three 1.8-kg batches (25% dry matter) were cooked in an autoclave at 121°C for 30 min, cooled, mixed with additives described below, and blended into a smooth puree. Each formulation was dried into flakes on a 12 × 19 inch, double drum dryer heated with steam at 80 psi. The flakes were retained on the drum for about 30 sec.

All formulations contained 0.3g sodium bisulfite, 0.1g sodium sulfite and 1.8g sodium acid pyrophosphate to prevent discoloration (Hoover, 1963). The soy-methionine formulation contained 129g defatted soy flour (Central Soya Company), 2.0g DL methionine, and 65g sucrose. The casein formulation contained 120g casein (NB Company) and 65g sucrose. The control formulation contained no other additives.

The flakes were ground to a size between 40- and 80-mesh, and 25-g samples sealed in air in #303 cans. Headspace volume was about 500 ml, thus assuring adequate oxygen levels for autoxidation. The cans were held at room temperature (21–23°C) until sampled. Each formulation was analyzed immediately after preparation and at 1, 2, 4, 6, 8, 12.25 and 16 months of storage.

Amino acid analysis

Duplicate samples of each formulation were acid hydrolyzed and amino acid content was measured with a Beckman Model 119, automatic amino acid analyzer (Walter et al., 1978). Data from the analyzer was integrated and reduced to the desired format by a Texas Instruments Model 960A minicomputer.

Analyses for cysteine and tryptophan were carried out on an enzymatic hydrolysate of the flakes, as previously described (Walter et al., 1978). This analysis was performed only on the freshly prepared formulations.

Carotene analysis

Duplicate 3.0-g samples of each formulation were reconstituted with 60 ml boiling water and set aside in the dark unitl cool. One gram of filter aid and 50 ml methanol were added and the mixture was blended in a high speed blendor. The slurry was filtered and the mat exhaustively extracted with hexane-acetone (1:1). The filtrate was placed in a 500 ml separatory funnel. The lower layer was passed into a second separatory funnel containing 200 ml of peroxide-free ethyl ether, then discarded. The upper layer in both separatory funnels was washed three times with 200 ml water. The lower layers were discarded and the organic extracts were combined and evaporated *in vacuo*. The residue containing carotenoids was redissolved and diluted to 250 ml with hexane. Since the solutions were cloudy, they were treated with 5g of anhydrous sodium sulfate and placed in the dark to clarify before the absorbancy was measured at 436 and 450 nm for calculation of the carotene concentration (Purcell, 1962).

Viscosity

Two 5-g samples of each formulation were reconstituted with 20 ml of boiling water and stirred for 1.0 min. Each sample was covered and held at room temperature for 3 hr. Apparent viscosity was determined on a Haake Rotovisko model RV-1 rotary viscometer (Walter et al., 1976).

Statistical methods

Effects of formulation on amino acid composition of flakes were determined by analysis of variance (ANOVA) and Duncan's multiple range test. Effects of storage time on amino acid levels were studied by regression analysis of data for individual formulations and of pooled data. Data were tested for both linear and quadratic regression. Similarly, the dependent variable apparent viscosity was subjected to zero time analysis of variance for determination of formulation effects, and to linear and quadratic regression analysis of variance for detection of viscosity changes over time.

Moisture and protein content

Moisture levels were determined by measuring weight changes occurring when the samples were dried to constant weight at 95°C. Protein content (N × 6.25) was determined by the Kjeldahl method with copper and selenium catalysts.

RESULTS & DISCUSSION

Flake properties

Initial moisture levels ranged from 4.02% for the control formulation to 5.30% for the soy-methionine formulation (Table 1). Pro-vitamin A (β-carotene) was lower in the fortified flakes than in the control due to the diluting effect of the additives. The casein formulation, although it contained the least amount of carotene, still provided three times the vitamin

All authors are with the Dept. of Food Science, North Carolina State University, Raleigh, NC 27607. Authors Walter and Purcell are also affiliated with the USDA Southern Region, ARS.

Table 1—Initial composition of control and fortified sweet potato flakes

Flake formulation	Protein[a] (%)	β-Carotene (mg/g flakes)	Moisture (%)
Control	6.21	0.43	4.02
Soy-methionine	13.25	0.32	5.30
Casein	19.36	0.29	4.61

[a] Kjeldahl nitrogen X 6.25

A RDA in a 100-g serving. As expected, fortification increased the protein two- to threefold.

Each formulation had its own characteristic amino acid pattern (Table 2). Amino acid balance, as manifested by the ratio of essential to nonessential amino acids for the control and soy-methionine formulations, has been reported previously (Walter et al., 1978) as 0.72 for control and 0.68 for soy-methionine formulations. This study has shown that the casein formulation ratio was slightly higher with a value of 0.75. Of the essential amino acids, only total sulfur is deficient with 97% of FAO/WHO (1973) reference value. Thus, both casein and soy flour plus methionine are suitable supplementary materials for sweet potato flakes with respect to amino acid balance and quality.

An examination of the apparent viscosities of the reconstituted formulations at several rotor speeds (Fig. 1) indicated that the control formulation had a greater water-binding capacity (i.e. higher apparent viscosity) than did either fortified formulation. The casein formulation bound slightly more water than the soy-methionine formulation and exhibited pseudoplastic flow patterns similar to the flow patterns of other fortified flake formulations previously reported (Walter et al., 1978).

Stored flakes

One measure of the progress of autoxidation in sweet potato flakes is the extent of carotene destruction. Loss of carotene (Fig. 2) began immediately in the control and casein formulations while loss from the soy-methionine flakes began after 19 days had elapsed. This evidence indicated that the soy flour contained an inhibitory substance which retarded lipid autoxidation for this initial period. The rate of destruction in all three formulations increased for 2 months, then moderated. After 4 months, destruction was very slow.

It has been reported previously (Walter and Purcell, 1974) that lipids of sweet potato flakes exist as surface and bound fractions. Surface lipids are oxidized first; of these, carotene is destroyed rapidly, whereas linoleic and linolenic acids of the surface fraction react more slowly. The bound lipid fraction is surrounded by a dense carbohydrate matrix which serves as an oxygen barrier and as a result, the carotene and fatty acids in this fraction are oxidized at an extremely slow rate. Thus, flakes exhibit a pattern of rapid carotene and fat destruction followed by a period of very slow loss. Fortification did not change the mechanism of carotene autoxidation except that the soy flour did cause a short induction period after which all formulations oxidized at about the same rate.

Amino acid changes. Regression analyses of data pooled over all formulations revealed only two instances of significant storage time effects. Lysine content changed according to a second order polynomial (quadratic) reaction rate (Table 3). The initial decrease indicated that lysine was more stable than the other amino acids during the 0 to 16 wk storage period. Phenylalanine content increased linearly with time (Table 3), again indicating high relative storage stability.

The levels of several amino acids (e.g. glutamic acid) displayed significant changes during storage for individual formulations, but not in pooled analyses for all samples. Linear models of these storage changes produced the best fit (Table 4). Since the amino acid levels were calculated on the basis of grams amino acid per 16g of amino acid nitrogen recovered, a

Table 2—Initial amino acid composition[a] of control and supplemented sweet potato flakes[b]

Amino acid	Formulation		
	Control	Soy-methionine	Casein
Aspartic acid	27.0a	19.1b	14.9c
Threonine	7.0a	5.4b	5.8c
Serine	7.6a	6.8b	7.5ab
Glutamic acid	14.1a	20.7b	28.4c
Proline	5.1a	5.5a	11.5b
Glycine	5.0a	4.6a	2.6b
Alanine	8.5a	6.1b	5.0c
Cysteine[c]	0.5a	0.3a	0.2a
Valine	8.3a	6.7b	8.6a
Methionine	1.9a	4.6b	3.2c
Isoleucine	6.0a	5.8a	6.7b
Leucine	9.4a	9.5a	11.9b
Tyrosine	4.5a	3.5b	5.7c
Phenylalanine	7.4a	6.2b	6.6ab
Lysine	6.1a	6.0a	6.6a
Histidine	2.1a	3.4a	2.0a
Arginine	4.0a	6.3b	3.3a
Tryptophan[c]	1.5a	1.1a	1.2a

[a] Grams of amino acid per 16g amino acid nitrogen recovered.
[b] Values followed by the same letter in a horizontal row are not significantly different ($P < 0.05$).
[c] Values determined colorimetrically on enzyme hydrolysate.

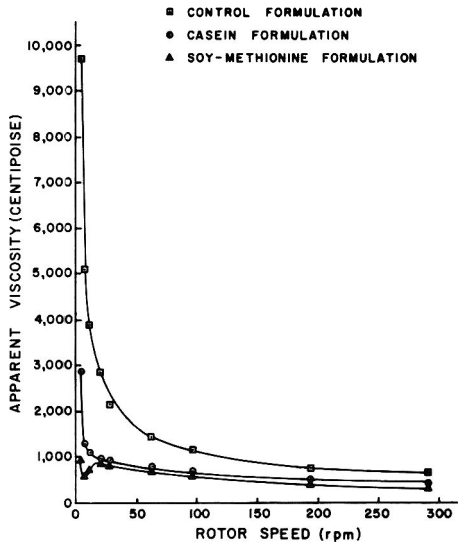


Fig. 1—Apparent viscosity of reconstituted sweet potato flake formulations as a function of rotor speed.

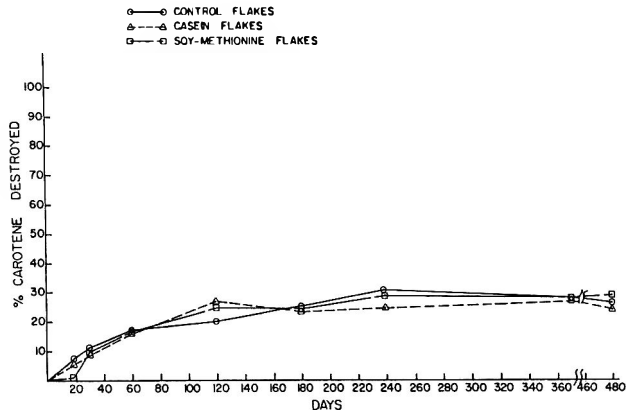


Fig. 2—Carotene loss from control and fortified sweet potato flakes stored in air.

Table 3—Quadratic and linear regression analyses of variance of lysine and phenylalanine composition of control and fortified (pooled) sweet potato flakes stored in air

		Lysine				Phenylalanine			
Source[a]	d.f.	MS	F	R^2	Slope[b]	MS	F	R^2	Slope[b]
Quadratic									
T	1	11.1405	15.55**	0.4211	0.0616**	2.4105	9.63**	0.1799	0.0121
T^2	1	11.7880	16.45**		−0.0013**	0.0052	0.02		−0.00003
Error	44	0.7165				0.2504			
Linear									
T	1	11.1405	11.57**	0.2046	−0.0222**	2.4105	9.84**	0.1795	0.0103**
Error	45	0.9625				0.2449			

[a] T = time (weeks)
[b] Slope = grams of amino acid · 16g amino acid nitrogen recovered^{-1} · week^{-1}.
** $P < 0.01$

Table 4—Regression analysis of variance of the amino acid composition of control, soy-methionine and casein formulation sweet potato flakes stored in air

			Control			Soy-methionine			Casein		
Amino acid	Source[a]	d.f.	MS	F	Slope[b]	MS	F	Slope[b]	MS	F	Slope[b]
Glutamic	T	1	5.6348	8.64*	−0.0273*	13.2487	16.59**	−0.0421**	27.466	17.51**	−0.0602**
acid	Error	14	0.6519			0.7984			1.568		
Isoleucine	T	1	0.2871	7.40*	−0.0062*						
	Error	14	0.0388								
Phenyl-	T	1				1.1708	11.06**	0.0125**	1.3855	9.978**	0.0135**
alanine	Error	14				0.1059			0.1389		
Lysine	T	1				5.6780	28.21**	−0.0275**	3.8233	5.77*	−0.0225*
	Error	14				0.2013			0.6626		
Glycine	T	1				0.5878	7.81*	0.0089*	0.2951	4.73*	0.0062*
	Error	14				0.0753			0.0624		
Arginine	T	1				1.0321	10.61**	0.0117**	0.9389	6.51*	0.0111*
	Error	14				0.0973			0.1442		
Tyrosine	T	1				0.6338	24.09**	0.0092**	0.5653	18.87**	0.0086**
	Error	14				0.0263			0.02996		
Histidine	T	1				1.4568	11.28**	−0.0152**	1.1233	5.41*	0.0122*
	Error	14				0.1291			0.2076		

[a] T = Time (weeks).
[b] Slope = grams amino acid · 16g amino acid nitrogen recovered^{-1} · week^{-1}.
* $P < 0.05$
** $P < 0.01$

positive regression coefficient indicated that the amino acid was more stable than the other amino acids. Conversely, a negative regression coefficient indicated a relatively less stable amino acid.

Storage changes were fewest in the control formulation with isoleucine decreasing only in this formulation. Glutamic acid was lost from all formulations. In the two fortified formulations, lysine decreased while phenylalanine, glycine, arginine, and tyrosine increased. This increase is probably the result of greater relative stability. Histidine decreased in soy-methionine but increased in casein.

Lipid autoxidation in foods generates chemical species such as hydroperoxides, carbonyls, epoxides, alcohols, and acids which are capable of reacting with proteins. Acidic and basic amino acid residues as well as those containing hydroxy and sulfur groups should be susceptible to reaction with autoxidation products. Our data however do not show any pattern of loss based on the functional groups of the amino acid residues. The only acidic amino acid lost was glutamic acid and it was lost from all formulations. Lysine and histidine, basic amino acids, were destroyed in one or both fortified formulations. None of the hydroxy or sulfur containing amino acids were destroyed.

Water-binding capacity. A previous study (Walter et al., 1978) on flakes stored under nitrogen at 23°C suggested that the water-binding capacity appeared to increase with time as indicated by an increase in viscosity of the reconstituted flakes. However, due to the nature of the data, there was some doubt as to whether the increase was real (i.e. statistically significant). The present study indicates no consistent significant changes of viscosity for either pooled data or those for individual formulations. Although the storage conditions of the two studies were not identical, we feel that our data strengthen the argument presented by Walter et al. (1978) that the water-binding capacity does not change when flakes are stored at 23°C.

REFERENCES

FAO/WHO. 1973. Energy and protein requirements. FAO Nutrition Meeting Report. Series no. 52, Rome.

Hoover, M.W. 1963. Preservation of the natural color in Processed Sweet Potato Products. 1. Flakes. Food Technol. 17: 128.

Labuza, T.P. 1972. Nutrient losses during drying and storage of dehydrated foods. CRC Critical Reviews in Food Technol. 3: 228.

Purcell, A.E. 1962. Carotenoids of Goldrush sweet potato flakes. Food Technol. 16: 99.

Purcell, A.E., Walter, W.M. Jr. and Giesbrecht, F.G. 1976. Distribution of protein within sweet potato roots. J. Agr. Food Chem. 24: 64.

Walter, W.M. Jr. and Purcell, A.E. 1974. Lipid autoxidation in precooked dehydrated sweet potato flakes stored in air. J. Agr. Food Chem. 22: 298.

Walter, W.M. Jr., Purcell, A.E. and Hoover, M.W. 1976. Changes in amyloid carbohydrates during preparation of sweet potato flakes. J. Food Sci. 41: 1374.

Walter, W.M. Jr., Purcell, A.E., Hoover, M.W. and White, A.G. 1978. Preparation and storage of sweet potato flakes fortified with plant protein concentrates and isolates. J. Food Sci. In press.

Ms received 10/22/77; revised 1/20/78; accepted 1/30/78.

Paper No. 5428 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh.

We thank Dr. Francis G. Giesbrecht of the Dept. of Statistics at North Carolina State University for his guidance and advice concerning the statistical analyses used in this study.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina State University, nor does it imply approval to the exclusion of other products that may be suitable.

HIGH PRESSURE LIQUID CHROMATOGRAPHIC ANALYSIS OF TYRAMINE, PHENYLETHYLAMINE AND TRYPTAMINE IN SAUSAGE, CHEESE AND CHOCOLATE

P. E. KOEHLER and R. R. EITENMILLER

ABSTRACT

High pressure liquid chromatography in conjunction with paired-ion chromatography provided a rapid, effective, sensitive tool for studying biogenic amines in food products. In a survey of 61 cheeses, 13 sausages and 10 chocolate samples for tyramine, phenylethylamine and tryptamine, tyramine was found to be most prevalent and in generally higher concentrations. Tyramine was present in 90% of the cheese and 85% of the sausage samples. Phenylethylamine was found in 38% of cheese and sausage samples while tryptamine occurred in only 18% of the cheeses and 38% of the sausages. None of the amines was detected in the chocolate samples.

INTRODUCTION

BIOLOGICALLY ACTIVE AMINES (biogenic amines) can be formed as a consequence of the metabolic process of animals, plants or microorganisms. Tyramine, tryptamine and phenylamine are included in the pressor amine group which are vasoactive amines that cause a rise in blood pressure. These amines arise primarily as a result of the enzymatic decarboxylation of the corresponding amino acids, tyrosine, tryptophan and phenylalanine. The enzyme, monoamine oxidase, oxidatively deaminates these amines and plays a major role in their degradation in man. Drugs known as monoamine oxidase inhibitors (MAOI) have been commonly prescribed for the treatment of mental depression and there have been numerous reports of hypertension crises of patients taking MAOI drugs (Ogilvie, 1955; Clark, 1961; Blackwell, 1963; Davies, 1963).

Although biologically active amines occur naturally in many foods (Lovenberg, 1973), they seldom present any hazard unless huge quantities are consumed or the normal routes of catabolism are inhibited or are genetically deficient. Tyramine and phenylethylamine have also been implicated in the onset of migraine headaches (Anonymous, 1968, 1974). There is, therefore, a need for reliable techniques for the determination of these amines in food products.

EXPERIMENTAL

Source of samples

All food samples were purchased from retail grocery stores in Athens, GA. The sausage samples were of types shown previously to contain detectable amounts of amines (Rice et al., 1975). The selection of cheese samples represented the different types commercially available in the area. The chocolate samples were representative of unsweetened, sweetened and milk chocolate types.

Extraction of amines

A modification of a procedure developed by Creveling and Daley (1971) was used to extract the amines. Ten grams of the sample were homogenized with 100 ml of 0.1N perchloric acid in 1 pint Mason jars using a Sorvall Omni-mixer (Du Pont Instruments, Newtown, CT). The homogenate was centrifuged 20 min at 10,000 × G at 4°C and the fat layer was removed. The supernatant was adjusted to pH 7.0 with KOH (approx 1.5g), left overnight at 4°C to allow precipitation, filtered through Whatman #40 filter paper, degassed under vacuum for 15 min, adjusted to pH 6.5 with 1N HCl and applied to a weak cation exchange column.

Purification of amines

Following extraction, all extracts were purified and concentrated by passing them through a (9 mm diam × 15 cm) weak cation exchange column (Bio-Rex 70, Bio-Rad Laboratories, Richmond, CA). To prepare the resin, it was washed with water, 1N HCl, and 1N NaOH, adjusted to pH 6.5 with 2N HCl and washed with 0.05M sodium phosphate buffer pH 6.5. The extract was applied to the column and 50 ml distilled water were added to wash the column. The absorbed bases were then eluted with 30 ml of 1N HCl.

Percent recovery of amines

Duplicate 10-g samples of cheddar cheese, summer sausage and unsweetened chocolate were spiked with sufficient quantities of standard solutions of tyramine, phenylethylamine and tryptamine in 0.1N HCl to give 0.4 mg/g of each amine in the samples. After extraction and purification, the percent recovery was determined by comparing the high pressure liquid chromatograph (HPLC) peak areas of each purified sample extract to the areas of the same standard solutions and calculating the recovery.

High pressure liquid chromatography

All extracts, eluted from the ion-exchange column in 30 ml of 1N HCl, were adjusted to pH 6.5 with 4N NaOH and the volume made to exactly 35 ml with distilled water prior to injection of 10–25 µl volumes into the HPLC column. A nonpolar µBondapak-C^{18} column (Waters Associates, Milford, MA) consisting of octadecylsilane coated on a solid support was used at ambient temperatures at a flow rate of 1.2 ml/min. A mobile phase of methanol-water (35:65) containing 0.005M 1-heptane sulfonic acid as a counter-ion was prepared by adding 20 ml of PIC-reagent B-7 (Water Associates) to 1000 ml of methanol:water (35:65). The amines were detected and quantitated by monitoring UV absorbance at 254 nm.

TLC confirmation of amines

The biogenic amine peaks collected from the HPLC column were evaporated with nitrogen, redissolved in 1N HCl and spotted on silica gel TLC plates. The plates were developed in a chloroform-methanol-conc ammonium hydroxide (12:7:1) solvent system. After development, the plates were air dried, sprayed with ninhydrin solution (0.3g ninhydrin, 100 ml butanol, 3 ml glacial acetic acid), and heated at 110°C for 10 min for visualization. The R_f values of the collected amine peaks were calculated and compared to the R_f values of tyramine, phenylethylamine and tryptamine standards to confirm the HPLC separations.

RESULTS & DISCUSSION

AFTER EVALUATING several procedures for efficiency in extracting biogenic amines based on recoveries of 0.1 µC of phenylethylamine-^{14}C blended into 10g of cheese, a modification of the procedure of Creveling and Daley (1971) was selected for extraction of the amines. This procedure extracted 89% of the added phenylethylamine.

Based on the use of this extraction procedure and the ion-exchange column clean-up procedure described, the overall

Authors Koehler and Eitenmiller are with the Food Science Dept., University of Georgia, Athens, GA 30602.

0022-1147/78/0004-1245$02.25/0
© 1978 Institute of Food Technologists

recoveries of tyramine, phenylethylamine and tryptamine from cheese, sausage and chocolate samples were determined. As seen in Table 1, recoveries of all three amines were greatest from cheese, but only slightly less from sausage samples. Recovery from chocolate samples was much lower, averaging only 50%. The percent recovery values in Table 1 were used as a correction factor in estimating the quantities of biogenic amines in the survey of cheese, sausage and chocolate.

HPLC has made major advances through the introduction of the technology to pack columns efficiently with extremely small particle supports. The resolving power of the new HPLC columns is usually greater than TLC and the separation times are often less. No chemical reactions are necessary to form volatile derivatives as may be necessary for gas chromatography. Since amines are basic and ionizable, ion-pair chromatography in conjunction with HPLC is feasible for biogenic amine separations. The association of the positively charged counter-ion (in this case, heptane sulfonic acid) results in a lipophilic ion-pair that can be separated by nonpolar (reversed phase) chromatography.

Mixtures of tyramine, phenylethylamine and tryptamine were separated by HPLC using a nonpolar octadecylsilane coated solid support with heptane-sulfonic acid counter-ion in the solvent. Figure 1 shows the separation of the three amines in an actual cheese extract.

Table 1—Percent recovery[a] of tyramine, phenylethylamine and tryptamine from cheese, sausage and chocolate

	Tyramine	Phenylethylamine	Tryptamine
Cheese	80 ± 9	73 ± 6	66 ± 6
Sausage	75 ± 8	71 ± 9	52 ± 6
Chocolate	48 ± 9	57 ± 8	44 ± 10

[a] Indicates total recovery including losses during both extraction and clean-up procedures expressed as mean of 3 samples ±1 standard deviation

Table 2—Resolution of tyramine, phenylethylamine and tryptamine by HPLC as a function of mobile phase ratio

	Resolution			
	MeOH:47[a] Water:53	MeOH:40[a] Water:60	MeOH:35[a] Water:65	MeOH:35[b] Water:65
TYR-PEA	1.00	1.75	3.00	2.25
PEA-TRY	0.17	0.42	0.93	0.92

[a] Represents flow rate of 1.0 ml/min
[b] Represents flow rate of 1.2 ml/min

Table 3—Tyramine content of cheese

Cheese		Tyramine content (μg/g cheese) Range[a]	Average
Extra sharp cheddar	(2)[b]	14–605	310 (100)[c]
Sharp cheddar	(7)	57–1118	353 (100)
Medium cheddar	(6)	28–490	114 (100)
Mild cheddar	(7)	ND–187	65 (86)
Processed	(9)	11–72	34 (100)
Ripened soft	(5)	ND–135	36 (80)
Semi-soft	(15)	ND–1320	401 (87)
Swiss	(5)	5–276	84 (100)
Very hard grating	(5)	ND–101	22 (60)

[a] ND: not detectable
[b] Number of samples examined
[c] Percent positive

The effectiveness of the separation (resolution) was calculated using the method suggested by Willard et al., 1974. Table 2 indicates the resolution obtainable with various solvent mixtures. The separation improved as the methanol:water ratio was reduced until it maximized at a ratio of about 35:65. The mobile phase ratio of methanol:water 35:65 at a flow rate of 1 ml/min gave a calculated resolution of 0.93 for the closest pair of amines—phenylethylamine and tryptamine. By the method used, a resolution, R, of 1.5 represents baseline resolution and R = 1.0 indicates that resolution is almost 94% complete or a 3% overlap of peaks. Since the resolution was virtually unchanged when the flow rate was increased to 1.2 ml/min but the time for the assay was reduced from 26 min to 15 min, the faster pumping rate was utilized for the remainder of the study.

Using the procedures developed, tyramine, phenylethylamine and tryptamine were determined in 61 cheese, 13 sausage and 10 chocolate samples. The 61 cheese samples were grouped into 9 categories. Tyramine was most prevalent, being found in 90% of the samples. Table 3 indicates the range of tyramine concentrations in the cheese samples and the average tyramine content of the tyramine-positive samples. The highest tyramine concentration (1320 μg/g) was found in a Port Salut cheese. The average level of tyramine-positive samples was 216 μg/g. For this sample and 3 others, including 2 sausage samples, the identities of the quantitated biogenic amine peaks from HPLC were confirmed by collecting each peak and spotting on silica gel TLC plates. After visualizaztion by reaction with ninhydrin spray, the R_f values of the suspected amines were compared to R_f values of known tyramine, phenylethylamine and tryptamine to confirm their identity.

Thirty-eight percent of all cheese sampled contained detectable levels of phenylethylamine. The average level of phenylethylamine (Table 4) was 102 μg/g. The highest phenylethylamine concentration (435 μg/g) was found in a natural brick cheese. The phenylethylamine levels are much lower than the tyramine levels and this fact coupled with the

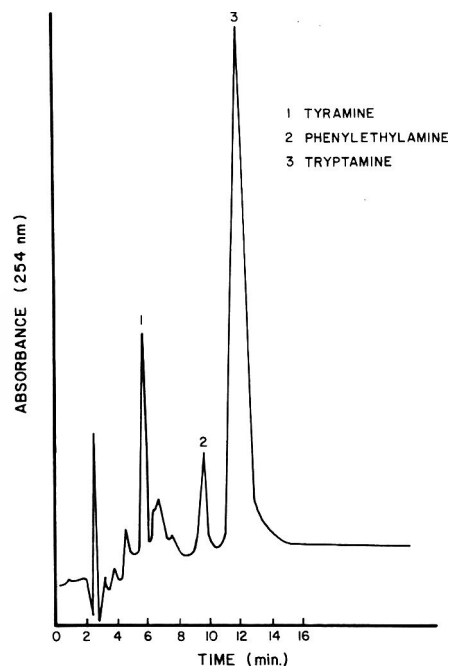


Fig. 1—HPLC separation of tyramine, phenylethylamine and tryptamine in typical cheese extract using octadecylsilane coated reversed phase column with heptane sulfonic acid counter-ion in the methanol-water (35:65) solvent.

Table 4—Phenylethylamine content of cheese

Cheese		Phenylethylamine content (μg/g cheese) Range[a]	Average
Extra sharp cheddar	(2)[b]	ND–35	17 (50)[c]
Sharp cheddar	(7)	ND	ND (0)
Medium cheddar	(6)	ND–140	59 (33)
Mild cheddar	(7)	ND–303	12 (43)
Processed	(9)	ND–155	45 (44)
Ripened soft	(5)	ND	ND (0)
Semi-soft	(15)	ND–435	102 (73)
Swiss	(5)	ND–78	16 (60)
Very hard grating	(5)	ND	ND (0)

[a] ND: not detectable
[b] Number of samples examined
[c] Percent positive

Table 5—Tryptamine content of cheese

Cheese		Tryptamine content (μg/g cheese) Range[a]	Average
Extra sharp cheddar	(2)[b]	ND	ND (0)[c]
Sharp cheddar	(7)	ND–0.04	0.02 (14)
Medium cheddar	(6)	ND–2	0.4 (17)
Mild cheddar	(7)	ND	ND (0)
Processed	(9)	ND–1	0.2 (11)
Ripened soft	(5)	ND–9	2 (20)
Semi-soft	(15)	ND–108	7 (40)
Swiss	(5)	ND	ND (0)
Very hard grating	(5)	ND–9	2 (20)

[a] ND: not detectable
[b] Number of samples examined
[c] Percent positive

fact that phenylethylamine has less pharmacological action (Asatoor et al., 1963) makes phenylethylamine less significant as far as hypertensive crises is concerned. However, its suspected role in migraine headache (Sandler et al., 1974) remains a less explored possibility. As Table 5 shows, the tryptamine levels in cheese were even lower. The average level of the positive samples was 20 μg/g and the highest level was 108 μg/g in a French Samsoe cheese. Only 18% of the cheese samples surveyed contained detectable amounts of tryptamine.

Within the cheddar cheese group, the average tyramine levels were highest in the sharp cheddar category (354 μg/g) and lowest in the mild group (65 μg/g). The concentrations of tyramine determined by HPLC in this study agree fairly well with other reports. Voigt et al. (1974), using a fluorescent derivative-TLC method, reported a tyramine value of 270 μg/g for extra-sharp cheddar while 310 μg/g were found in this study. In the cheddar class, the highest phenylethylamine levels were found in the medium class (59 μg/g) while none was found in the sharp class. Apparently, there is no correlation between aging and phenylethylamine levels.

Table 6 indicates the tyramine, phenylethylamine and tryptamine content of 13 samples of commercial sausages. Again, tyramine occurs more often in sausages and the tyramine levels are generally higher than either phenylethylamine or tryptamine. Eighty-five percent of all sausages surveyed contained tyramine. The average level was 133 μg/g. The highest tyramine concentration (374 μg/g) was in Danish smoked salami. This is equivalent to nearly 0.2g per pound. Phenylethylamine was not found in as many sausages. The average level in positive samples was 184 μg/g. The average level of tryptamine in sausages was less than 29 μg/g and it was present in only 38% of the samples. None of the 10 chocolate samples showed any detectable levels of tyramine, phenylamine or tryptamine. The poor recovery of amines found using spiked chocolate samples (Table 1) could have contributed to the inability to detect them in the samples surveyed.

When one considers that Sandler et al. (1974) reported that 3 mg of phenylethylamine caused migraine headaches in susceptible individuals, cheese and sausage samples containing the higher levels of phenylethylamine might provide sufficient phenylethylamine to trigger migraine headaches in susceptible individuals during consumption of normal amounts of these products. Since Blackwell and Mabbitt (1965) reported that 6 mg of tyramine taken orally triggered hypertensive attacks in patients on MAOI, it can be seen that several cheeses sampled could contribute to hypertensive crises. It would be difficult to speculate on the effect of tyramine, in the amounts found, on migraine headache since Hanington (1967), in his study, used quite large doses of tyramine to induce migraine episodes in susceptible patients. Tryptamine would not be expected to pose any problem if normal amounts of cheese or sausage are consumed.

Table 6—Tyramine, phenylethylamine and tryptamine content of commercial sauseges

Sausage	Amine content (μg/g sausage) TYR[a]	PEA[a]	TRY[a]
1. Pepperoni	193	55	31
2. Hard salami	77	ND	ND
3. Smoked beef sausage	62	ND	ND
4. Smoked salami	263	68	ND
5. Lebanon bologna	54	ND	23
6. Lebanon bologna	63	ND	ND
7. Cotto salami	103	696	ND
8. Cooked salami	46	57	3
9. Danish smoked salami	374	48	66
10. Genoa	215	ND	18
11. Hard salami	10	ND	ND
12. Party salami	ND	ND	ND
13. Thuringer	ND	ND	ND

[a] ND: not detectable

REFERENCES

Anonymous. 1968. Headache, tyramine, serotonin, and migraine. Nutr. Rev. 26: 40.
Anonymous. 1974. Chocolate, cheese and migraines. Science News 106(6): 85.
Asatoor, A.M., Levi, A.J. and Milne, M.D. 1963. Tranylcypromine and cheese. Lancet ii: 733.
Blackwell, B. 1963. Tranylcypromine. Lancet i: 167.
Blackwell, B. and Mabbitt, L.A. 1965. Tyramine in cheese related to hypertension crises after monoamine-oxidase inhibition. Lancet i: 938.
Clark, J.A. 1961. Side effects of tranylcypromine. Lancet i: 618.
Creveling, C.R. and Daley, J.W. 1971. The use of dansyl derivatives for the identification and quantitation of amines. In "Methods in Entymology," Vol. 17b, p. 846. Academic Press, New York.
Davies, E.B. 1963. Tranylcypromine and cheese. Lancet ii: 691.
Hanington, E. 1967. Preliminary report on tyramine headache. Br. Med. J. 2: 550.
Lovenberg, W. 1973. Some vaso- and psychoactive substances in food: amines, stimulants, depressants, and hallucinogens. In "Toxicants Occurring Naturally in Foods," p. 170. National Academy of sciences, Washington, D.C.
Ogilvie, C. 1955. Treatment of pulmonary tuberculosis with iproniazid and isoniazid. Q.J. Med. 24: 175.
Rice, S.R., Eitenmiller, R.R. and Koehler, P.E. 1975. Histamine and tyramine content of meat products. J. Milk & Food Technol. 38: 256.
Sandler, M., Youdin, M.B.H. and Hanington, E. 1974. A phenylethylamine oxidizing defect in migraine. Nature (London) 250: 335.
Voigt, M., Eitenmiller, R.R., Koehler, P.E. and Hamdy, M.K. 1974. Tyramine, histamine, and tryptamine content of cheese. J. Milk & Food Technol. 37: 377.
Willard, H.H., Merritt, L.L. and Dean, J.A. 1974. "Instrumental Methods of Analysis," p. 540. D. Van Nostrand Col. New York.

Ms received 6/21/77; revised 12/4/77; accepted 12/8/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5–8, 1977.

SYNTHESIS OF SOME UNSATURATED LACTONES AND THEIR RELATIONSHIP TO DEEP-FAT FRIED FLAVOR

W. A. MAY, R. J. PETERSON and STEPHEN S. CHANG

ABSTRACT

Seven unsaturated lactones, viz., the lactones of 4-hydroxy-nonenoic acid, with the double bond at the 2 and 3 position, respectively, 4-hydroxy-octenoic acid with the double bond at the 2 and 3 position, respectively, 5-hydroxy-4-nonenoic acid, 5-hydroxy-2-octenoic acid, and 5-hydroxy-2-undecenoic acid, were synthesized. Their chemical structures were confirmed by their infrared and mass spectra. Organoleptic evaluation indicated that γ-lactones with unsaturation at the 2 or 3 positions imparted a characteristic deep-fat fried flavor to cottonseed oil when added at 2.5 ppm. Furthermore, γ-lactones with unsaturation at the 3 position could improve the flavor of margarine and snack foods.

INTRODUCTION

LACTONES have been known as volatile flavor components of dairy products for a long time.

Boldingh and Taylor (1962) isolated a number of saturated lactones and one unsaturated γ-lactone from oxidized butterfat and suggested that these compounds may contribute to the flavor of butter. Patton (1961) suggested that saturated γ-lactones played an important role in the natural flavor of milk. The Flavor and Extract Manufacturers' Association included several saturated lactones in its list of the Second FEMA Survey of Flavoring Ingredients (1975).

Recently, a number of unsaturated lactones were identified in the volatile decomposition products produced by corn oil (Krishnamurthy and Chang, 1967) and hydrogenated cottonseed oil (Reddy et al., 1968) during deep-fat frying. They were also identified by Thompson et al. (1977) in thermally oxidized trilinolein.

The present paper reports the synthesis of a number of unsaturated lactones and the organoleptic evaluation of their flavor characteristics.

EXPERIMENTAL

Synthesis of 5-hydroxy-4-nonenoic acid lactone

The synthesis of 5-hydroxy-4-nonenoic acid lactone was carried out by a modification of the aldol condensation method of Lardelli et al. (1967), used for the synthesis of saturated γ-lactones (Fig. 1).

Cyclopentanone (35.0g) was added to a 2.5% aqueous solution of KOH (300g) with cooling by cold water. To this mixture was added dropwise, a mixture of cyclopentanone (44.0g) and butanal (82.0g) with vigorous stirring at a temperature of 5–12°C. The reaction was allowed to proceed for 1 hr. The reaction mixture was neutralized to pH 7 and extracted with ethyl ether. The solvent and any unreacted cyclopentanone was removed at 82°C and 100 Torr. A 2% anhydrous oxalic acid aqueous solution (15 ml) was added to the reaction product, 2-(1-hydroxylbutyl)-cyclopentanone (I). The water of dehydration and oxalic acid were then removed at 150°C and 200 Torr. The resulting 2-butylidenecyclopentanone (II) was then purified by fractional distillation at 110–112°C and 10 Torr; II was obtained in yields of 47–55%.

Hydrogenation of II was carried out by slurrying a 5% palladium on charcoal catalyst (2.50g) with absolute ethanol (40 ml). To this mixture was added II (4 ml) and hydrogen gas was allowed to flow through the reaction mixture for 90 min at room temperature. Upon completion of the reaction, the catalyst was filtered out and the solvent removed under slight vacuum. The product, 2-butylcyclopentanone (III), was obtained in a greater than 90% yield.

III (22.2g), followed by the dropwise addition of CrO_3 (24g) in 34% sulfuric acid was added to 34% sulfuric acid (125 ml) in 30 min with constant stirring. The reaction temperature was maintained at 20–40°C with a cold water bath and the stirring was continued for 1 hr after the dropwise addition. The reaction product, 5-ketononoanoic acid (IV) was extracted with ethyl ether and the solvent removed by distillation. IV was purified by recrystallization from petroleum ether at −10°C. The yield of IV was 50–60%.

IV (14.5g) and redistilled acetic anhydride (125.0g) were refluxed for 3 hr in a dry N_2 atmosphere. Anhydrous sodium acetate (0.54g) was then added and the mixture refluxed for 3 hr more under dry N_2. The reaction mixture was filtered to remove excess sodium acetate and the acetic anhydride was then removed at 30 Torr. The residue was then dissolved in ethyl ether (225 ml) and extracted three times each with 5% aqueous sodium bicarbonate (60 ml), distilled water (60 ml), and aqueous, saturated sodium chloride solution (60 ml). The ether extract was then dried over anhydrous sodium sulfate and the ether removed under vacuum. The residue was vacuum distilled at 8–10 Torr to give the final product, 5-hydroxy-4-nonenoic acid lactone.

Synthesis of 5-hydroxy-2-octenoic acid lactone and 5-hydroxy-2-undecenoic acid lactone

The synthesis of the δ-lactones unsaturated in the 2 position of the ring was carried out by the application of the Doebner synthesis (House, 1972) for preparation of dienoic acids. The lactones were obtained from this reaction as by-products (Fig. 2). Malonic acid (30.0g) and pyridine (30g) were added to 2-hexenal (15.0g). The reaction mixture was heated with constant stirring at 128°C for 5 hr. The reaction was accompanied by evolution of a large amount of CO_2. The reaction mixture was then cooled in an ice bath and 50% sulfuric acid (30 ml) was added until the mixture was acidic to the Congo Red indicator. After storage at −10°C for 24 hr, a white precipitate (2,4-octadienoic acid) was formed which was filtered off. The filtrate was extracted with ethyl ether. The ether extract was further extracted with a 10% aqueous sodium carbonate solution to insure complete removal of the 2,4-octadienoic acid. The ether solution was dried over anhydrous sodium sulfate and the solvent removed under vacuum. The residue was then fractionally distilled at 8–10 Torr with the pyridine distilling off at 78°C and the desired product, 5-hydroxy-2-octenoic acid lactone, distilling off at 103°C.

Malonic acid (30.0g) and pyridine (30g) were added to 2-nonenal (15.0g). The reaction mixture was heated with constant stirring at 154°C for 5 hr. The isolation of the desired product, 5-hydroxy-2-undecenoic acid lactone, from the reaction mixture was carried out in the same manner as described above. Under fractional distillation, the lactone distilled off under 8–10 Torr at 120°C.

Synthesis of the 4-hydroxy-2 and 3-nonenoic acid lactones

The synthesis of the unsaturated γ-lactones consisted of two principal parts: preparation of a 4-keto-2-alkenoic acid by reacting chloral with a methyl ketone (Breusch and Keskin, 1948) and the selective hydrogenation to the 4-ketoalkanoic acid with subsequent lactonization by an enolization reaction (Fig. 3).

Anhydrous chloral (18.4g) was refluxed with 2-heptanone (14.0g) and glacial acetic acid (15.0g) for 15 hr. The reaction mixture was

Authors Peterson and Chang are with the Dept. of Food Science, Cook College, Rutgers–The State University of New Jersey, New Brunswick, NJ 08903. Author May, formerly with Rutgers Univ., is now employed by Fritzche – Dodge & Olcott, Inc. 76 Ninth Ave. New York, NY 10011

0022-1147/78/0004-1248$02.25/0
© 1978 Institute of Food Technologists

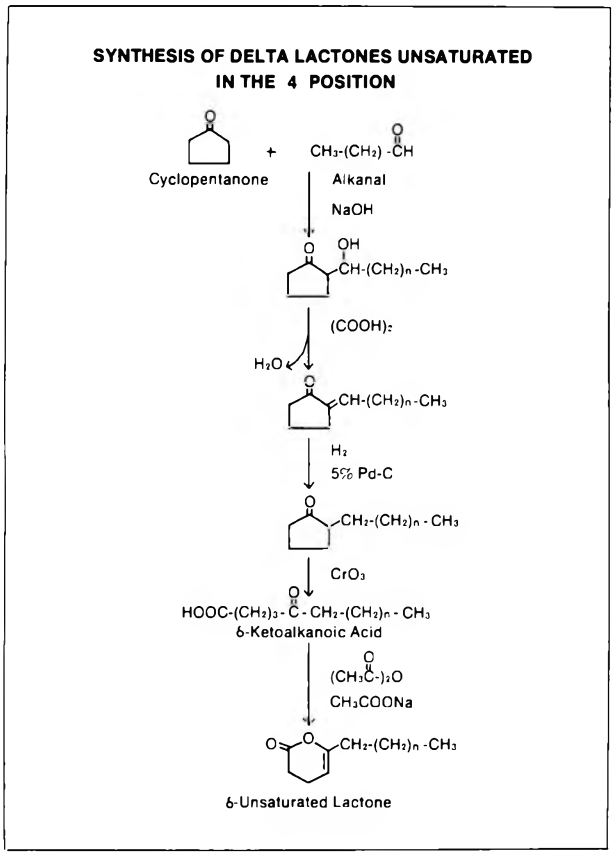


Fig. 1—Synthesis of Δ lactones unsaturated in the 4 position.

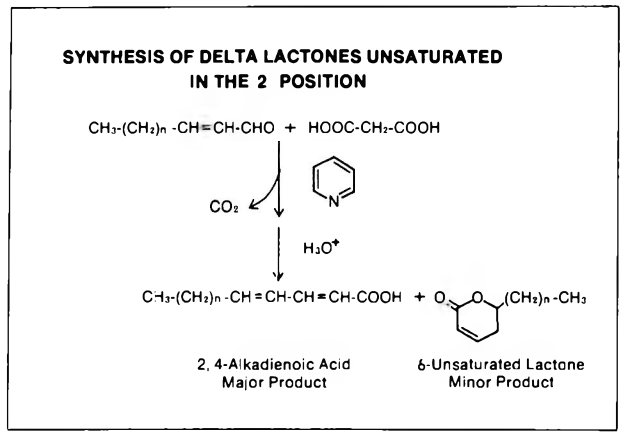


Fig. 2—Synthesis of δ lactones unsaturated in the 2 position.

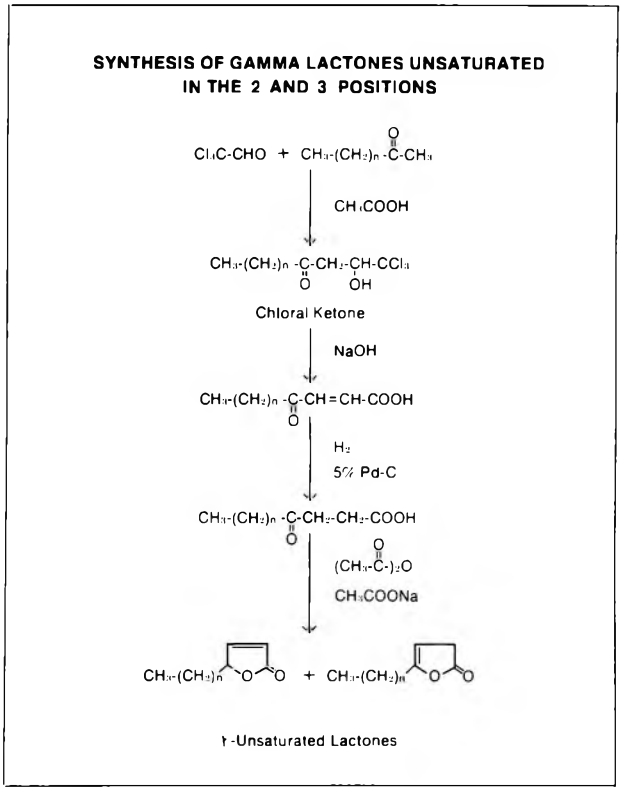


Fig. 3—Synthesis of γ lactones unsaturated in the 2 and 3 positions.

cooled to room temperature and added to an excess of 4% aqueous sodium hydroxide solution previously cooled to 0°C. This mixture was extracted with ethyl ether and then the solvent and the excess acetic acid and 2-heptanone were distilled off under vacuum. The residue was then twice distilled at 160°C and 10 Torr to yield the chloral ketone (I). I was then held at −10°C for 24 hr to promote complete crystallization. The yield of I was 55–60%.

Crystallized I (2.5g) was saponified by adding it to 4% aqueous potassium carbonate (120 ml) and refluxing for 15 min. The unsaponified I remaining, was removed by extraction with ethyl ether. The aqueous alkaline solution was filtered and acidified by addition of concentrated hydrochloric acid. The 4-keto-2-nonenoic acid (II) produced, was extracted with ethyl ether and then washed with a saturated, aqueous sodium chloride solution. The ether was removed under vacuum and II was recrystallized twice from hexane.

Hydrogenation of II was carried out by slurrying a 5% palladium on charcoal catalyst (2.50g) with absolute ethanol (40 ml). To this slurry was added II (4.0g) and hydrogen gas was allowed to flow through the reaction mixture for 90 min at room temperature. Upon completion of the reaction, the catalyst was filtered out and the solvent removed under slight vacuum. The product, 4-ketononanoic acid (III), was obtained in a greater than 90% yield.

III (7.5g) and redistilled acetic anhydride (65.0g) were refluxed for 3 hr in a dry N_2 atmosphere. Anhydrous sodium acetate (0.30g) was then added and the mixture refluxed for 3 hr more under dry N_2. The reaction mixture was filtered to remove excess sodium acetate and the acetic anhydride was then removed at 30 Torr. The residue was dissolved in ethyl ether (225 ml) and extracted three times each with 5% aqueous sodium bicarbonate (60 ml), distilled water (60 ml), and aqueous, saturated sodium chloride solution (60 ml). The ether solution was then dried over anhydrous sodium sulfate and the ether removed under vacuum. The residue was vacuum distilled at 122°C and 10 Torr to yield a mixture of 4-hydroxy-2 and 3-nonenoic acid lactones.

Synthesis of 4-hydroxy-2 and 3-octenoic acid lactones

These unsaturated γ-lactones were synthesized in the same manner as described above except 2-hexanone was used as the starting ketone. In the final vacuum distillation, the mixture of unsaturated γ-lactones distilled at 115–116°C and 10 Torr.

Purification of the synthesized unsaturated lactones

For all synthesized unsaturated lactones, the fraction collected from the final vacuum distillation step was gas chromatographed with an Aerograph A-90P using a 10 ft × ¼ in. i.d. aluminum column packed with 15% SE-30 on 70/80 mesh Anakrom ABS. An initial isothermal holding time of 2 min at 50°C was followed by a nonlinear temperature program to 200°C where the temperature was held. The He flow rate was 80 ml/min. A series of 100 μl injections were made for each lactone fraction and all major and minor peaks were accumulatively collected in "hairpin" traps (Thompson et al., 1977).

To insure the purity of the synthesized unsaturated lactones, each of them, collected as described above, was rechromatographed with an Aerograph 1520 using a 20 ft × 1/8 in. i.d. stainless steel column packed with 10% DEGS on 60/70 mesh Anakrom ABS. The single peak from the rechromatography was collected in a "hairpin" trap for IR and mass spectral analysis.

—Text continued on page 1250

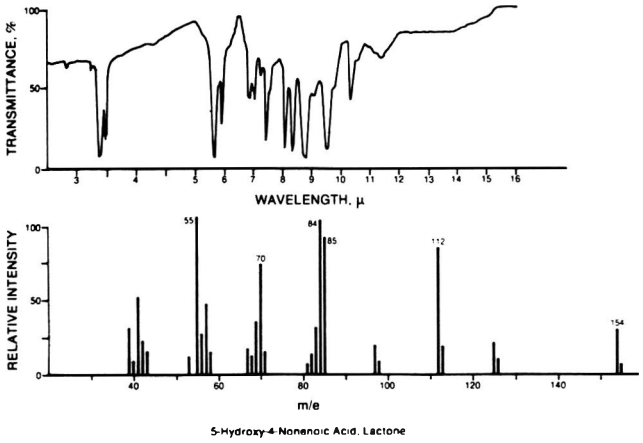


Fig. 4—Infrared and mass spectra of 5-hydroxy-4-nonenoic acid, lactone.

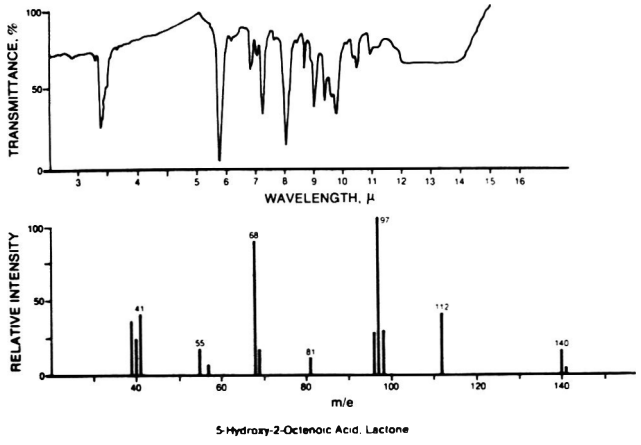


Fig. 5—Infrared and mass spectra of 5-hydroxy-2-octenoic acid, lactone.

Organoleptic evaluation of the unsaturated lactones

Prior to studying the organoleptic properties of the unsaturated lactones, optimum levels of addition of the lactones to a bland cottonseed oil were established by a five member expert panel. The optimum addition levels were based on testing only for a well balanced flavor and not on a specific type of flavor contribution. The expert panel indicated that the unsaturated δ-lactones should be tested at 1.0 ppm and the unsaturated γ-lactones at 2.5 ppm.

A triangle test was used to determine whether a panel, not specifically trained to evaluate fats and oils, could distinguish between a bland, untreated cottonseed oil and a cottonseed oil which had a characteristic deep-fat fried flavor. In order to produce the characteristic deep-fat fried flavor, cottonballs, to each of which 1.5 ml of distilled water were added, were fried in cottonseed oil at 185°C for 2 min. This frying was repeated five times at 30 min intervals. At that time, the oil had acquired a deep-fat fried flavor, according to an expert panel. The triangle test was conducted by presenting each panelist with three samples maintained at approximately 60°C, two of which were identical. The panelists were able to identify the difference between samples at the 0.01 level.

Once it was established that the panel could recognize the characteristic flavor of a deep-fat fried oil, the unsaturated lactones were evaluated for possible contribution to this flavor. A paired comparison test was used for this purpose in which a control (a cottonseed oil with the characteristic deep-fat fried flavor) and a test sample (a bland cottonseed oil with added unsaturated lactones) were submitted to each panelist. The panelists were asked to taste the samples and indicate the degree of flavor difference between the test sample and the control and describe the test sample's flavor.

Since a number of panelists described the flavor of the bland cottonseed oil plus certain added unsaturated lactones as buttery, the contribution of such compounds to the flavor of other food products was evaluated.

A number of unsaturated lactones at 2.5 ppm were added to Chiffon margarine (Anderson Clayton Foods, Dallas, Texas), obtained from a local store. The lactones were thoroughly whipped into the margarine to insure uniform distribution and the control sample of margarine was also thoroughly whipped to insure a uniform texture of both samples. A paired comparison test was then conducted. The panelists were asked to indicate the degree of flavor acceptability of the test sample in terms of being better than, poorer than, or comparable to the control margarine.

The contribution of certain unsaturated lactones to the flavor of a commercial snack product was also evaluated. The product chosen was Chipsters (Nabisco Inc., Fairlawn, NJ) made by the extruding and baking of dehydrated potatoes and modified starch. The test sample was prepared by painting onto the Chipsters a solution of 2.5 ppm of 4-hydroxy-3-nonenoic acid lactone in bland cottonseed oil. To another batch of Chipsters, bland cottonseed oil with no additive was painted on the product as the control. The samples were then presented to the panel in a paired comparison test. The panelists were asked to indicate the degree of flavor difference between samples and to indicate the flavor acceptability of the test sample in terms of being better than, poorer than, or comparable to the control sample.

RESULTS & DISCUSSIONS

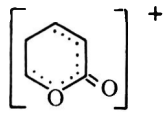
Confirmation of the structures of the synthesized unsaturated lactones

Seven unsaturated lactones were successfully synthesized, viz.

5-Hydroxy-4-nonenoic acid, lactone
5-Hydroxy-2-octenoic acid, lactone
5-Hydroxy-2-undecenoic acid, lactone
4-Hydroxy-2-nonenoic acid, lactone
4-Hydroxy-3-nonenoic acid, lactone
4-Hydroxy-2-octenoic acid, lactone
4-Hydroxy-3-octenoic acid, lactone

The IR and mass spectra of the synthesized 5-hydroxy-4-nonenoic acid lactone are shown in Figure 4. The absorption band at 5.67μ is due to the carbonyl group within the ring and adjacent to the unsaturated ether type linkage (Conley, 1972). The absorption band at 5.90μ is due to the double bond of the latter grouping. The C–O–C stretching bands are apparent in the 8.0–10.0μ region. The mass spectrum shows a molecular ion at m/e 154 and a base peak at m/e 55. The large peak at m/e 112 is due to the loss of 42 mass units from the molecular ion, necessitating a hydrogen rearrangement during fragmentation of the molecular ion. Other major fragment ions occur at m/e 85, 84 and 70.

The IR and mass spectra of the synthesized 5-hydroxy-2-octenoic acid lactone are given in Figure 5. The absorption at 5.78μ is due to the conjugated, unsaturated carbonyl group within the ring (Conley, 1972). The relatively weak absorption at 6.16μ is due to the conjugated *cis* double bond of the ring. The strong series of bands between 8.0 and 10.0μ is, at least in part, due to the C–O–C stretching vibrations. The mass spectrum shows a molecular ion at m/e 140 and the base peak at m/e 97. The latter peak is probably due to cleavage of the alkyl side chain with charge retention on the resonance stabilized ring fragment.



Other major fragment ions occur at m/e 69, 68, 41 and 39. The mass spectrum of this reaction product agrees very well with the published mass spectrum of 5-hydroxy-2-octenoic acid lactone (Urbach et al., 1972).

The infrared and mass spectra of the synthesized 4-hydroxy-2-nonenoic acid lactone are shown in Figure 6. The γ-lactones unsaturated in the 2,3 position are characterized by a strong carbonyl absorption at 5.71μ and a weak double bond

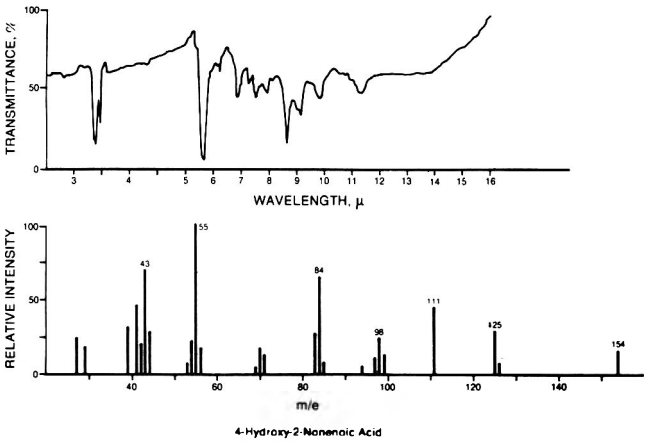


Fig. 6—Infrared and mass spectra of 4-hydroxy-2-nonenoic acid.

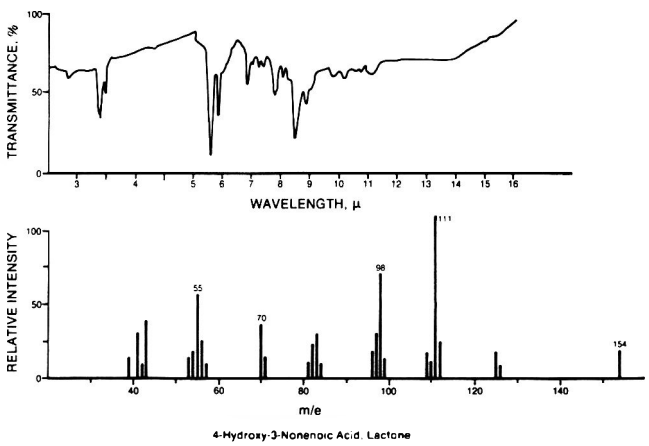


Fig. 7—Infrared and mass spectra of 4-hydroxy-3-nonenoic acid, lactone.

absorption at 6.20μ as shown in this figure (Nakanishi, 1962). Again, strong absorptions due to C—O—C stretching are apparent between 8.0 and 10.0μ. A molecular ion at m/e 154 and a base peak at m/e 55 are indicated. The peaks at m/e 126 (M-28) and 125 (M-29) may be due, at least in part, to loss of CO and COH, respectively, from the molecular ion, analogous to the situation in 6-membered ring unsaturated lactones (Urbach et al., 1972). The peaks at m/e 84 and 83 are most likely due to cleavage of the alkyl side chain with or without hydrogen rearrangement, respectively, with charge retention on the lactone ring. Other major peaks occur at m/e 111, 98, 43, 41 and 39.

The infrared and mass spectra of the synthesized 4-hydroxy-3-nonenoic acid lactone are given in Figure 7. The γ-lactones unsaturated in the 3,4 position are characterized by a strong carbonyl absorption at 5.56μ with a relatively strong absorption doublet between 5.80 and 6.00μ (Conley, 1972). The longer wavelength peak of this doublet is probably due to the double bond of the ring. A series of relatively strong bands, at least partly due to C—O—C stretching vibrations, is apparent between 8.0 and 10.0μ. The mass spectrum shows a molecular ion at m/e 154 and a base peak at m/e 111. The base peak at m/e 111 is probably due to cleavage of part of the alkyl side chain with charge retention on the ring fragment. The large peak at m/e 98 (M-56) indicates that a hydrogen rearrangement takes place during fragmentation leading to this ion. Other major fragment ions occur at m/e 97, 83, 70, 55 and 43.

Organoleptic evaluation of the aroma and flavor of unsaturated lactones

The results of the paired comparison test of deep-fat fried cottonseed oil vs bland cottonseed oil plus unsaturated lactone are given in Table 1. The statistical analysis of the pooled panel's responses was performed using the tables of probabilities given in Kramer and Twigg (1962). In evaluating the results of the paired comparisons, the responses to degree of flavor difference between samples of none, slight and moderate were considered to be a positive contribution to deep-fat fried flavor, whereas responses of large and extreme were considered to be negative.

The results indicate that 5-hydroxy-2-octenoic acid lactone

Table 1—Organoleptic evaluation of the relationship of unsaturated lactones with deep fat fried flavor unsaturated lactones with deep fat fried flavor

Product tested	Degree of flavor difference-response #					Level of statistical significance
	None	Slight	Moderate	Large	Extreme	
Deep fat fried oil (B,C) vs bland cottonseed oil (Control-A)[a]	3	–	–	–	11	99
Deep fat fried oil (Control) vs cottonseed oil + 1.0 ppm 5-hydroxy-2-octenoic acid, lactone	–	2	3	1	3	–
Deep fat fried oil (Control) vs cottonseed oil + 2.5 ppm 4-hydroxy-2-nonenoic acid, lactone	–	4	6	–	1	95
Deep fat fried oil (Control) vs cottonseed oil + 2.5 ppm 4-hydroxy-3-nonenoic acid, lactone	–	8	2	–	1	95

[a] Triangle test of samples A, B and C. Others are paired comparison tests.

Table 2—Organoleptic evaluation of the effect of unsaturated lactones upon the flavor of margarine and a snack food

Product tested[a]	Degree of flavor difference-response #					Product preference response number			Level of statistical significance
	None	Slight	Moderate	Large	Extreme	Poorer	Comparable	Better	
Margarine (Control) vs margarine + 2.5 ppm 4-hydroxy-3-nonenoic acid, lactone	1	5	6	2	–	–	3	11	95
Margarine (Control) vs margarine + 2.5 ppm 4-hydroxy-2-nonenoic acid, lactone	–	7	3	5	–	7	7	1	–
Margarine (Control) vs margarine + 2.5 ppm 4-hydroxy-3-octenoic acid, lactone	–	5	9	2	–	–	3	13	95
Margarine (Control) vs margarine + 2.5 ppm 4-hydroxy-2-octenoic acid, lactone	–	2	5	6	–	6	2	5	–
Chipsters + bland cottonseed oil vs chipsters + cottonseed oil w/2.5 ppm 4-hydroxy-3-nonenoic acid, lactone	1	4	5	3	–	1	1	11	95

did not significantly contribute to the characteristic deep-fat fried flavor of oil at 1.0 ppm. However, the γ-lactones with unsaturation at the 2 or 3 position, viz 4-hydroxy-2-nonenic acid, lactone and 4-hydroxy-3-nonenoic acid, lactone, did impart a characteristic deep-fat fried flavor to cottonseed oil when added at 2.5 ppm (Table 1). The responses of the panel to the description of the cottonseed oil plus 4-hydroxy-2-nonenoic acid lactone included a number indicating nutty, fried fat notes plus a few indicating a butter-like note.

This observation helps explain some previously published results. 4-Hydroxy-2-nonenoic acid, lactone was found by Krishnamurthy and Chang (1967) in corn oil after it was used in simulated deep-fat frying. However, such lactones were not found by Reddy et al. (1968) in hydrogenated cottonseed oil after it was used in simulated deep-fat frying under identical conditions. Since such lactones are now shown to play an important role in deep-fat fried flavor, they might be the reason why Blumenthal et al. (1976) found that vegetable oils such as corn oil had a more desirable flavor than hydrogenated fat such as hydrogenated soybean oil after both were subject to simulated deep-fat frying under identical conditions.

More recently, Thompson et al. (1977) isolated, fractionated and identified the volatile flavor compounds produced from trilinolein, and May et al. (1977) identified those from triolein when each was subjected to simulated deep-fat frying, separately. They found more unsaturated lactones in the decomposition products of trilinolein, but less in those of triolein; both in number and in amount. This may indicate that some linoleic acid is necessary in frying fat if the characteristic deep-fat fried flavor is desired.

Organoleptic evaluation also showed that 4-hydroxy-2-nonenoic acid, lactone, and 4-hydroxy-2-octenoic acid, lactone had an adverse effect upon the flavor of margarine (Table 2). However, addition of 2.5 ppm of 4-hydroxy-3-nonenoic acid and 4-hydroxy-3-octenoic acid improved the flavor of margarine. The former could also improve the flavor of a snack food.

REFERENCES

Blumenthal, M.M., Trout, J.R. and Chang, S.S. 1976. Correlation of gas chromatographic profiles and organoleptic scores of different fats and oils after simulated deep-fat frying. J. Amer. Oil Chem. Soc. 53: 496.

Boldingh, J. and Taylor, R.J. 1962. Trace constituents of butterfat. Nature 194: 909.

Breusch, F.L. and Keskin, H. 1948. Neure Feitsauren und Fette. Arch. Biochem. 18: 305.

Conley, R.T. 1972. "Infrared Spectroscopy," 2nd ed. Allyn and Bacon, Inc., Boston, MA.

FEMA, 1975. "Results of Second FEMA Survey of Flavoring Ingredients." Flavor and Extract Manufacturers' Association of the United States.

House, H.O. 1972. "Modern Synthetic Reactions," 2nd ed. W.A. Benjamin, Inc., Menlo Park, CA.

Kramer, A. and Twigg, B.A. 1962. "Fundamentals of Quality Control for the Food Industry." Avi Publishing Co., Westport, CT.

Krishnamurthy, R.G. and Chang, S.S. 1967. Chemical reactions involved in the deep-fat frying of foods. 3. Identification of nonacidic volatile decomposition products of corn oil. J. Amer. Oil Chem. Soc. 44: 136.

Lardelli, G., Lamberti, V., Weller, W.T. and DeJongh, A.P. 1967. Synthesis of lactones. 1. Saturated δ- and γ-lactones. Rec. Trav. Chim., Holland 86: 481.

May, W.A., Peterson, R.J. and Chang, S.S. 1977. Chemical reactions involved in the deep-fat frying of foods. 8. Identification of the volatile decomposition products of triolein. J. Amer. Oil Chem. Soc. (Submitted for publication).

Nakanishi, K. 1962. "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, CA.

Patton, S. 1961. Gas chromatographic analysis of flavor in processed milks. J. Dairy Sci. 44: 207.

Reddy, B.R., Yasuda, K., Krishnamurthy, R.G. and Chang, S.S. 1968. Chemical reactions involved in the deep-fat frying of foods. 5. Identification of nonacidic volatile decomposition products of hydrogenated cottonseed oil. J. Amer. Oil Chem. Soc. 45: 629.

Thompson, J.A., May, W.A., Paulose, M.M., Peterson, R.J. and Chang, S.S. 1977. Chemical reactions involved in the deep-fat frying of foods. 7. Identification of volatile decomposition products of trilinolein. J. Amer. Oil Chem. Soc. (Submitted for publication).

Urbach, G., Start, W. and Nobuhara, A. 1972. Low resulution mass spectra of some unsaturated δ-lactones. Agr. Biol. Chem. 36: 1217.

Ms received 6/25/77; revised 11/21/77; accepted 12/1/77.

Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers—The State University of New Jersey, New Brunswick, NJ.

Supported in part by Public Health Research Grant HE-07610 from the National Heart Institute.

EFFECT OF NITROGEN SOURCE ON GROWTH AND EXOPROTEASE PRODUCTION BY Clostridium perfringens

H. P. BLASCHEK and M. SOLBERG

ABSTRACT

16 strains of *Clostridium perfringens* were screened for their growth and exoprotease activity in a synthetic amino acid medium (R + S) and in media containing either vitamin-free casein, spray-dried whole egg or ground frankfurter as the protein source. Growth, measured by gas production, was determined manometrically under standard conditions. Quantitative enzymatic assay of sterile culture filtrates was carried out at pH 7.0 using Azocoll general proteolytic substrate. Strains grown in protein-containing media usually produced more exoprotease per unit of growth than strains grown in synthetic amino acid medium. Strains which constitutively produce high levels of exoprotease were identified.

INTRODUCTION

ONE OF MANY FACTORS that contributes to the potential use of *C. perfringens* for protein quality evaluation includes its proteolytic capacity. Previously investigated microbiological methods toward simplified protein quality evaluation use the protozoan flagellate *Tetrahymena pyriformis* (Fernell and Rosen, 1956) or the bacterium *Streptococcus zymogenes* (Ford, 1960). Both of these microbiological procedures depend on enzymic predigestion of the test samples to obtain results that are in reasonable agreement with rat assay values. The dependence on enzymic predigestion of the test material is an inherent disadvantage, since the extent of hydrolysis to which a test protein is subjected is critically important in determining the test results. A further disadvantage lies in the possible utilization by the microorganism of the added enzyme protein.

Maschmann (1937) described an enzyme in young culture filtrates of *C. perfringens (welchii)* which could attack gelatin extensively and he suggested the name "collagenase" for it. Weil et al. (1939) indicated that the exocellular proteinase of *C. perfringens* hydrolyzed gelatin, casein, and Witte peptone. Oakley et al. (1946) observed that *C. perfringens* type A filtrates contain kappa toxin, a collagenase which breaks down muscle by attacking its collagen scaffolding. This proteolytic enzyme was specific in attacking only collagen and gelatin (Bidwell and van Heyningen, 1948). Oakley et al. (1948) found that type B strains and some type D strains produce a substance that will attack Azocoll and hide powder but not muscle or collagen paper. This new substance was antigenic and was referred to as lambda toxin. It was a proteinase. These results were substantiated by Bidwell (1950) who found that lambda toxin attacked hide powder, gelatin, casein and hemoglobin but had no effect on native collagen.

The studies of Bidwell and van Heyningen (1948) and Oakley et al. (1946) suggested that all five types of *C. perfringens* produce exoproteases of one type or another. The occurrence of collagenase and proteinase in filtrates of *C. perfringens* types were summarized by Oakley and Warrack (1953).

The purpose of this study was to examine the effect of various nitrogen sources on the growth and exoprotease production by 16 strains of *C. perfringens* to obtain strains with high overall proteolytic activity and thereby avoid the need for protein predigestion of the test sample.

Authors Blaschek and Solberg are with the Dept. of Food Science, Rutgers–The State University, Cook College, New Brunswick, NJ 08903.

0022-1147/78/0004-1253$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Microorganisms

A total of 16 *C. perfringens* type A strains were used throughout the experiments. All but two of these strains were isolates either from feces of food poisoning cases or from incriminated foods. The food poisoning strains included NCTC 8237, 8797, 8238, 8679, 8235, 8232, 841, 7867, 691 and 1451. Strains 1362, S-80, S-88 and 6867 were obtained from Dr. H.E. Hall, Robert A. Taft Sanitary Engineering Center, Cincinnati, OH. The other two strains, ATCC 3624 and BP6K, are medically significant strains isolated from pathological specimens.

Maintenance of cultures

Stock cultures were carried in cooked meat medium (BBL) at 4°C. Every 3–4 wk the cultures were transferred by passing them twice through fresh fluid thioglycolate medium (Difco) incubated at 37°C for 24 hr or until vigorous foaming became apparent. Cultures were then transferred into freshly prepared cooked meat medium incubated at 37°C for 24 hr and stored at refrigerated temperature.

Preparation of inocula

One-half ml from a stock culture tube was inoculated into 20 ml of freshly prepared R + S medium (Riha and Solberg, 1971). The inoculated culture tubes (25 × 100 mm) were incubated in a water bath at 37°C for 9–10 hr. Two ml of a 10-hr culture were then transferred into 18 ml of fresh R + S medium. This cell suspension was incubated in 25 × 105 mm cuvettes (Coleman Instruments Div.) at 45°C until an absorption of 0.6–0.7 was attained using a Coleman Junior Spectrophotometer (Model 6A, Coleman Instruments, Inc., Maywood, IL) at 600 nm. The suspension was then poured into sterile stainless steel centrifuge tubes which were placed in a Servall superspeed type SS-1 centrifuge (Ivan Sorvall, Inc., Norwalk, CT) and centrifuged for approximately 5 min at 14,350 relative centrifugal force units. Following centrifugation, the supernatant was decanted and 10 ml sterile tempered (40°C) R + S base (R + S medium without amino acids) was added to the cell pellet. This was shaken vigorously on a vortex shaker until the cells no longer clumped together. This cell washing procedure was repeated two more times for each strain being tested. After the third washing, part of the cell suspension was transferred into sterile "Spectronic 20" spectrophotometer tubes. The absorbance of the washed cells was adjusted with tempered base medium to approximately 1.4 using a "Spectronic 20" spectrophotometer (Bausch and Lomb) set at 600 nm. One-half ml of this cell suspension was inoculated into 20 ml of test growth medium in a Pyrex culture tube. Inoculations were carried out in duplicate for each strain in a particular test medium. Experimental controls were inoculated with 0.5 ml of sterile tempered base medium. Plate counts to determine the inoculum levels of each strain were carried out using TSC agar (Harmon et al., 1971).

Composition of test growth media

Five test growth media were used throughout the experiments. These included the chemically defined R + S medium, three types of protein-containing media and R + S base. R + S medium was adjusted to pH 7.0 with 0.1N HCl followed by filter sterilization using a 0.22 micron micropore filter (Millipore Corp., Bedford, MA) in an O_2 free N_2 stream. The medium was stored overnight at 4°C and filtered in an O_2 free N_2 stream once again just prior to use the following morning. The protein-containing test media were prepared with spray dried whole egg, vitamin-free casein or ground frankfurter added to R + S base at a concentration of 0.26% N, which is equivalent to the amino nitrogen in R + S medium. Percent nitrogen in each of these proteins was determined using a Coleman Nitrogen Analyzer (Model 29A, Coleman Industries, Inc., Maywood, IL). Specifically, 3.2500g ground frankfurter, 0.7447g spray-dried whole egg or 0.3667g casein were added to 20 ml of R + S base. The resulting suspensions were adjusted to pH 7.0 and stirred gently to disperse the protein throughout the medium.

–Text continued on page 1254

Conditions and measurement of gas production

The inoculated culture tubes were incubated in a water bath at 45°C. Duplicate controls inoculated with 0.5 ml sterile tempered base medium were run for each medium.

The gas evolution of each strain of *C. perfringens* in a particular medium was followed manometrically. The apparatus used consisted of a Fisherbrand manometer (Fisher Scientific Co., Pittsburgh, PA) connected to a culture tube via rubber tubing and a #3 rubber stopper (Fisher Scientific Co.) (see Fig. 1). The gas produced was quantitated by observing the displacement of the manometer oil in the manometer tube. Each centimeter increment on the manometer scale was called a manometer unit. A cubic centimeter of gas was equivalent to 3.4 manometer units. For each experiment, a control culture tube was connected to a manometer to permit volume corrections resulting from minor temperature fluctuations and barometric pressure changes. Gas evolution for each strain was followed until the growth measured by gas production plotted on semi-logrithmic paper began to demonstrate that the stationary phase had been reached.

Preparation of sterile filtrates

Sterile filtrates were prepared by centrifuging stationary phase cultures. The supernatant was passed through diatomaceous earth (Celite Analytical Filter-Aid, John's-Manville Corp.) and sterilized using 0.45 micron micropore filters contained in a multiple sample mainifold (Model 1225, Millipore Corp., Bedford, MA). The pH of the filtrate was adjusted to 7.0 using 0.1N NaOH. Duplicate 5 ml filtrates were refrigerated overnight.

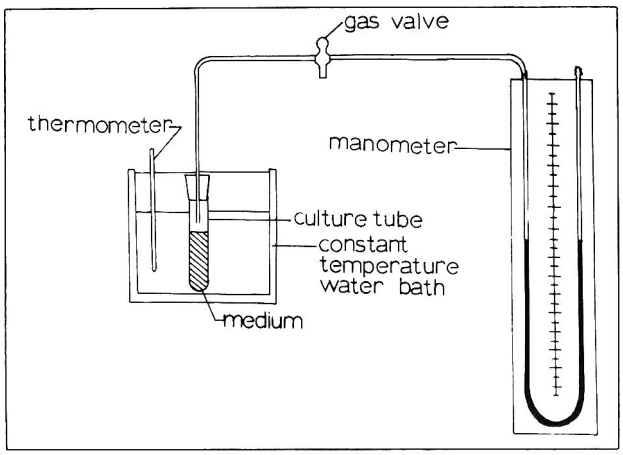


Fig. 1—Gas measurement system.

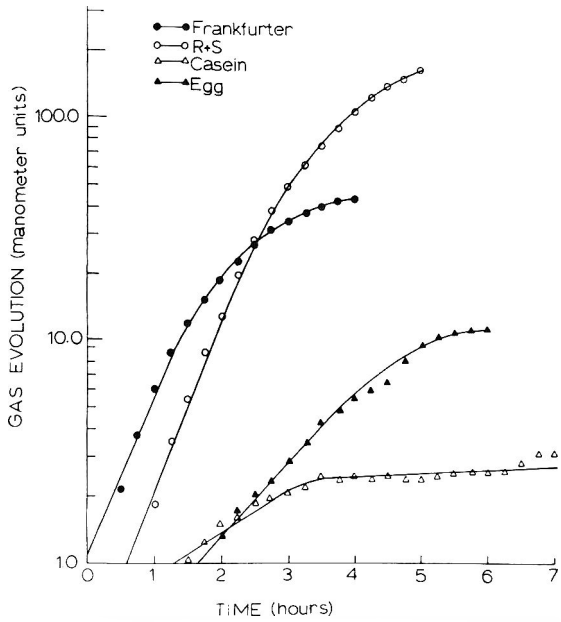


Fig. 2—Mean gas production curves for C. perfringens strain S-80 in frankfurter, R + S, whole egg and casein media.

Determination of pH optimum

The pH optimum for assay of proteolytic activity was determined by taking sterile pH adjusted R + S filtrates of strain 1451 and subjecting them to quantitative enzymic assay using Azocoll general proteolytic substrate (Cat. #19493, 50–100 mesh, Calbiochem, San Diego, CA). In this procedure, a single filtrate from *C. perfringens* strain 1451 was divided into 7 duplicate 5-ml samples. The pH of the samples was adjusted with either 0.1N NaOH or HCl to a range between pH 4.0 and 10.0. Duplicate samples at each integer value pH were tested for enzymic activity.

Procedures for enzyme assay

Azocoll (25 mg) was added to 5 ml of sterile culture filtrate. The reaction mixture was incubated in a water bath at 37°C for 4 hr. It was shaken at 15 min intervals to prevent clumping of the Azocoll and to permit uniform release of the attached dye. The digestive action of the proteolytic enzymes on Azocoll was terminated by filtering out the substrate using Whatman #541 paper. To the substrate-free filtrate was added 1 ml of 0.1 mg/ml trichloroacetic acid to precipitate the remaining protein out of solution. The filtrate was then hand centrifuged using a Boekel hand powered centrifuge. Centrifugation resulted in a clear dye containing supernatant and a protein precipitate. The supernatant was transferred via syringe into a "Spectronic 20" tube and the absorbance was measured at 520 nm using a "Spectronic 20" spectrophotometer.

Establishment of an enzyme activity conversion unit

Duplicate samples containing 5 ml of a 1.9M trypsin solution (8.0×10^{-5} mg/ml) in 0.1M phosphate buffer at pH 7.0 and 25 mg of Azocoll were incubated at 37°C. The absorbance of the reaction mixture was read at 10 min intervals for nearly 5 hr. The mixture was shaken periodically after which the Azocoll was allowed to settle to the bottom of the tube before a reading was made. This prevented possible interference of the Azocoll particles with spectrophotometric measurements.

Data analysis

Growth curves were obtained by plotting gas evolution in manometer units vs. time on semi-logrithmic paper. This was done in duplicate for each of 16 *C. perfringens* strains in four different media. Linear regression was used to determine log slope of the gas production curves during their exponential phase. Generation times were calculated from slopes using the formula G = log 2/slope. Analysis of variance in addition to Tukey's Test at the 95% level (Steel and Torrie, 1960) were utilized for analysis of dependent variables, mean exoprotease activity and mean ratio: exoprotease activity/unit gas production.

RESULTS & DISCUSSION

A TYPICAL PLOT of gas evolution as a function of time for one strain of *C. perfringens* (S-80) grown in four different media is presented in Figure 2. The term "gas evolution" is used synonymously here with growth of the organism. The validity of gas production as a measurement of *C. perfringens* growth was evaluated by comparing plate counts with gas evolution data in cooked meat medium (Berkowitz, 1977). The results as plotted on semi-logarithmic paper indicated that although the slopes were different, the adjustment phase duration was equivalent, thereby making gas evolution a feasible measurement of growth response.

As a further test of gas evolution as a measure of growth response, experiments were carried out in nonreplicating R + S base medium. Results indicated no measurable gas production during the four hour test period, further reinforcing the argument that gas production is a valid growth response measurement.

As seen in Figure 2, growth in ground frankfurter medium demonstrated the shortest adjustment phase and often the steepest slope representing exponential growth. There was more total gas evolution by most strains in R + S, followed in decreasing order by ground frankfurter, whole egg and casein, respectively. The means of duplicate total gas production volumes and the slopes of the gas production curves for each strain in each medium can be seen in Table 1. A maximum of 5% variation occurred between replicate determinations of both exponential slope and total volume of gas produced. In terms of total gas production, the media exerted significant

Table 1—Exponential slope and total volume of gas produced by strains of Clostridium perfringens in various culture media during 45°C incubation

	Medium							
	R + S		Casein		Egg		Frankfurter	
Strain	Slope	(cc)	Slope	(cc)	Slope	(cc)	Slope	(cc)
S-80	0.0129	48.1	0.0020	0.4	0.0080	3.4	0.0115	13.1
BP6K	0.0158	20.0	0.0015	0.6	0.0068	7.2	0.0115	8.2
8238	0.0089	26.1	0.0017	0.7	0.0071	3.9	0.0084	8.2
841	0.0152	54.2	0.0014	1.0	0.0126	7.3	0.0170	36.8
8232	0.0111	14.0	0.0055	3.7	0.0105	10.8	0.0112	18.8
S-88	0.0018	0.8	0.0000	−0.2	0.0000	0.2	0.0103	14.9
8235	0.0071	6.8	0.0027	0.7	0.0067	3.1	0.0116	8.4
7867	0.0151	30.0	0.0031	1.0	0.0065	12.7	0.0138	19.6
6867	0.0211	41.6	0.0054	1.2	0.0108	11.4	0.0144	18.9
3624	0.0142	56.8	0.0032	0.5	0.0069	7.5	0.0134	23.6
691	0.0152	39.8	0.0007	0.2	0.0067	11.9	0.0098	16.6
8797	0.0082	31.3	0.0043	1.2	0.0065	6.9	0.0145	24.4
8679	0.0119	21.3	0.0036	1.1	0.0095	2.9	0.0121	10.4
1451	0.0067	25.4	0.0055	3.3	0.0091	13.7	0.0172	12.7
1362	0.0178	34.8	0.0013	0.5	0.0076	13.7	0.0167	22.8
8237	0.0105	39.1	0.0032	7.5	0.0100	13.1	0.0176	22.9

effects on the majority of strains tested. There were also significant differences in total gas production among different strains grown in the same medium. Specifically, within R + S medium total gas evolution ranges from a high of 56.8 cubic centimeters for strain 3624 to a low of 0.8 cubic centimeters for strain S-88. Despite this rather large range, most strains were capable of producing high levels of gas in this medium.

Of all 16 strains inoculated into casein medium, only 3 strains—8232, 1451 and 8237—were able to produce any significant quantity of gas. The low gas production levels by most strains in casein medium may have been caused by poor solubility of casein and the presence of inhibitory factors. Jayko and Lichstein (1959) reported that native casein was unable to support growth by *C. perfringens* strain BP6K. Table 1 shows that strain BP6K produced only 0.6 cc of gas in casein medium. Busta and Schroder (1971) reported that sodium caseinate and certain soy proteins were inhibitory to the growth of *C. perfringens* strain S40. Inhibition may also have been due to chelation of cations essential for growth or inhibition of the *C. perfringens* protease system as suggested by Schroder and Busta (1973).

The total gas production by most strains in ground frankfurter was higher than that in whole egg and less than that in R + S medium. *C. perfringens* strain differences with respect to amino acid and vitamin requirements as reported by Fuchs and Bonde (1957) could account for variations in total gas production among strains within a given medium.

The slopes of the gas production curves for most strains in ground frankfurter were higher than in any of the other media. Angelotti et al. (1962) reported that *C. perfringens* grew well in meat and meat products and Busta and Schroder (1971) indicated that the rate of growth in liquid medium, in which beef was the sole protein source, was extremely rapid. Strain S-88, which was unable to produce any significant gas in casein, whole egg or R + S media, was able to grow well in ground frankfurter medium. There seems to be some essential growth factor in the meat medium which is not found in the other media. Busta and Schroder (1971) suggested the presence of readily available *C. perfringens* growth stimulating factors in beef.

The pH optimum for protease activity in culture filtrates from *C. perfringens* strain 1451 was 7.0. There was a gradual decline in activity at pH 6.0 and pH 8.0. As the pH dropped below 6.0 or rose above 8.0 the enzyme activity decreased drastically.

The averages of the exoprotease activities for 16 strains of *C. perfringens* in four different media can be seen in Table 2.

Table 2—Exoprotease activity expressed in relative equivalents of trypsin in cell free extracts from cultures of Clostridium perfringens grown in various culture media

	Medium			
Strain	R + S	Casein	Whole egg	Frankfurter
S-80	0.0200	0.0000	0.1100	0.0570
BP6K	0.0014	0.0082	0.4800	0.5500
8238	0.0027	0.0000	0.0450	0.0620
841	0.0170	0.0000	0.2100	0.3200
8232	0.3100	0.3000	0.3900	0.0750
S-88	0.0000	0.0027	0.0055	0.0930
8235	0.0014	0.0014	0.0830	0.0082
7867	0.0000	0.0082	0.0810	0.0000
6867	0.0160	0.0260	0.7200	0.0087
3624	0.2100	0.0000	0.2700	0.0007
691	0.1800	0.0009	0.3300	0.0350
8797	0.0100	0.0000	0.0530	0.2300
8679	0.0068	0.0055	0.0660	0.0140
1451	0.9000	0.5100	0.6000	0.3900
1362	0.0055	0.0041	0.0630	0.0870
8237	0.2100	0.5800	0.6200	0.4000

Exoprotease activity is expressed as Relative Equivalents of trypsin (RET). The standard curve prepared by incubation of 1.9M trypsin at pH 7.0 and 37°C showed a linear A_{520} response for more than 260 min. The absorbance value at 240 min was 1.83. Since all enzyme activity tests were incubated for 240 min, a RET was defined as the A_{520} for the test filtrate divided by 1.83 (the A_{520} for the standard 1.9M trypsin solution).

As seen in Table 2, the extracellular proteolytic activity of *C. perfringens* varies widely as a function of both medium and strain. Strains 1451 and 8237 constitutively produced high levels of proteolytic enzyme in all four media tested. Other strains, such as S-88, 7867, 8238, 8235, 8679 and 1362 produced only low levels of exoprotease in all four media. The amount of exoprotease activity of the majority of other strains varied as a function of medium. The proteolytic activity of these strains is selectively activated or repressed by the medium in which a particular strain is grown.

The effect of medium on the proteolytic activity of the strains was examined by averaging the enzymatic activities in each of four different media over the 16 strains. Analysis of variance in addition to Tukey's Test at the 95% level indicated that all four media enzyme activity means were significantly different from each other. The most enzyme activity when

averaged over 16 strains was associated with whole egg medium, followed in descending order by ground frankfurter, R + S and Casein media, respectively. The low exoproteolytic activity of most strains in casein appears to be related to growth inhibition of these strains by this medium. This is in agreement with the work of Schroder and Busta (1973) who indicated that sodium caseinate had some inhibitory effect on the proteinase system of *C. perfringens*. The relatively low exoprotease activity value as averaged over 16 strains in R + S medium may be explained in terms of the report by Pivnick et al. (1965) who observed that amino acids derived from peptides and free amino acids entered different bacterial pools and therefore could be incorporated into different proteins either cellular or exocellular. Jayko and Lichstein (1959) also suggested that increases in amino acid concentration appeared to mask the stimulatory effect of peptides on lecithinase production. It seems quite likely therefore, that the presence of free amino acids in R + S and ground frankfurter medium may somehow repress the formation of exoproteases by most *C. perfringens* strains.

The higher proteolytic enzyme activity levels associated with strains grown in whole egg medium when compared to the other three media may be related to the presence of an ample supply of peptides in this medium which stimulate enzyme formation. The importance of peptides for exotoxin synthesis was reported by Jayko and Lichstein (1959) and Tsukamoto et al. (1963). These authors indicated that toxin synthesis in cultures of *C. perfringens* was dependent on the supply of peptides which could not be replaced with free amino acids.

The presence or absence of TCA-precipitable macromolecules in all four test media was examined using TCA addition. While TCA was able to precipitate out protein components in casein and whole egg media, there was no precipitation when equivalent amounts of TCA were added to ground frankfurter or R + S media filtrates. This indicated that there were protein-precipitable macromolecules present in the whole egg and casein media, while R + S and frankfurter filtrates contained nonprecipitable components. Specifically, R + S contains only free amino acids, while the frankfurter filtrates may contain free amino acids as well as non-precipitable peptides.

Exoprotease activity for each of 16 strains in four different media was subsequently evaluated after normalization for growth differences (total gas production). This was accomplished by dividing exoprotease activity in relative equivalents of trypsin by the respective amount of gas produced by a given strain in a given media. The greatest enzyme activity/unit gas production for most strains usually developed in whole egg medium and occasionally in casein medium while smaller ratios were associated with frankfurter and R + S media. Analysis of variance in addition to Tukey's Test at the 95% level indicated that all four mean ratios were significantly different from each other. The most exoprotease activity per unit gas production when averaged over 16 strains was associated with whole egg medium, followed in descending order by casein, frankfurter and R + S media, respectively. These results indicate that in many of these strains exoprotease activity is inducible by media containing TCA-precipitable macromolecules. This may be as a result of peptide stimulation or derepression of extracellular proteolytic activity brought about by the lack of free amino acids in whole egg and casein media. The presence of free TCA soluble amino acid pools in R + S and TCA, soluble components in frankfurter may repress the genetic material of the bacterial cell in producing extracellular proteolytic enzyme. These results are supported by the work of Boyd et al. (1948) who found that a synthetic amino acid medium did not support formation of alpha toxin or any other toxins or exoenzymes by *C. perfringens* BP6K. However, Murata et al. (1965) were able to produce alpha toxin in synthetic medium by modifying the nutritional balance.

Strains 3624, 6867, 691 and 8797 were tested for their gas production in R + S base medium. Four hours after inoculation there was no significant gas production by any of these four strains in this medium. Less than 0.3 cc of gas was produced by these strains and there was no significant enzyme activity in any of the respective culture filtrates. Based on these results, the enzyme activity appears related not to glucose induced metabolism of resting cells, but to active growth of cells in amino acids or protein nitrogen-containing media. Gas is produced in significant quantities only in the presence of an organic amino acid or protein nitrogen source.

From the data presented it appears that most strains of *C. perfringens* produce exoproteases after induction or derepression caused by the presence of TCA-precipitable macromolecules (i.e. proteins, peptides) or the absence of free amino acids while a few strains produce exoprotease constitutively. Nonprecipitable peptides such as those which may be present in frankfurter medium do not induce exoprotease activity.

Those constitutively high exoprotease producing strains discovered in this study may be useful in protein quality evaluation if their amino acid requirements prove to be comparable with those of humans and rats.

REFERENCES

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

Berkowitz, K.A. 1977. The development of a method for the determination of protein quality using Clostridium perfringens. Master's thesis, Rutgers University, New Brunswick, NJ.

Bidwell, E. 1950. Proteolytic enzymes of Clostridium welchii. Biochem. J. 46: 589.

Bidwell, E. and van Heyningen, W.E. 1948. The biochemistry of the gas gangrene toxins. 5. The kappa-toxin (collagenase) of Clostridium welchii. Biochem. J. 42: 140.

Boyd, M.J., Logan, M.A. and Tytell, A.A. 1948. The growth requirements of Clostridium perfringens (welchii) BP6K. J. Biol. Chem. 174: 1013.

Busta, F.F. and Schroder, D.J. 1971. Effect of soy proteins on the growth of Clostridium perfringens. Appl. Microbiol. 22: 177.

Fernell, W.R. and Rosen, G.D. 1956. Microbiological evaluation of protein quality with Tetrahymena pyriformis W. 1. Characteristics of growth of the organism and determination of relative nutritive values of intact proteins. Brit. J. Nutrit. 10: 143.

Ford, J.E. 1960. A microbiological method for assessing the nutritional value of proteins. Brit. J. Nutrit. 14: 485.

Fuchs, A.R. and Bonde, G.J. 1957. The nutritional requirements of Clostridium perfringens. J. Gen. Microbiol. 16: 317.

Harmon, S.M., Kautter, D.A. and Peeler, J.T. 1971. Improved media for enumeration of Clostridium perfringens. Appl. Microbiol. 22: 688.

Jayko, L.G. and Lichstein, H.C. 1959. Nutritional factors concerned with growth and lecithinase production by Clostridium perfringens. J. Infect. Dis. 104: 142.

Maschmann, E. 1937. Über Bakterienproteasen 2. Biochem. Zeit. 294: 1.

Murata, R., Yamamoto, A., Soda, S. and Ito, A. 1965. Nutritional requirements of Clostridium perfringens BP6K for alpha toxin production. Jap. J. Med. Sci. Biol. 18: 189.

Oakley, C.L. and Warrack, G.H. 1953. Routine typing of Clostridium welchii. J. Hyg. Camb. 51: 102.

Oakley, C.L., Warrack, G.H. and van Heyningen, W.E. 1946. The collagenase (kappa-toxin) of Cl. welchii type A. J. Path. Bacteriol. 58: 229.

Oakley, C.L., Warrack, G.H. and Warren, M.E. 1948. The kappa and lambda antigens of Clostridium welchii. J. Path. Bacteriol. 60: 495.

Pivnick, H., Hauschild, A.H.W., Gorenstein, B. and Habeeb, A.F.S.A. 1965. Effect of controlled pH on toxinogenesis by Clostridium perfringens type D. Can. J. Microbiol. 11: 45.

Riha, W.E. and Solberg, M. 1971. Chemically defined medium for growth of Clostridium perfringens. Appl. Microbiol. 22: 738.

Schroder, D.J. and Busta, F.F. 1973. Growth of Clostridium perfringens in food proteins previously exposed to proteolytic bacilli. Appl. Microbiol. 26: 675.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw Hill Book Co., NY.

Tsukamoto, M., Yamamoto, A. and Murata, R. 1963. Effect of some synthetic peptides on the alpha-toxin production by Clostridium perfringens BP6K. Jap. J. Med. Sci. & Biol. 16: 213.

Weil, K., Kochalaty, W. and Smith, L.D. 1939. Studies on proteinases of some anaerobic and aerobic microorganisms. Biochem. J. 33: 893.

Ms received 8/4/77; revised 12/23/77; accepted 12/30/77.

Presented in part at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

This work was supported in part by Contract (#223-74-2087) from the Food & Drug Administration.

The authors thank Dr. Bill Swallow for his help in setting up the statistical analysis computer programs.

GROWTH OF Bifidobacterium bifidum VAR. Pennsylvanicus IN LABORATORY MEDIA SUPPLEMENTED WITH AMINO SUGARS AND SPENT BROTH FROM Escherichia coli

Y. C. JAO, E. M. MIKOLAJCIK and P. M. T. HANSEN

ABSTRACT

Growth stimulation of *B. bifidum* var. *pennsylvanicus* by filter-sterilized, unheated amino sugars was studied utilizing an ATCC medium 76 assay system. The order of stimulatory effect was N-acetylglucosamine (GlcNAc) > N-acetylgalactosamine > N-acetylmannosamine > N-acetylneuraminic acid. When GlcNAc was heated at 121°C for 15, 30 and 45 min, depression of growth occurred and was directly related to the heat treatment applied. The presence of *E. coli B* organisms in the assay system did not influence the growth stimulatory effect of the amino sugars on *B. bifidum*. Spent broth, prepared by growth of *E. coli B* in the assay medium followed by filter sterilization, was found to stimulate the subsequent growth of *B. bifidum*. The spent broth contained small quantities of amino sugars. *E. coli B* failed to grow in spent broth prepared from *B. bifidum* without pH adjustment. Growth of *E. coli B* was observed when this spent medium was neutralized and fortified with amino sugars, however, without any specific stimulatory pattern attributable to the type of amino sugar.

INTRODUCTION

Bifidobacterium bifidum was originally introduced by Tissier (1899) as *Bacillus bifidus*. The organism has been described by Norris et al. (1950), Schuler et al. (1968), Sandine et al. (1972), Poupard et al. (1973) and Kandler and Lauer (1974). It is an anaerobic, gram positive organism, often Y-shaped or clubbed at the end, but exhibiting pronounced pleomorphism when grown in artificial media (Bauer et al., 1975).

A large number of strains of *B. bifidum* which require a specific bifidum factor for growth have been isolated from the stools of infants (György, 1953) and from the vaginal secretions of pregnant women (Harrison et al., 1953). Such strains have been given the variety name, *pennsylvanicus*, by György et al. (1954). This variety, generally recognized as requiring human milk for growth, is not capable of synthesizing its cell wall without external sources of suitable growth factors (Lambert and Zilliken, 1965). Highly active primary bifidum growth factors are required. These include N-acetyl-D-glucosamine and its derivatives, particularly β-ethyl-N-acetyl-D-glucosamine, as well as some novel growth factors, such as N-benzoyl-D-glucosamine and N-carboethoxy-D-glucosamine (Lambert and Zilliken, 1965).

The supplementary growth factors, described by György and Rose (1955), appear to be in the class of proteins and peptides. For example, peptones from degraded insulin and other proteins such as strepogenin (Haurowitz, 1950) show a bifidum positive effect. Nichols et al. (1974) isolated seven fractions of M-1-glycoproteins from human colostrum whey and two fractions from bovine milk which they showed to be stimulatory for *B. bifidum*. The degree of growth stimulation was proportional to the total carbohydrate content. We have previously found a degradation product of bovine casein which showed a stimulatory effect on the growth of *B. bifidum* var. *pennsylvanicus* and named it a casein bifidum factor (CBF) (Kehagias et al., 1977). Demonstration of a highly active bifidum stimulant from casein, derived by mild acid hydrolysis, confirms the observation by György and Rose (1955) of a particular growth factor which they found to be present in the enzymatic digest of casein but which was destroyed by complete acid hydrolysis.

It was reported by György et al. (1974) that undialyzable sialic-acid-containing oligosaccharides and glycoprotein from human milk and body fluid did not exhibit any *B. bifidum* growth promoting effect, unless pretreated with neuraminidase. von Nicolai and Zilliken (1972) have demonstrated that *B. bifidum* var. *pennsylvanicus* possesses a neuraminidase but that sialic-acid-bound oligosaccharides from human milk are not bifidum growth promoting factors because the enzyme showed fairly slow scission of the sialic acid from these oligosaccharides.

B. bifidum comprises nearly 100% of the bacteria cultured from the stools of breast-fed infants and may approach a level of approximately 10^{11}/g feces (Mata et al., 1969). In contrast, Haenel et al. (1971) have reported that only 30–40% of the intestinal flora of formula-fed infants are *B. bifidum*. Mata et al. (1969) and Mata and Urritia (1971) reported that the intestinal microflora in breast-fed infants plays a role in resistance to *Shigella* and enteropathogenic *Escherichia coli* infections. Bullen and Willis (1971) suggested that the predominance of *B. bifidum* which lowers the pH of feces from breast-fed infants may also suppress *E. coli* in the gut. Mayer (1965) indicated that the disappearance of bifidobacteria from the intestine increased infant susceptibility to enteric viral infections. In contrast, Hewitt and Rigby (1976) found no consistent relationship between high counts of bifidobacteria and low counts of *E. coli* in the stools of new-born infants and suggested that implantation of bifidobacteria in the large intestine of artificially fed infants may be largely ineffective in influencing the *E. coli* population. Muting et al. (1968) and Mayer (1969) suggested that the development of *B. bifidum* flora in the human gut plays a detoxification role in the case of chronic liver disease. Commercial processes for production of fermented dairy foods containing the organism have been developed (e.g., Schuler et al., 1968; and Schuler, 1973).

The purpose of this study was to investigate microbial interactions between *B. bifidum* var. *pennsylvanicus* and *E. coli B* in systems containing amino sugars as growth factors.

MATERIALS & METHODS

B. bifidum var. *pennsylvanicus* (ATCC 11863) was obtained from American Type Culture Collection, Rockville, MD. *Escherichia coli B* was from the Microbiology Department of The Ohio State University.

Fresh broth systems

Elliker broth (Difco, 0974) and ATCC medium 76 (containing peptone) were used for cell preparation and growth assay, respectively. Elliker broth with 1.5% agar was used for plate counts of *B. bifidum* and mixed cultures. Violet Red Bile medium was used for selective plating of *E. coli B*. Incubation was at 37°C ± 0.001 in a water bath equipped with a proportional controller. Turbidity of cell suspensions was determined at 660 nm in a Spectronic-20 Colorimeter (Bausch & Lomb). Stock solutions of amino sugars (Calbiochem) were prepared by dissolving 25 mg amino sugars in 4 ml double distilled water, adjust-

All authors are with the Dept. of Food Science & Nutrition, Ohio Agricultural Research & Development Center and The Ohio State University, Columbus, OH 43210.

0022-1147/78/0004-1257$02.25/0
© 1978 Institute of Food Technologists

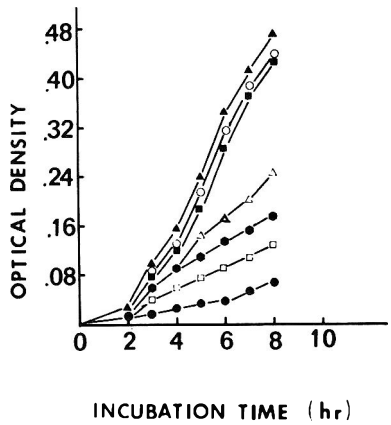


Fig. 1—Effects of different GlcNAc levels on the growth rate of B. bifidum at 37°C in ATCC medium 76 as determined turbidometrically. GlcNAc conc (ppm): ■ 1000, ○ 500, ▲ 250, Δ 100, ⬢ 50, □ 10 and ● 0.

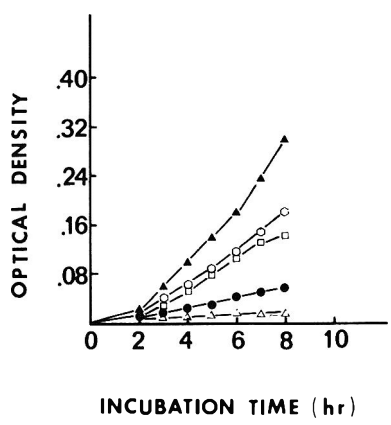


Fig. 2—Effect of heat treatment of GlcNAc on the growth rate of B. bifidum ATCC medium 76 at 37°C. GlcNAc (250 ppm) heated at 121°C: ▲ 0 min, ○ 15 min, □ 30 min, Δ 45 min and ● Control: No GlcNAc.

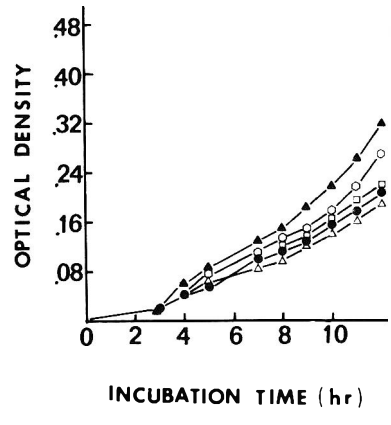


Fig. 3—Growth effects of heat treated GlcNAc on mixed cultures of B. bifidum and E. coli. GlcNAc (250 ppm) heated at 121°C: ▲ 0 min, ○ 15 min, □ 30 min, Δ 45 min and ● Control: No GlcNAc.

ing the pH to 6.5 and the final volume to 5 ml, followed by filter sterilization through a Gelman 0.2 micron membrane. The desired concentration of the sterile amino sugars was added to the broth previously sterilized at 121°C for 15 min. Heat treatment of solutions of GlcNAc was at 121°C for up to 45 min.

Spent broth systems

"Spent broth" was obtained by culturing either *E. coli B* or *B. bifidum* in the semi-synthetic broth (pH 6.5) for 24 hr, followed by membrane filter sterilization. The pH of one set of the *B. bifidum*-spent broth (pH 5.47) was readjusted to 6.5 prior to filter sterilization. The control was spent broth without pH treatment. The pH of *E. coli B*-spent broth was 6.25 and was not readjusted. Sterility of both spent broths was verified by agar plate count.

Culture propagation

Young active cells were sedimented from a 14–16 hr Elliker broth culture by centrifugation at 2000 rpm for 10 min, followed by washing with sterile saline and recentrifugation. The washing and centrifugation steps were repeated twice. The cells were then added to fresh or spent semi-synthetic broth and the percent transmission adjusted to 75 at 660 nm. Bacterial counts for single and mixed cultures were conducted in oval tubes in which sufficient anaerobic conditions were created by sealing with 1.5% agar containing 0.1% sodium thioglycollate. Selective plate counts for *E. coli B* in mixed cell systems were run on Violet Red Bile agar.

Evaluation of growth stimulation

The assay method consisted of the following steps: (a) pipette 4 ml cell stock solution into sterile Spectronic-20 cuvette for single culture study or 2 ml from each cell stock solution for mixed culture study; (b) add 0–1 ml amino sugar stock solution to desired concentration; (c) add 0–1 ml sterile water to final volume of 5 ml; (d) incubate at 37°C; (e) record transmittance at selected time intervals up to 10 hr using reference blank of 4 ml semi-synthetic broth plus 1 ml sterile water; and (f) convert percent transmittance into absorbance unit (OD) with correction for change in OD value (Δ OD).

N-acetylhexosamine

Analysis was performed according to the method of Kumar and Hansen (1972b).

RESULTS

TO EVALUATE the effect of different levels of N-acetylglucosamine (GlcNAc) on the growth of *B. bifidum*, the growth rate in ATCC medium 76 was determined turbidimetrically. The growth rate of *B. bifidum* increased with increasing GlcNAc concentration up to 250 ppm (Fig. 1). Beyond, there was a decrease in growth rate. Similar results were obtained when the growth rate was measured by the agar plate count method.

When aqueous GlcNAc solutions received a heat treatment of 121°C for 15, 30 and 45 min prior to addition to the assay system, the growth stimulation of *B. bifidum* was reduced when compared with the unheated GlcNAc system (Fig. 2). The reduction was related to the rigor of the heat treatment. The growth rate of a mixture of *B. bifidum* and *E. coli B* in the presence of heated GlcNAc was also studied. Results shown in Figure 3 reveal that the loss of the growth stimulating effect of heated GlcNAc was similar to that observed in *B. bifidum* alone.

The growth profile for the two species in the mixed culture system containing heat-treated GlcNAc was examined by a selective medium (Violet Red Bile Agar) for coliform counts and total counts by Elliker agar medium in anaerobic oval tubes.

Total count data shown in Figure 4 indicate that the higher the final total count, the lower the final pH of the system. The growth profiles for each of the organisms in the different systems are presented in Figure 5. For *E. coli B*, exponential growth was evident during the first 4 hr of incubation in all systems. With unheated GlcNAc, *E. coli* continued to increase in number for an additional 4 hr. Only slight increases in population were noted in the other systems with the exception of the system containing GlcNAc heated for 45 min. The number of *B. bifidum* organisms (Fig. 5) increased slowly for the first 8 hr with additional increases between 8 and 12 hr. While it is difficult to associate the growth pattern of *E. coli* in the presence of *B. bifidum* with any specific metabolite, it is likely that the acidic environment produced by *B. bifidum* played a major role in the suppression.

Although not shown, *E. coli B* cultured alone in the various systems continued to increase in numbers up to 17 hr of incubation. The generation time between 8 and 17 hr of incubation ranged from 3.1 hr in the control to 7.2 hr in the system containing GlcNAc heated at 121°C for 45 min.

Sasaki and Yamashita (1969) reported increased growth of *B. bifidum* in the spent media of *E. coli*. Their methods were adopted for this part of the study. *E. coli B*-spent broth was prepared by inoculating *E. coli B* into the semi-snythetic broth and incubating at 37°C for 24 hr, followed by filter sterilization of the spent broth. To this system was added GlcNAc which had been heated for 0–45 min at 121°C and an active culture of *B. bifidum*. The growth curves (Fig. 6) indicate that components stimulatory to *B. bifidum* may be produced by *E. coli B*. The results in Table 1 indicate an elevated level of N-acetylhexosamines in the *E. coli*-spent medium in contrast to the depletion of this factor in the *B. bifidum*-spent medium.

The findings with GlcNAc suggested that other amino

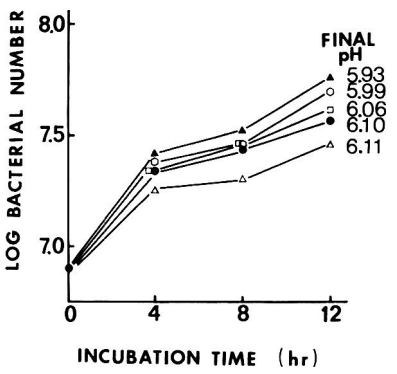


Fig. 4—Total plate counts for the growth effects of heat treated GlcNAc on mixed cultures of B. bifidum and E. coli. GlcNAc (250 ppm) heated at 121°C: ▲ 0 min, ○ 15 min, □ 30 min, △ 45 min and ● Control: No GlcNAc.

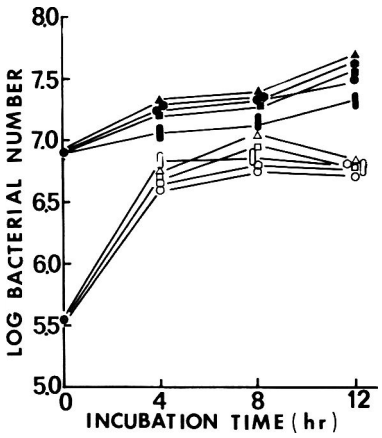


Fig. 5—Plate count of individual bacteria in the mixed cultures under the growth effects of heat treated GlcNAc. GlcNAc (250 ppm) heated at 121°C: solid symbols, B. bifidum; hollow symbols E. coli: ▲ △ 0 min, ● ○ 15 min, ■ □ 30 min, ● ○ 45 min and ● ○ Control: No GlcNAc.

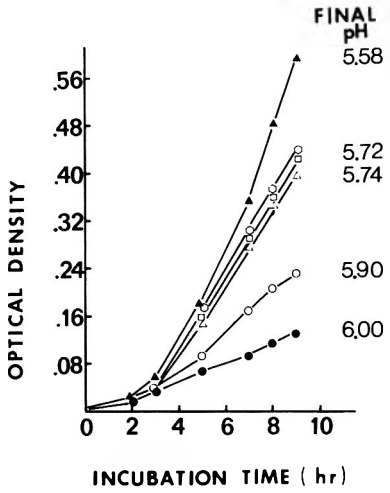


Fig. 6—Growth effects of heat treated GlcNAc on B. bifidum in E. coli-spent broth. GlcNAc (250 ppm) heated at 121°C: ▲ 0 min, ○ 15 min, □ 30 min, △ 45 min, ● Fresh broth control and ○ Spent broth control.

sugars may have similar effects on the growth of *B. bifidum* var. *pennsylvanicus*. The results in Figure 7 show that in all cases, the level of growth achieved was higher in systems containing added amino sugars than in the control. Growth stimulatory effects of the selected amino sugars was in the order of GlcNAc > GalNAc > ManNAc > NeuNAc. The biological activity of the various amino sugars was evaluated also in a mixed system consisting of *B. bifidum* and *E. coli B* with similar results.

The effect of selected amino sugars on the growth rate of *B. bifidum* in *E. coli B*-spent broth was also investigated. The data shown in Figure 8 demonstrate the consistency of the growth stimulation pattern of the selected amino sugars. In order to clarify the growth effect of amino sugars on the bacteria in the spent broth assay system, the same level of glucose was added as a supplemental energy source. This addition did not affect the growth rate of *B. bifidum* when compared with the spent broth control, showing that the stimulatory effect of the amino sugars is unique in this context.

Table 1—Concentration of N-acetylhexosamine in growth media[a]

Medium	GlcNAc equivalents (ppm)
Fresh — not fortified	16
E. coli — spent	64
B. bifidum — spent	0

[a]ATCC medium 76

An experiment was conducted to investigate possible amino sugar stimulation of *E. coli B* when cultured in a *B. bifidum*-spent broth. The pH of the *B. bifidum*-spent broth was 5.47 and was readjusted to 6.5 prior to filter sterilization. Spent broth without pH adjustment but filter sterilized served

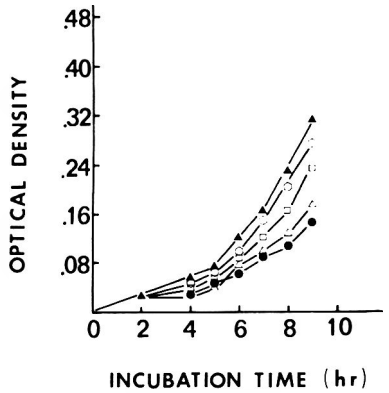


Fig. 7—Growth effects of selected amino sugars on B. bifidum in ATCC medium 76 at 37°C (dosage 250 ppm). ▲ GlcNAc, ○ GalNAc, □ ManNAc, △ NeuNAc and ● Control.

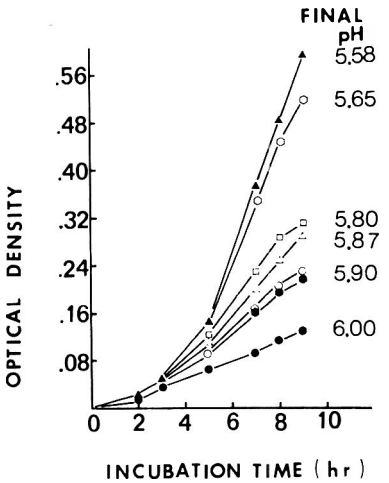


Fig. 8—Growth effects of amino sugars on B. bifidum in E. coli-spent broth (dosage 250 ppm). ▲ GlcNAc, ○ GalNAc, □ ManNAc, △ NeuNAc, ○ Spent control, ● Glucose and ● Fresh control.

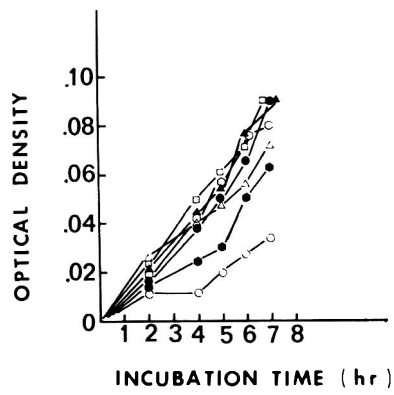


Fig. 9—Effect of various amino sugars on the growth rate of E. coli in B. bifidum-spent broth (dosage 250 ppm). ▲ GlcNAc, ○ GalNAc, □ ManNAc, △ NeuNAc, ○ Spent control: pH 5.47, ● Spent control: pH 6.50 and ● Fresh control.

as a control. The data (Fig. 9) show that all of the amino sugars tested stimulated the growth of *E. coli B* equally well when compared to the controls. The absence of any specific stimulatory pattern for this organism is an indication that possibly the added amino sugars simply served as a supplementary energy source.

As part of this experiment and to further clarify the influence of pH of *B. bifidum*-spent broth on growth of *E. coli B*, the growth rate of *E. coli B* in the unadjusted control (pH 5.47) was compared with growth in the system where pH was adjusted to 6.5 (Fig. 9). There was a definite depression in growth of *E. coli B* at pH 5.47, further confirmation that acid development by *B. bifidum* plays a major role in the suppression of *E. coli B*.

DISCUSSION

THIS RESEARCH was undertaken to study the growth of *B. bifidum* var. *pennsylvanicus* in a semi-synthetic medium, fortified with heated and unheated amino sugars, and to determine the extent of interaction between this organism and *E. coli B*.

The growth requirements of *B. bifidum* are complex. According to Gyögy and Rose (1955), strains of prototype *B. bifidum* and *B. bifidum* var. *pennsylvanicus* have an absolute requirement for pantethine and cannot utilize pantothenic acid. In addition, some strains of *B. bifidum* var. *pennsylvanicus* require a specific bifidum factor found in human milk, as well as a supplementary factor, present in human and bovine milk, and in insulin and pancreatic extracts. György and Rose (1955) identified N-acetylglucosamine and N-acetyllactosamine as the specific bifidum factors in human milk, while the independent, supplementary factor(s) may be derived from an enzymatic bovine casein hydrolysate (NZ case peptone) or from casein-free whey.

György and Rose (1955) have emphasized that in assaying for specific bifidum factors it would be important to enrich the medium with pantethine and an adequate source of the supplementary factor.

In the present study, the basal medium (ATCC medium 76) contained one percent of an enzymatic digest of casein (NZ case peptone, Sheffield) and results have demonstrated that this system is satisfactory for evaluation of the microbiological growth activity of amino sugars on *B. bifidum* var. *pennsylvanicus* (Mikolajcik et al., 1973). In addition to supplying the supplementary growth factor, the peptone also served as the source of the essential nutrient, pantethine. According to György and Rose (1955), one hundred mg of peptone supplies one μg of pantethine which is in excess of that required for maximum growth.

Results from this research have demonstrated that amino sugars provide growth stimulation of *B. bifidum* var. *pennsylvanicus*, both in pure culture and in a mixed culture system containing *E. coli B*. Different amino sugar isomers varied in the magnitude of stimulation with N-acetylglucosamine exerting the highest activity.

The growth stimulatory effect of heat sterilized amino sugars on the organism was less than that of unheated controls. The loss of stimulatory effect may be a result of the heat-induced alteration of N-acetylhexosamines (HexNAc) into heterocyclic compounds which are detectable by their reaction with p-dimethylaminobenzaldehyde (Kumar and Hansen, 1972a, b). In fact, these heat-altered forms were found to be somewhat inhibitory to the growth of *B. bifidum*. György and Rose (1955) related the diminished activity of heated N-acetyl-D-glucosamine to interactions of these sugars with constituents of the basal medium causing a reduction in the amount of N-acetyl-D-glucosamine as precursor of the bifidum factor. However, there is evidence that the principal reaction of heat on these amino sugars is not interactions but the formation of furan derivatives, including mono- and dianhydro derivatives of N-acetyl-D-glucosamine, containing one or two double bonds, respectively (Kumar and Hansen, 1972a).

Information was obtained about the growth profile of *B. bifidum* var. *pennsylvanicus* and *E. coli B* in mixed culture systems. The bifidum organisms grew well in a medium which had first supported the growth of the coliforms. The reason was apparently that *E. coli B* produced a small but measurable quantity of N-acetylhexosamines which supplied the needed bifidum factor. In a separate study (Jao, 1974), it has been determined that the bifidum factor in the *E. coli B*-spent medium is heat labile, as would be expected from N-acetylhexosamines. These observations support the findings by Sasaki and Yamashita (1969) who reported that an *E. coli*-spent medium has a stimulatory effect on the growth of bifidobacterium.

The increased growth rate of *E. coli B* observed in neutralized *B. bifidum*-spent broth would support the conclusion that a major portion of the suppression of *E. coli B* proliferation is associated with acid development. However, the growth rate of *E. coli B* in the neutralized *B. bifidum*-spent broth was not to the level found in the amino sugar fortified systems. Other factors in addition to acid production may be involved. Two of these which come to mind are (a) depletion of nutrients and/or (b) antibiotic production by *B. bifidum*. In relation to the former, the stimulation of *E. coli B* growth by amino sugars is related apparently to a need for an energy source, not to the amino sugar *per se*, since *E. coli B* produce an excess of N-acetylhexosamines during growth. In relation to antibiotic production, this area is in need of study. Other lactic acid bacteria, for example *Lactobacillus acidophilus*, has been shown by Hamdan and Mikolajcik (1974) to produce an antibiotic, acidolin, which is inhibitory for *E. coli B*.

REFERENCES

Bauer, H., Sigarlakie, E. and Faure, J.C. 1975. Scanning and transmission electron microscopy of three strains of Bifidobacterium. Can. J. Microbiol. 21: 1305.

Bezkorovainy, A. 1977. Human milk and colostrum proteins. J. Dairy Sci. 60: 1023.

Bullen, C.L. and Willis, A.T. 1971. Resistance of the breast-fed infant to gastroenteritis. British Medical J. 3: 338.

György, P. 1953. A hitherto unrecognized biochemical difference between human milk and cow's milk. Pediatrics 11: 98.

György, P., Jeanloz, R.W., von Nicolai, H. and Zilliken, F. 1974. Undialyzable growth factors for Lactobacillus bifidus var. pennsylvanicus: protective effect of sialic acid bound to glycoproteins and oligosaccharides against bacterial degradation. Eur. J. Biochem. 43: 29.

György, P., Norris, R.F. and Rose, C.S. 1954. Bifidus. 1. A variant of Lactobacillus bifidus requiring a special growth factor. Arch. Biochem. Biophys. 48: 193.

György, P. and Rose, C.S. 1955. Further observations on the metabolic requirements of Lactobacillus bifidus var. pennsylvanicus. J. Bact. 69: 483.

Haenel, H., Muller-Beuthow, W. and Gritte, F.K. 1971. Faecal microecology of young infant in relation to nutrition composition of microflora and occurrence of types of Lactobacillus bifidus. Dairy Sci. Abs. 33: 3025.

Hamdan, I.Y. and Mikolajcik, E.M. 1974. Acidolin: An antibiotic produced by Lactobacillus acidophilus. J. Antibiotics 27: 631.

Harrison, W., Stahl, R.C., Maganvian, J., Sanders, M., Norris, R.F. and György, P. 1953. The incidence of Lactobacillus bifidus in vaginal secretions of pregnant and non-pregnant women. Am. J. Obstet. Gynecol. 65: 352.

Haurowitz, F. 1950. "Chemistry and Biology of Proteins," p. 315. Academic Press, N.Y.

Hewitt, J.H. and Rigby, J. 1976. Effect of various milk feeds on numbers of Escherichia coli and Bifidobacterium in the stools of newborn infants. J. Hyg. (Camb.) 77: 129.

Jao, Y.C. 1974. Growth effect of N-acetylhexosamines on Lactobacillus bifidus var. pennsylvanicus. Ph.D. dissertation, The Ohio State University.

Kandler, O. and Lauer, E. 1974. Neuere Vorstellungen zur taxonomie der bifidobacterien. Zbl. Bakt. Hyg., Abt. Orig. A 228: 23.

Kehagias, C., Jao, Y.C., Mikolajcik, E.M. and Hansen, P.M.T. 1977. Growth response of Bifidobacterium bifidum to a hydrolytic product isolated from bovine casein. J. Food Sci. 42: 146.

Kumar, S. and Hansen, P.M.T. 1972a. Reactions of heated milk and amino sugars with p-Dimethylaminobenzaldehyde. Agr. Food Chem. 20: 1141.

Kumar, S. and Hansen, P.M.T. 1972b. New reaction mixture for spectrophotometric determination of N-acetylhexosamine. Anal. Chem. 44: 398.

Lambert, R. and Zilliken, F. 1965. Novel growth factors for Lactobacillus bifidus var. pennsylvanicus. Arch. Biochem. Biophys. 110: 544.

—Continued on page 1263

ISOLATION, CHARACTERIZATION AND ENZYMIC HYDROLYSIS OF PINEAPPLE GUM

K. L. CHENCHIN and H. Y. YAMAMOTO

ABSTRACT

Pineapple gum was isolated and characterized. The gum was a cold-water soluble neutral polysaccharide composed predominantly of galactomannans. Solutions of pineapple gum formed viscous solutions which tended to foam. Studies on the enzymic hydrolysis showed that some commercial enzymes, specifically pectinases, cellulases and hemicellulases, were effective in degrading pineapple gum.

INTRODUCTION

PINEAPPLE contains a gum (Dull, 1958) which appears to contribute to the viscosity, suspension of solids, and foaming of pineapple juice. Bates (1964) has attributed the ability of pineapple concentrate to foam suitably for foam-mat drying without the addition of stabilizers to the presence of galactomannans and pectic substances. According to Chan (1970) centrifuged pineapple juice underflow contains 0.05% gum and pectic substances.

Although the presence of gums may be desirable for solids suspension and mouthfeed, it can be undesirable in operations such as fermentation or clarification where foaming reduces tank capacity and viscosity makes filtration difficult. Since pineapple gum has important roles in pineapple products and processes, it was of interest to prepare purified pineapple gum and to characterize its chemical and physical properties. Chemical composition, foaming and foam stability, and viscosity properties were determined. In addition, several commercially available enzyme preparations were examined for effectiveness in hydrolyzing purified gum.

EXPERIMENTAL

Isolation

Pineapple gum was isolated from the foam of centrifuged pineapple juice or pineapple juice concentrate. Pineapple foam was collapsed with ethanol or silicone antifoam or both and concentrated to approximately 65° brix in a centrifugal concentrator (Centritherm 1 B, Alfa Laval) or a rotary evaporator (Buchi Rotavapor). The gum was extracted by the general method of Robinson (1967). Pineapple foam concentrate or juice concentrate in 80% ethanol was settled for several hours after which the clear yellow supernatant was decanted and the precipitate was dissolved in water at 45°C. The mixture was then filtered and the resulting filtrate was reprecipitated in 80% ethanol. The precipitate, collected by centrifugation, was dissolved in water and trichloroacetic acid was added to 10% final concentration to precipitate the proteins. Proteins were removed by centrifugation, and the resulting opalescent solution was dialyzed against several changes of distilled water at 5°C for 5 days and then freeze dried.

Gum composition

Protein was determined according to Potty (1969) using a crystalline bovine albumin standard. The moisture content was determined by drying to constant weight under 29 in. vacuum at 70°C. Total ash was determined by first charring the gum in crucibles and then ashing at 500°C for 18 hr in a muffle furnace. Total sugars were determined by the anthrone method as described by Bailey (1958) with glucose standard. Total phenols were determined by the method reported by Swain and Hillis (1959) using a Folin-Ciocalteau reagent (Phenol Reagent, Fisher Scientific Company).

Identification of monosaccharides

The purified gum was hydrolyzed with 1N H_2SO_4 in sealed tubes at 100°C for 8 hr. The resulting hydrolysate was neutralized with $Ba(OH)_2$ to pH 5.0. The hydrolysate was filtered and deionized by passing through a Dowex 50W-X4 (200–400 mesh, H^+ form) cation exchange column and then through a Dowex 1X8 (100–200 mesh, formate form) anion exchange column followed by 4–5 volumes of distilled water wash. The effluent and wash were concentrated at 40–45°C under 29 in. vacuum to a thick syrup.

The gum hydrolysate and sugar standards were silylated as described by Kline et al. (1970). The sugar standards were dissolved in 65% ethanol and the internal standard, myoinositol was dissolved in water. The hydrolysate and standards were placed in a 7-ml screw cap vial along with 0.1g celite and evaporated to dryness under vacuum at 30°C. After drying, one ml of TRI-SIL (Pierce Chemical Co.) was added; the vial was capped with a butyl rubber septum, mixed vigorously for 30 sec and incubated at 45°C for 15 min.

A Varian Aerograph 200 gas chromatograph with flame ionization detector was used. The nitrogen carrier gas flow rate was 25 cm^3 per min and the hydrogen flow rate was 44 cm^3 per min. Two different columns were used for retention time studies: a 7-ft, 0.093-in. i.d. stainless steel column packed with 3.0% OV-17 on Chromasorb G A/W HMDS 60/80 and a 7-ft, 0.093-in. i.d. stainless steel column packed with 3.8% SE-30 on Chromasorb W HMDS 60/80. The OV-17 column was operated isothermally at both 145° and 190°C and the SE-30 column at 170°C. Injector and detector temperature for both columns were 225° and 250°C respectively. The OV-17 column was used for the quantitative determination of arabinose, galactose, mannose and glucose and the SE-30 column was used for xylose.

Mineral composition

The qualitative mineral composition of pineapple gum was determined by X-ray fluorescence using a VXQ 72,000 Quantometer (Applied Research Laboratories) by the Soil Science Dept., Hawaii Agricultural Experiment Station, University of Hawaii.

Viscosity

Relative viscosity of pineapple gum, locust bean gum and gum tragacanth gum (Stein Hall and Co.) was calculated from the ratio of flow time of sample to that of distilled water. Flow times were measured in six replications in an Ostwald viscometer (Cannon Fenske Viscometer, size 200) with 10 ml samples at 50°C.

Foaming

Foaming ability was determined as overrun from the volume of foam produced after blending 50 ml gum solution in a Waring Blendor for 30 sec. Overrun was calculated as percent volume of foam obtained in excess of the volume of gum solution. Drainage, used as an index of foam stability, was determined as the percent liquid separated during the first 3 min after blending.

Enzyme hydrolysis

Five enzymes were surveyed for their ability to hydrolyze isolated pineapple gum. The enzymes were Cellzyme 225, a cellulase; Kleerzyme HT, a pectinase; Amigase, an amyloglucosidase; Papain 400, a protease (Wallerstein Co.); and Rhozyme HP-150, a hemicellulase (Rhom and Haas Co.). Enzymic hydrolysis as indicated by relative viscosity change was carried out as follows: 0.01 ml of 10% enzyme solutions were added to 9.9 ml of 0.2% pineapple gum in an Ostwald viscometer at

Authors Chenchin and Yamamoto are with the Dept. of Food Science & Technology, University of Hawaii, 1920 Edmonson Road, Honolulu, HI 96822.

0022-1147/78/0004-1261$02.25/0
© 1978 Institute of Food Technologists

Table 1—Quantitative determination of sugars in pineapple gum hydrolysate by GLC

Sugar	% of gum hydrolysate
Arabinose	18.3
Glucose	6.9
Galactose	21.4
Mannose	48.2
Xylose	6.9

Table 2—Qualitative mineral composition of pineapple gum

Silicon	Magnesium	Zinc
Chlorine	Phosphorus	Manganese
Sodium	Sulfur	Aluminum
Calcium	Iron	Copper
	Potassium	

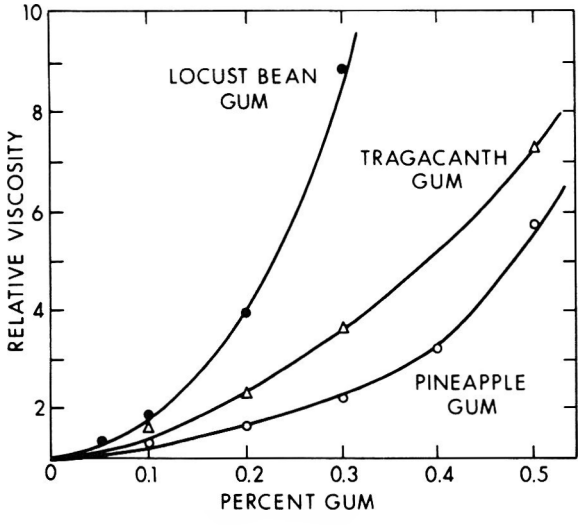


Fig. 1—Relative viscosity of locust bean, tragacanth and pineapple gum solutions. Flow times were measured at 50°C with an Ostwald viscometer.

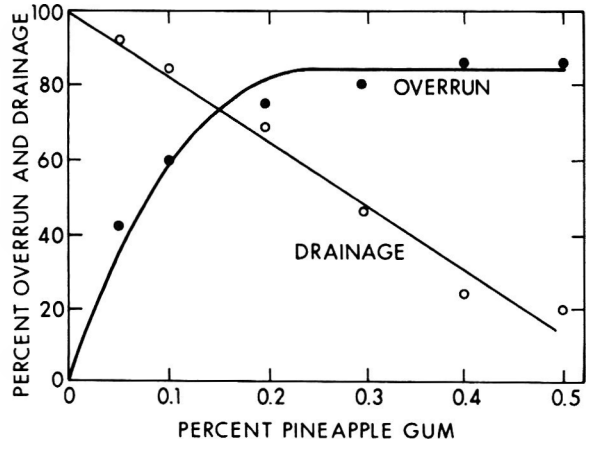


Fig. 2—Effect of pineapple gum concentration of foaming and foam stability.

50°C. Final enzyme concentration was 1000 ppm which represented 2 AG units (Amigase), 2 WCA units (Cellzyme 225) and 2.7×10^{-3} activity factor (Rhozyme HP-150) as defined by the manufacturers.

RESULTS & DISCUSSION

THE YIELD OF GUM from pineapple foam liquid (15° brix) and reconstituted pineapple juice (15° brix) was approximately 0.054%. A similar yield was obtained by Chan (1970) from centrifuged pineapple juice underflow. The freeze-dried isolate, which was white, fluffy and hygroscopic, contained 79–92% sugars by the anthrone test, 7–12% moisture, 1.5% protein, 1.5–3.7% ash and 0.5% phenols.

The presence of five sugars, arabinose, glucose, galactose, mannose and xylose was established by comparison of relative retention times of known sugar standards on both the OV-17 and SE-30 columns. Quantitative analysis (Table 1) showed that mannose was the predominant monosaccharide followed by galactose and arabinose. Mannose and galactose comprised nearly 70% of the sugars in pineapple gum. Sugar composition of pineapple gum was similar to other galactomannans in that they usually contain a higher proportion of mannose than galactose (Smith and Montgomery, 1959). Pineapple gum had a mannose:galactose ratio of 2.25 to 1 similar to that of guar gum, a commercial galactomannan extracted from seeds of guar plant. The hydrolysate was also tested for amino and acidic sugars but none was detected. Levand and Heinicke (1968) identified neutral sugars of a polysaccharide present in bromelain preparations as glucose, galactose, mannose, arabinose and xylose. However, they also reported the presence of glucuronic and galacturonic acids.

A qualitative mineral analysis of pineapple gum by X-ray flourescence showed the presence of 13 minerals as listed in Table 2. These findings were similar to the mineral content in pineapple fruit (Dull, 1971) with the exception of zinc, copper, sulfur and aluminum which have not been reported previously.

Gum is defined as any polymeric material that can be dissolved or dispersed in water to give a thickening effect (Klose and Glicksman, 1972). Pineapple gum was soluble in cold water, forming an opaque and viscous solution. The effect of pineapple gum concentration on viscosity is shown in Figure 1. Typical of gums, the viscosity of pineapple gum solutions increased logarithmically with concentration. The effect of locust bean gum and tragacanth gum concentration on viscosity is also shown for contrast in Figure 1. Locust bean gum is a galactomannan and tragacanth gum is a complex mixture of polysaccharides. Both are known for their ability to form highly viscous solutions at 1% concentration.

There was little effect of pH on pineapple gum viscosity. Pineapple gum viscosity was constant over pH 3–9, indicating the gum to be a neutral polymer.

In addition to increasing viscosity, gums have other functional effects, one of which is foaming. Solutions of pineapple gum foamed readily as shown in Figure 2 where overrun and drainage is plotted against gum concentration. Overrun increased with increasing gum concentration reaching a maximum at about 0.4% gum concentration. Foaming occurred at relatively low concentrations, consistent with the fact that pineapple juice which contains less than 1/10% gum foams readily. Drainage decreased with increasing gum concentration indicating development of a more stable foam.

A variety of commercial enzymes were examined for their ability to reduce the viscosity of pineapple solutions. Figure 3 shows typical results with each type of enzyme investigated. The enzymes shown are cellulase, pectinase, amylase, protease and hemicellulase. As shown, hemicellulase, cellulase and pectinase reduced pineapple gum viscosity at a higher rate and to a greater extent than amylase and protease. Viscosity was reduced approximately 80–95% and hydrolysis appeared to be complete within 10 min. It should be noted that since the

enzymes are commercial preparations, they are not necessarily homogeneous. However, a number of other commercially available enzymes were evaluated and generally, the pectinases, cellulases and hemicellulases were more effective in hydrolysing pineapple gum than the amylase and proteases.

These results suggest enzyme treatment may be a potentially useful means of removing gums in processing of pineapple products. Possible benefits of enzymic treatment would include increased juice fermentation capacity, elimination of antifoams, lowering of viscosity with resulting improvements in filtration, removal of suspended pulp, improved mouthfeel of juice products, and improvement in the clarity of pineapple wine. The effects of enzyme treatment of pineapple juice will be reported in a later paper.

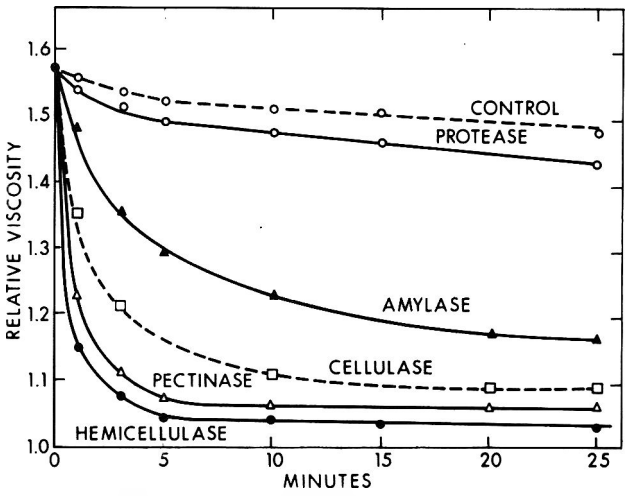


Fig. 3—Hydrolytic effect of enzymes on pineapple gum. Pineapple gum solutions (0.2%) were treated at 50

REFERENCES

Bailey, R.W. 1958. The reaction of pentoses with anthrone. Biochem. J. 68: 669.
Bates, R.P. 1964. Factors affecting foam production and stabilization of tropical fruit products. Food Technol. 18: 93.
Chan, J.K.C. 1970. Characterization and utilization of centrifuged pineapple juice underflow. MS thesis, University of Hawaii, Honolulu, HI.
Dull, G.G. 1958. "Selected Data on Chemical Composition of the Pineapple Plant and Fruit," p. 25. Pineapple Research Institute, Hawaii.
Dull, G.G. 1971. The Pineapple: General. In "The Biochemistry of Fruits and Their Products," Vol. 2, p. 308, Ed. Hulme, A.C. Academic Press, London and New York.
Kline, D.A., Fernandes-Flores, E. and Johnson, A.R. 1970. Quantitative determination of sugars in fruits by GLC separation of TMS derivatives. J. Assoc. Off. Agr. Chem. 53: 1198.
Klose, R.E. and Glicksman, M. 1972. Gums. In "Handbook of Food Additives," p. 295, Ed. Furia, T.E. CRC Press, Cleveland, OH.
Levand, O. and Heinicke, R.M. 1968. Ferulic acid as a component of a complex carbohydrate polymer of bromelain. Phytochemistry 7: 1659.
Potty, V.H. 1969. Determination of proteins in the presence of phenols and pectins. Anal. Biochem. 29: 535.
Robinson, T. 1967. "The Organic Constituterts of Higher Plants," p. 28. Burgess Publishing Co., Minneapolis, MN.
Smith, F. and Montgomery, R. 1959. "The Chemistry of Plant Gums and Mucilages," p. 105. American Chemical Society Monograph Series, Reinhold Publishing Corporation.
Swain, T. and Hillis, W.E. 1959. The phenolic constitutents of Prunus Domestica I. The quantitative analysis of phenolic constituents. J. Sci. Food Agr. 10: 63.
Ms received 8/29/77; revised 12/21/77; accepted 12/30/77.

Journal Series No. 2180 of the Hawaii Agricultural Experiment Station, Honolulu, HI 96822.
This research was supported in part by the Pineapple Growers Association of Hawaii.
The authors thank Wallerstein Co., Novo Enzyme Corporation and Rohm and Haas Co. for enzyme samples. The authors also thank Drs. H. Chan and T.O.M. Nakayama for their assistance.
No endorsement of products named in this paper is implied.

GROWTH OF B. Bifidum IN LAB MEDIA . . . From page 1260

Mata, L.J. and Urrutia, J.J. 1971. Intestinal colonization of breast-fed children in a rural area of the low socioeconomic level. In. "Neonatal Enteric Infection Caused by E. Coli," Ed. Tennat, B. Ann. N.Y. Acad. Sci. 176: 93.
Mata, L.J., Carrillo, C. and Villaton, E. 1969. Fecal microflora in healthy persons in a preindustrial region. Appl. Microbiol. 17: 596.
Mayer, J.B. 1965. Viren und Darmflora. Paediat. Paedol. 1: 131.
Mayer, J.B. 1969. Interrelationships between diet, intestinal flora and viruses. Dairy Sci. Abs. 32: 2964.
Mikolajcik, E.M., Jao, Y.C. and Hansen, P.M.T. 1973. Effect of heat on biological activity of N-acetylglucosamine. J. Dairy Sci. 56: 631.
Muting, D., Eschrich, W. and Mayer, J.B. 1968. The effect of Bacterium bifidum on intestinal bacterial flora and toxic protein metabolites in chronic liver disease. Amer. J. Proctol. 19: 336.
Nichols, J.H., Bezkorovainy, A. and Landau, W. 1974. Human colostral whey M-1 glycoproteins and their L. bifidus var. penn. growth promoting activities. Life Sciences 14: 967.
Nicolai, H. von and Zilliken, F. 1972. Neuraminidase aus Lactobacillus bifidus var. pennsylvanicus. Hoppe-Seyler's Z. Physiol. Chem. 353: 1015.
Norris, R.F., Flanders, T., Tomarelli, R.M. and György, P. 1950. The isolation and cultivation of Lactobacillus bifidus: A comparison of branched and unbranched strains. J. Bact. 60: 681.
Poupard, J.A., Husain, I. and Norris, R.F. 1973. Biology of the bifidobacteria. Bacteriol. Rev. 37: 136.
Sandine, W.E., Muralikara, K.S., Elliker, P.K. and England, D.C. 1972. Lactic acid bacteria in food and health: A review with special reference to enteropathogenic Escherichia coli as well as certain entric diseases and their treatment with antibiotics and lactobacilli. J. Milk Food Technol. 35: 691.
Sasaki, M. and Yamashita, M. 1969. Growth stimulation by Escherichia coli of Lactobacillus bifidus. Medicine & Biology (Japan) 79: 79.
Schuler, R. 1973. Method for the production of a food for infants. Dairy Sci. Abs. 35: 2229.
Schuler, V.R., Ruppert, A. and Muller, F. 1968. Die milkro-organismen der Bifidusgruppe. 2. Mitteilund: die technologie der Bifiduskulture in milchverarbeitenden betrieb. Milchwissenchaft. 23: 554.
Tissier, H. 1899. La réaction chromophile d'Escherich et le Bacterium coli. Comptes Rendus des seances de la Société de Biologie. 51: 943. (cited by Bauer et al., 1975).
Ms received 8/11/77; revised 12/31/77; accepted 1/4/78.

Journal Article No. 123-77 of the Ohio Agricultural Research & Development Center, Wooster. Supported in part by Public Health Service Research Grants FD-00117 and FD-00462, Office of Research & Training Grants, FDA.

MEASUREMENT OF GEL WATER–BINDING CAPACITY BY CAPILLARY SUCTION POTENTIAL

THEODORE P. LABUZA and PIOTR P. LEWICKI

ABSTRACT

A new simple method has been developed which may be applicable in the measurement of the water-binding capacity (WBC) of moist foods and gels which easily deform under pressure. This study was designed to establish the procedure conditions using gels. The material to be measured is placed in a special cup, layered with filter paper of a predetermined moisture content and held at 6°C for 72 hr. From the change in moisture content of the filter paper after equilibration, the suction potential in N/m^2 of the measured material is read off a standard curve. This curve was prepared by measuring the water lost from filter paper in a standard soil test cell as a function of applied pressure. On this basis, the suction pressure as a function of moisture content can be determined at very high water contents.

INTRODUCTION

WATER is an important component of food. It comprises 75–95% of many food products. As such a major constituent, it affects shelf life and organoleptic properties. The rate and occurrence of chemical reactions, enzyme activity and microbial growth are dependent on the presence and state of water in food. From the physical point of view, water is related to mechanical strength, elasticity, plasticity and flow of food material.

Water present in foods is immobilized in some way, because if the food is cut, the water does not flow out. It is known that a small portion of water in foods is immobilized in mono- and multi-molecular layers as reviewed by Labuza (1968). Some of the water can also be contained in capillaries present in food materials (Labuza and Rutman, 1966). This water corresponds usually to 20–30g per 100g of dry matter. However, food products contain about 400–2,000g of water per 100g dry matter, and gels can imbibe as much as 5,000–9,000g of water per 100g dry matter. The bulk of this water contained in foods demonstrates almost the same properties as normal water.

The water imbibing power of foods is called either water-holding capacity (WHC) or water-binding capacity (WBC). These terms are typically used to mean the strength by which water is held in foods or gels but really are the water content under some given condition. Methods used to measure WBC are quite arbitrary, and they do not give any physical chemical property to express the immobilized part of water. Consequently, WBC should be defined in terms of a particular method of measurement, so that the results obtained in different laboratories may be comparable.

Common methods used to measure WBC are based mostly on the application of either pressure (Grau et al., 1953; Grau and Hamm, 1953, 1957 and Wierbicki and Deatherage, 1958) or centrifugal force (Marsh, 1952; Hamm, 1958; Penny et al., 1963; Miller et al., 1968; Bouton et al., 1971; and Sollars, 1973) to the product, with the subsequent measurement of water loss. In both cases the product undergoes deformation. It is not known whether water loss is entirely due to the force equilibrium or if it originates in part from the alterations in the internal structure of the material. Therefore, one must be very careful in interpreting WBC data. Moreover, none of the above-mentioned methods can be used with fairly elastic materials which deform easily without any water loss. Deformation beyond a certain level leads to the formation of cracks within the material and to destruction of its internal and external structure. These properties are inherent in many food products, i.e., cheeses, meat emulsions and food gels. Hence, there is a need for a method which would enable us to measure WBC of different types of food, such as food gels, and which would provide a means to express WBC quantitatively in defined physical chemical units. Other methods are available such as NMR, DTA, freezing point, etc.; however, these lead to much misinterpretation of the meaning of bound water (Labuza, 1974). In all cases using these methods the water can be defined as bound; however, it may still be available for reaction.

Another method of measuring water-binding is thermodynamically. The relative partial molar free energy of water immobilized in food may be calculated from measurements of osmotic pressure, of suction height, or of relative vapor pressure in accordance with the equations:

$$\Delta F = \bar{F} - \bar{F}_o = -\bar{V}\pi = -Mgh = RT\ln P/P_o \qquad (1)$$

where: $\bar{F} - F_o$ = molar free energy in measured and standard state; $\bar{V}$ = partial molar volume of water in food; π = osmotic pressure; M = molecular weight of water; h = suction head; R = gas constant; T = absolute temperature; P = water vapor pressure over food surface; and P_o = water vapor pressure over pure water.

Measurement of relative vapor pressure (water activity) of foods containing large amounts of immobilized water is difficult and imprecise (Labuza, 1974; Labuza et al., 1976). In addition, membranes in osmotic pressure measurements are not completely impermeable to all dissolved solutes, and the composition of the hypertonic solution can change in the course of measurement. However, the measurement of suction head can be used as one easy method of determining water-binding capacity.

Hoffman (1975) has proposed the use of gypsum porous plates to withdraw solution from meat tissue. The amount of air displaced in the porous plate by meat juice was considered as a measure of WBC. The method provides a means to measure WBC of different food products; however, the porosity of gypsum plates cannot be controlled and each batch of plates can be different from the capillary suction potential point of view. Moreover, to facilitate movement of meat juice from the tissue to the plate a compression force is used. Measurement of the suction head should be done with the aid of a standard porous material which can be easily calibrated, and which withdraws water from the material fast enough that application of any force will not be necessary.

The purpose of this work was to develop a method which could be used to measure water-binding capacity of gels as well

Author Labuza is with the Dept. of Food Science & Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108. Author Lewicki is with the Dept. of Food Technology, Warsaw Agricultural University (S.G.G.W.), Warszawa ul. Rakowiecka 26/30, Poland.

0022-1147/78/0004-1264$02.25/0
© 1978 Institute of Food Technologists

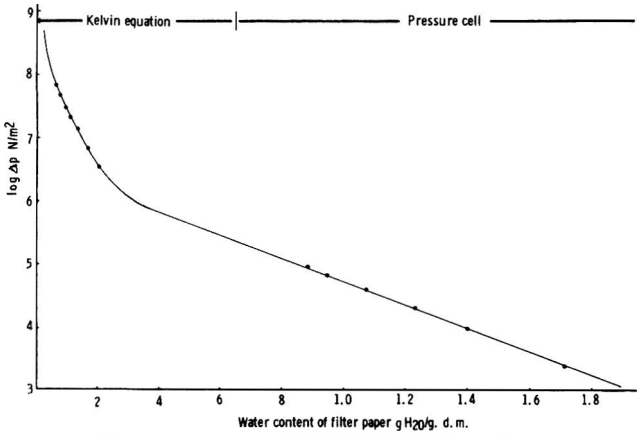


Fig. 1—Relationship between logarithm of suction potential and water content of Whatman No. 1 filter paper.

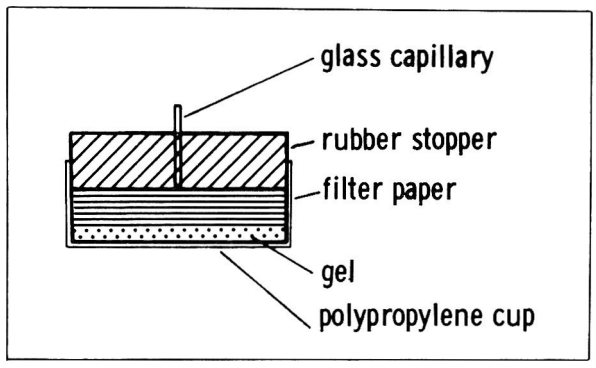


Fig. 2—Container for capillary suction measurement.

as moist food products. The method should provide a means to express WBC quantitatively in absolute units, and should be simple and based on the use of a standard porous material which can be easily calibrated. The suction potential of a material can be determined by allowing it to contact a capillary standard material. If done at a range of moisture contents for the standard, a value of moisture content for which no transfer would occur can be extrapolated and thus the standard suction potential of the original material can be calculated. This was the basis for the procedure developed; however, this paper presents only the experimental evidence to establish the conditions under which equilibrium is attained in a reasonable time.

METHODS

Gel preparation

Gelatin (General Gelatin Co.) of 250 Bloom and water content of 10% was suspended in an appropriate amount of deionized distilled water and warmed to 55°C with continuous stirring. The solution was cooled to 40°C, and at that temperature poured into containers for capillary suction potential measurement.

Potato starch was mixed with an appropriate amount of deionized distilled water, heated to 100°C with continuous stirring, and maintained at 100°C for 2 min. Thereafter, the container was immersed in a boiling water bath, and the solution dispensed into containers for the capillary suction potential measurement.

Carrageenan (Gelcarin HWG, Marine Colloids, Inc.) was mixed with water and brought to boiling with continuous stirring. Subsequent handling of the solution was exactly the same as for potato starch gels.

The special polypropylene containers used to measure capillary suction were filled with the gelling agent solution, placed into a desiccator with relative humidity kept at 100%, and stored for 24 hr at 6°C.

Determination of suction potential standard curve

Filter paper Whatman No. 1 was used as the standard material. It was dried under a vacuum of 750 mm Hg at 60°C for 24 hr before use. In order to vary the initial water content of the filter paper, the following procedure was used. Dry filter paper was placed in small glass jars, wetted with a predetermined amount of water. The glass jars were then tightly sealed and held at room temperature for 24 hr of equilibration.

The curve for suction potential vs moisture content at high moisture content for the capillary standard filter paper was done using the Tempe Pressure Cell (Soil Moisture Equipment Co.). Twenty pieces of completely wet filter paper were placed in the cell, and air under pressure was introduced into the system in a step-wise manner. At each pressure the amount of water pressed out of filter paper was measured. When the pressure reached the maximum level possible for the cell, the filter paper was removed from the cell and dried under vacuum. The water content of filter paper at each pressure was calculated on the basis of final filter paper wetness and the amount of water expressed from filter paper at each pressure and the relationship to capillary suction potential is shown in Figure 1.

An adsorption isotherm of the filter paper was obtained with the isopiestic method (Vos and Labuza, 1974). The sorption isotherm data in the range of water activities 0.60–0.95 were used to calculate the capillary suction from the Kelvin equation:

$$\Delta p = \frac{P_o \cdot \rho \cdot \ln a_w}{\rho_v} \qquad (2)$$

where: Δp = capillary suction, N/m^2; P_o = water vapor pressure, N/m^2; ρ = density of water, kg/m^3; a_w = water activity; and ρ_v = of water water vapor, kg/m^3. Again, the relationship between water content of filter paper and its capillary suction was obtained (Fig. 1) for the lower a_w range.

Determination of dry matter content of a gel

About 1g of a gel was dried at room temperature for 24 hr. Further drying was done under vacuum of 750 mm Hg at 60°C for 24 hr.

Measurement of capillary suction potential of gels

To achieve fast movement of water from the gel to the filter paper, a large contact surface and small thickness of the gel layer must be assured. Moreover, no large void spaces in the container for capillary suction potential measurement should be present, since water condensation on the surface of the void as well as saturation of air with water vapor will contribute to the random error of the measurement. In addition the relative masses can vary in a determination which would change the moisture at equilibrium for a determination. Thus the test was designed to vary the water content of the standard material and project the value to that moisture at which no exchange would occur.

Measurement of the capillary suction potential of gels was done in the polypropylene containers shown in Figure 2. A glass capillary was inserted in each rubber stopper in order to avoid pressure formation during stopping of the containers. Preliminary studies had shown that a capillary bore of 0.3 mm inserted into the rubber stopper was small enough to prevent any evaporation of water from the containers.

Filter paper was layered on the surface of gels set in the containers. The containers were tightly closed with rubber stoppers and stored at a given temperature until an equilibrium was attained. The equilibrium water content in the filter paper was then determined. This was done in triplicate for filter paper initially soaked to different moisture contents. At least 10 moisture values were used. Because the bottom paper would stick to the gel surface and carry off solids, only the papers above this were weighed and it is assumed the last one is the same. Knowing the initial filter paper weight and moisture content, the change in water content is determined by weighing. Then, plotting weight change in filter paper vs initial filter paper moisture content and extrapolating to zero change, the moisture content of paper in thermodynamic equilibrium with the gel could be found for a constant value of C. From that and the standard curve, the suction potential could be read or the value could be computed from Eq (3):

$$\Delta p = 10^{(6.402 - 1.730 \cdot U_i)} \qquad (3)$$

where U_i is the projected moisture at equilibrium and fits Eq (3) in the

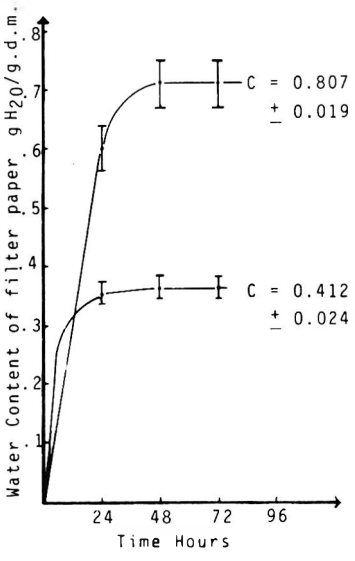


Fig. 3—Effect of the ratio of gel mass to filter paper mass (C) on the equilibration time of 4.51% gelatin gel and completely dry filter paper at 22° C.

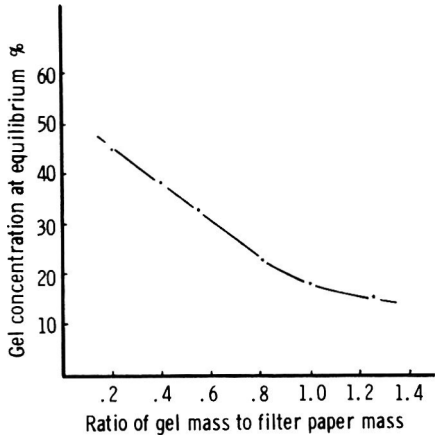


Fig. 4—Effect of the ratio of gel mass to filter paper mass on the equilibrium concentration of gelatin gels. Initial gel concentration 4.5% and completely dry filter paper. Equilibration done at 22° C for 48 hr.

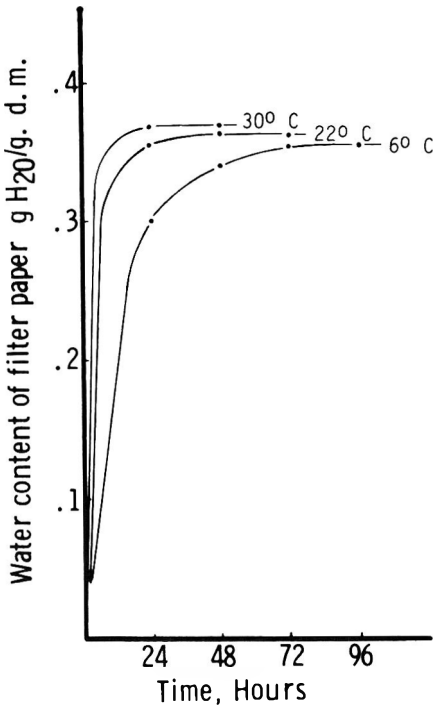


Fig. 5—Effect of temperature on the equilibration time of 4.6 gelatin gel and completely dry filter paper. The ratio of gel mass to filter paper. The ratio of gel mass filter paper mass is 0.400.

range of $0.4 < U_1 < 4.0$. At lower values of U_1 than 0.4, the capillary suction potential was read directly from the standard curve.

RESULTS & DISCUSSION

Ratio of gel mass to filter paper mass

In the development of the method, the mass of a gel in the container for capillary suction potential measurement was kept constant, whereas the number of filter paper circles placed on the surface of a gel were varied from 1 to 30. At a large ratio of gel mass to filter paper mass, a small amount of water withdrawn from a gel substantially increases the water content of filter paper and strongly decreases the driving force in the system. Therefore, at large ratios of gel mass to filter paper mass, a long equilibration time will be experienced, while at small ratios equilibrium should be attained at relatively short times.

Measurement of the kinetics of water uptake by filter paper at two different ratios of C which is gel dry mass to filter dry paper mass (Fig. 3) shows clearly that there is a definite effect of the ratio on the time of equilibration. Under the same experimental conditions, equilibrium is attained within 24 hr at C = 0.412, and within about 48 hr at C = 0.807. Moreover, as can be seen in Figure 4, the gel concentration at equilibrium is strongly dependent on the ratio of gel mass to filter paper mass. To dehydrate gelatin gel with an initial solids concentration of 4.5% to a final concentration of 45% the ratio of 0.2 is required for gel to dry filter paper mass. It is the final gel concentration that is at equilibrium and the first value that should be used. This could be calculated from the water difference between the paper and the initial gel. However, drawing water out of a gel changes its structure and thus its potential. Thus the proposed method uses varying intital filter paper water contents and projects the plot of increase in water content versus initial filter paper moisture to the moisture gain at which no gain takes place. An example will be given.

Temperature effect on the equilibration time

The gel preparation method applied in this study does not assure sterility of the gel. Moreover, gel formation and its structure are affected by the temperature in storage (Ferry, 1948; Izmailova et al., 1966; Aussel et al., 1965). Under these circumstances an effect of equilibration temperature on the equilibration time might be expected, and low temperatures at which microbial proliferation is very slow might be preferable for capillary suction potential measurement.

There is a pronounced effect of temperature on the equilibration time (Fig. 5 and Table 1). At 30°C equilibrium is attained within 24 hr while at 6°C, 75 hr are required. Moreover, the final water content of filter paper at equilibrium is different for each temperature. This effect can be attributed either to the change in gel structure or to the change in surface

Table 1—Effect of temperature on the equilibration time of 5% gelatin gel

Temp (°C)	Initial gel conc (%)	C	Water content[a] of filter paper in g H_2O/g d.m. at 24 hr	48 hr	72 hr	96 hr
6	4.99	0.400 ± 0.014	0.301 ± 0.010	0.341 ± 0.015	0.354 ± 0.011	0.353 ± 0.020
22	5.00	0.412 ± 0.024	0.356 ± 0.026	0.366 ± 0.022	0.361 ± 0.025	–
30	4.98	0.411 ± 0.009	0.370 ± 0.010	0.365 ± 0.012	–	–

[a] Results of 10 measurements

tension of water and the change in diameter of capillaries. Volume contraction, as well as the increase in surface tension at lower temperatures, both increase capillary suction potential of a gel and of filter paper as well. Therefore, it can be asssumed that the observed differences in water content of filter paper at equilibrium at different temperatures are mainly due to changes in gel structure. Clifford and Child (1971) suggested that lowering the temperature of gelatin gel slows down polymer chain mobility. In such a situation an average lift of small spacings between polymer chains should be longer at lower temperatures than at higher ones. In consequence, capillary suction potential of a gelatin gel should be inversely related to temperature. In fact, this is exactly what has been observed in this study.

Effect of initial water content of filter paper on the equilibration time

Filter paper made of cellulose fibers is composed of capillaries with a spectrum of radiuses. According to the Kelvin equation, the suction potential of a capillary is inversely proportional to its radius. Therefore, wetting of filter paper with different amounts of water produced material in which a portion of pores is filled with water. At low water contents of filter paper, the capillaries with the smallest radiuses are filled with water, while at high water contents only the large capillaries are accessible to water from the gel.

Filter paper with low initial water content has a large capillary suction potential and takes an appreciable amount of water from the gel. On the other hand, wet filter paper has a small capillary suction potential and withdraws a small volume of water from the gel. As a result of this difference, the relationship observed between increased initial water content of filter paper and equilibration time may be expected as seen in Figure 6. It is seen that the equilibration time decreases with increased initial water content of filter paper.

Effect of initial gel concentration on the Equilibration Time

Figure 7 shows that there is less effect between the initial gelatin gel concentration and the required equilibration time. For gelatin gels of solids concentration about 5, 10 and 22%, a time of 72 hr was required to attain equilibrium at 6°C as calculated by standard diffusion methods. The initial concentration of potato starch gel (Table 2) and Gelcarin gel (Table 3) affects equilibration time to a small degree. For potato starch gel at concentrations of 8, 16 and 33% an equilibration time between 54 and 72 hr is necessary at 6°C Gelcarin gels lose their water fast, and within 24 hr an equilibrium is attained at 6°C.

Effect of Filter paper Quality on the Capillary Suction Potential

Filter paper composed of cellulose fibers contains long fibers intermingled to form a matrix in which short pieces of fibers are loosely held. Therefore, it might be expected that washing of filter paper with water will remove loosely held fibers giving a product with fixed structure and constant capillary suction potential.

An experiment was conducted in which fresh filter paper as well as washed filter paper was used. Results (Fig. 8) show that

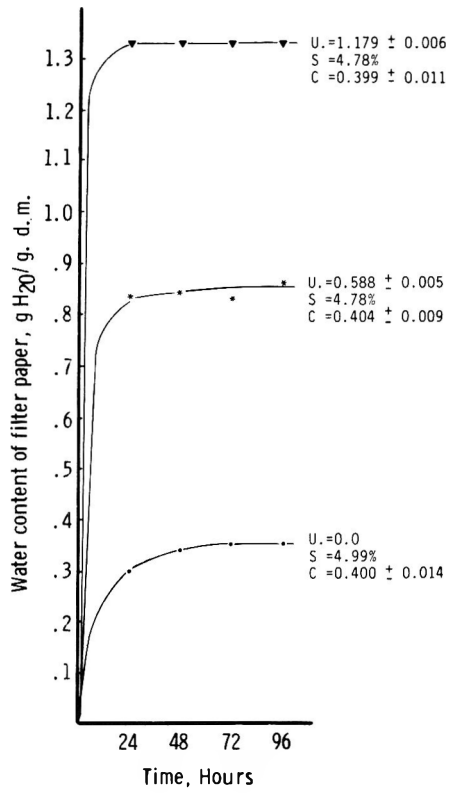


Fig. 6—Effect of initial water content of filter paper ($U_\bullet$) on the equilibration time of gelatin gels at 6° C of initial concentrations S and a gel to filter paper ratio of C.

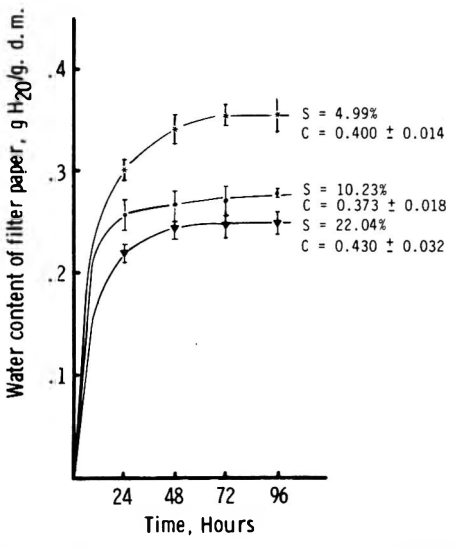


Fig. 7—Effect of initial gel concentrations on the equilibration time of gelatin gels and completely dry filter paper at 6° C. C is gel mass to filter paper ratio.

Table 2—Effect of the initial potato starch gel concentration on the equilibration time

Temp (°C)	Initial gel conc (%)	C	Water content[a] of filter paper in g H_2O/g d.m. at 25 hr	54 hr	72 hr	101 hr
6	8.11	0.409 ± 0.010	0.352 ± 0.012	0.347 ± 0.001	0.367 ± 0.007	0.365 ± 0.011
	15.99	0.412 ± 0.008	0.226 ± 0.008	0.272 ± 0.020	0.278 ± 0.010	–
	32.88	0.366 ± 0.038	0.169 ± 0.002	0.203 ± 0.031	0.201 ± 0.004	–

[a] Results of 10 measurements

Table 3—Effect of the initial carrageenan gel concentration on the equilibration time

Temp (°C)	Initial gel conc (%)	C	Water content[a] of filter paper in g H_2O/g d.m. at 24 hr	48 hr	72 hr
6	1.00	0.361 ± 0.011	0.356 ± 0.008	0.360 ± 0.008	0.351 ± 0.011
	2.02	0.372 ± 0.028	0.347 ± 0.010	0.352 ± 0.013	0.349 ± 0.012
	2.79	0.356 ± 0.011	0.322 ± 0.008	0.327 ± 0.012	0.328 ± 0.012

[a] Results of 10 measurements

Table 4—Effect of washing filter paper on the average mass of the filter paper circle (Statistical Analysis)

Quality of filter paper	Wt of filter paper circle[a] (grams)	Degrees of freedom	t_{calc}	t_{tabl} at P = 0.05
Fresh	0.12194 ± 0.00348	20	2.21	2.09
Washed	0.12004 ± 0.00180			

[a] Results of 20 measurements

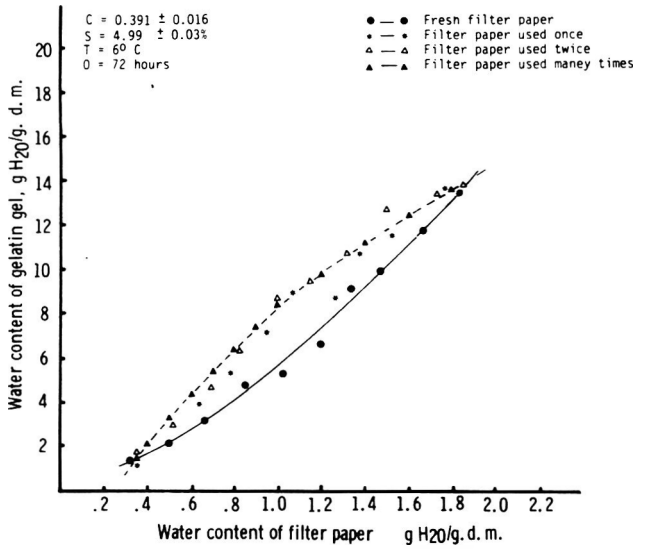


Fig. 8—Effect of washing filter paper on the equilibrium. Water content after 72 hr at 6°C.

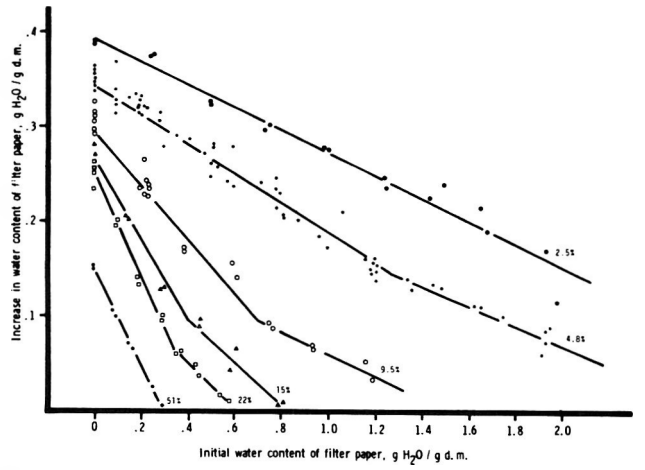


*Fig. 9—Relationship between the initial water content of filter paper and increase in water content of filter paper during the equilibration at 6°C for 72 hr for gelatin gels. (●—● 2.4%; ★—★ 4.83%; ○—○ 9.46%; △—△ 15.21%; □—□ 22.04%; *—* 50.67%)*

there is a pronounced difference between fresh and washed filter paper as far as capillary suction potential is concerned. Furthermore, it has been found that there is no significant difference between washed filter papers used once, twice and several times. Hence, it can be concluded that washed filter papers can be used many times for capillary suction potential measurement.

Washing of a filter paper circle in water at a temperature of about 50°C reduces its average weight from 0.12194g to 0.12004g. This difference is statistically significant (Table 4).

Reproducibility of the capillary suction potential measurement

Figure 9 shows the data for gelatin from Table 5 plotted in the manner to determine suction potential (i.e., increase in filter paper moisture content versus initial moisture content). As seen for several concentrations, a break exists in the line which is due to an abrupt physical change. Lewicki et al. (1976) have explained this with respect to possible crystallinity changes. However, the important point is the value projected to zero moisture increase. This would be the filter paper moisture content needed to cause no change in moisture. These values converted to suction potential are shown in Figure 10.

Statistical analysis of the data collected in this study have shown that reproducibility is a function of temperature, initial gel concentration and initial water content of filter paper. At higher temperatures the variability of the final water content in filter paper and final gel concentration is much larger than at low temperatures. The average standard deviation of final water content in filter paper at 22 and 6°C amounts to ± 0.020 and ± 0.018g H_2O/g d.m., respectively. For final gel

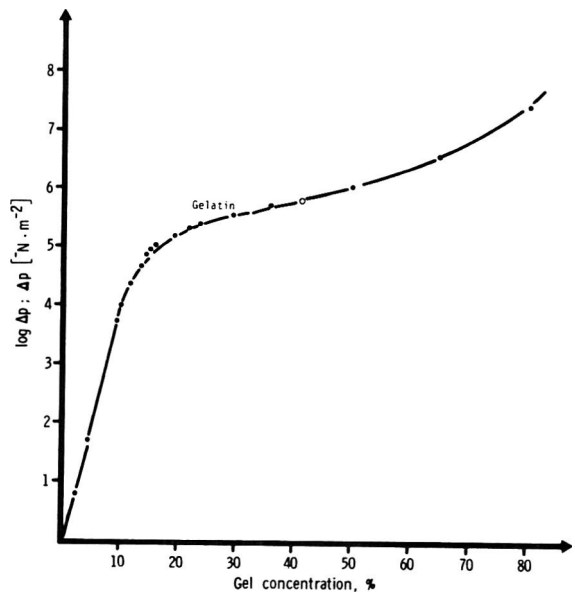


Fig. 10—Log suction potential Δp [N/m²] as a function of gelatin gel concentration.

Table 5—Effect of the initial gelatin gel concentration and teh initial water content of filter paper on the state of equilibrium

Temp (°C)	Initial gel conc (%)	Initial water content of filter paper g H_2O/g d.m.	C	Final water content of filter paper g H_2O/g d.m.	Final gel conc (%)
30	5.00	0.0	0.411 ± 0.010	0.365 ± 0.012	43.98 ± 7.03
22	5.00	0.0	0.412 ± 0.024	0.361 ± 0.025	50.33 ± 6.45
	5.00	0.0	0.412 ± 0.024	0.372 ± 0.025	51.09 ± 6.61
	4.92	0.0	0.411 ± 0.010	0.370 ± 0.010	51.39 ± 3.37
6	2.47	0.0	0.405 ± 0.011	0.392 ± 0.002	60.05 ±10.18
	2.47	0.240 ± 0.005	0.405 ± 0.011	0.614 ± 0.006	23.26 ± 6.25
	2.47	0.250 ± 0.004	0.405 ± 0.011	0.625 ± 0.003	22.87 ± 5.82
	2.47	0.495 ± 0.001	0.405 ± 0.011	0.821 ± 0.001	12.62 ± 1.67
	2.47	0.740 ± 0.008	0.405 ± 0.011	1.038 ± 0.012	9.72 ± 0.16
	2.47	0.991 ± 0.011	0.405 ± 0.011	1.268 ± 0.009	7.78 ± 0.21
	2.47	1.244 ± 0.002	0.405 ± 0.011	1.484 ± 0.005	5.84 ± 0.35
	2.47	1.439 ± 0.002	0.405 ± 0.011	1.663 ± 0.004	5.63 ± 0.28
	2.47	1.499 ± 0.003	0.405 ± 0.011	1.737 ± 0.002	5.58 ± 0.31
	2.47	1.672 ± 0.007	0.405 ± 0.011	1.875 ± 0.012	4.93 ± 0.42
	2.47	1.928 ± 0.003	0.405 ± 0.011	2.096 ± 0.008	4.42 ± 0.39
	2.47	1.979 ± 0.005	0.405 ± 0.011	2.094 ± 0.006	3.43 ± 0.40
6	4.99	0.0	0.400 ± 0.014	0.354 ± 0.015	40.85 ± 3.50
	4.49	0.193 ± 0.004	0.408 ± 0.013	0.521 ± 0.020	23.11 ± 2.90
	4.85	0.279 ± 0.006	0.408 ± 0.013	0.577 ± 0.016	18.30 ± 3.03
	4.94	0.504 ± 0.005	0.408 ± 0.013	0.773 ± 0.019	14.84 ± 1.47
	4.78	0.588 ± 0.005	0.401 ± 0.010	0.844 ± 0.016	13.12 ± 1.34
	4.85	1.006 ± 0.007	0.408 ± 0.013	1.289 ± 0.016	15.72 ± 1.51
	4.78	1.179 ± 0.006	0.401 ± 0.010	1.331 ± 0.002	7.72 ± 0.24
	4.96	1.491 ± 0.032	0.408 ± 0.013	1.645 ± 0.040	7.74 ± 2.12
6	9.45	0.0	0.411 ± 0.010	0.313 ± 0.007	39.86 ± 2.12
	9.44	0.200 ± 0.004	0.410 ± 0.014	0.424 ± 0.008	20.99 ± 2.49
	9.60	0.239 ± 0.005	0.411 ± 0.010	0.478 ± 0.012	22.99 ± 0.73
	9.44	0.391 ± 0.005	0.410 ± 0.014	0.560 ± 0.007	16.00 ± 0.09
	9.44	0.598 ± 0.012	0.410 ± 0.014	0.746 ± 0.001	14.69 ± 0.82
	9.44	0.757 ± 0.005	0.410 ± 0.014	0.847 ± 0.001	12.17 ± 0.28
	9.44	0.936 ± 0.005	0.410 ± 0.014	1.003 ± 0.004	11.38 ± 0.01
	9.44	1.175 ± 0.016	0.410 ± 0.014	1.217 ± 0.001	10.55 ± 0.42
6	22.04	0.0	0.422 ± 0.022	0.266 ± 0.008	50.31 ± 1.30
	22.04	0.095 ± 0.002	0.422 ± 0.022	0.293 ± 0.004	40.50 ± 0.10
	22.04	0.193 ± 0.004	0.422 ± 0.022	0.332 ± 0.001	32.67 ± 0.68
	22.04	0.294 ± 0.002	0.422 ± 0.022	0.392 ± 0.002	28.36 ± 0.11

concentrations respective values of average standard deviation with respect to 22 and 6°C are: ± 5.48 and ± 3.50%.

The effect of initial gel concentration on the reproducibility of the capillary suction potential measurement is very pronounced. Average standard deviations of final water content in filter paper at initial gel concentrations of 5, 10 and 22% are equal to ± 0.018, ± 0.004 and ± 0.004g H_2O/g d.m., respectively. On the other hand, average standard deviations of final gel concentration amount to ± 3.5, ± 2.12 and ± 1.30% at initial gelatin gel concentrations of 5, 10 and 22% respectively. These results may suggest that the initial gelatin concentration determines the internal structure of the gel. At low initial gel concentration either the structure formed upon cooling is quite random, or mobility of macromolecules in a gel is large, which in effect produces a system of low reproducibility. On the other hand, higher concentrations of gelatin promote formation of definite structures or restrict mobilities of macromolecules in a gel. As a result, better reproducibility of capillary suction potential is obtained.

Data contained in Table 5 show also that variability in final gel concentration is affected by the initial water content of filter paper. Wet filter paper has a small capillary suction potential, and therefore, the amount of water withdrawn from a gel varies within a narrow range. At high capillary suction potentials variability is strongly affected by gel structure, i.e., the initial gel concentration. In a subsequent paper we will present the data for the suction potential of the various gels as a function of concentration.

CONCLUSION

THE PROPOSED METHOD to measure water-binding capacity of gels by capillary suction potential is very simple and requires only elementary laboratory equipment. The standard porous material can be easily calibrated and water-binding capacity can be expressed in defined units of pressure. The measurement is affected by initial gel concentration, initial water content of filter paper and temperature of equilibration. For three gels studied the time of 72 hr at 6°C was appropriate to attain equilibrium at the highest suction potential differential. The increase of driving forces decreases the equilibration time substantially.

Reproducibility of measurement is very good. The water-binding capacity of gels can be measured with a coefficient of variation of ± 2.5%. More variable and less reproducible is the gel concentration attained at the equilibrium state. For low initial gel concentrations the coefficient of variation amounts to ± 15% of the measured value, while at high initial gel concentrations it is equal to ± 3%. It is suggested that at low gel concentrations the internal structure of a gel is very random and cannot be reproduced with a standard gel formation procedure.

REFERENCES

Aussel, P., Chanal, J.L. and Marignan, R. 1965. A study of the diffusion of water in macromolecular gels. Trav. Soc. Pharm. Montpellier. 25(2): 145.

—Continued on page 1273

EMULSIFICATION PROPERTIES OF GROUNDNUT PROTEIN

G. RAMANATHAM, LEE HEA RAN and LEELA N. URS

ABSTRACT

The ability of groundnut protein to emulsify soyabean oil was studied under various experimental conditions. The emulsification capacity (EC) of groundnut protein isolate was highest at 5 mg/ml protein concentration. The EC vs pH profile resembled a typical protein solubility curve and the EC of groundnut protein isolate was greatest at pH 10.0. Sodium chloride at low concentrations (0.05–0.2M) increased the EC of protein dispersed, in the pH range 7–10. Groundnut protein isolate showed poor emulsifying properties in water (pH 6.8) in contrast to casein or soyabean protein. Results with groundnut flour indicated that it was a less effective emulsifier than the protein isolate. EC increased with the fineness of the flour, indicating the influence of particle size. Less amount of oil was emulsified at higher mixing speeds.

INTRODUCTION

PROTEINS are surface active substances. In a system consisting of water, oil and proteins, they facilitate the formation of emulsions by lowering the surface tension. The emulsifying capacity (EC) of meat, milk and vegetable proteins has been studied (Swift et al., 1961; Pearson et al., 1965; Inklaar and Fortuin, 1969; Acton and Saffle, 1971).

Many physical and chemical factors are involved in formation, stability and rheology of the protein emulsions. Efficiency of emulsification varies with the type of protein, its concentration, pH, ionic strength, viscosity of the system, temperature and the method of preparation of emulsion (Saffle, 1968). Several methods for the determination of emulsification capacity of protein have been described (Swift et al., 1961; Carpenter and Saffle, 1964; Pearson et al., 1965; Borton et al., 1968; Ivey et al., 1970; Hegarty et al., 1963; Crenwelge et al., 1974).

In the present investigation, the EC of groundnut flour and groundnut protein isolate has been measured. The effect of variables such as protein concentration, pH, presence of sodium chloride, speed of mixing, etc. has been studied. The EC of groundnut protein has been compared with casein and soyabean protein, which are known for their good emulsifying properties (Pearson et al., 1965).

EXPERIMENTAL

Groundnut flour

Edible groundnut flour obtained by expeller process (Subrahmanyan et al., 1957) was solvent extracted with hexane to remove the residual oil. The solvent extracted flour was ground and the flour passing through 60, 100 and 200 mesh were used in the study.

Groundnut protein isolate

The protein was prepared according to the process developed at CFTRI (Bhatia et al., 1966). The wet protein isolate was freeze dried and stored. Protein dried by spray drying and roller drying was also used to study the effect of processing on EC. The protein content of the dry protein isolate was 90.0%.

Casein

Skim milk powder was dispersed in water and the protein was precipitated by isoelectric precipitation at pH 4.8. The precipitate was separated by centrifugation, washed with water and freeze dried. The material had a protein content of 80%.

Soyabean protein

Defatted soy flour was dispersed in aqueous medium at pH 8.5 and centrifuged to remove the insoluble carbohydrate fraction. The supernatant was adjusted to pH 4.8 when the protein precipitated. The precipitate was centrifuged, washed with water and freeze dried. The protein content of the material was 71.5%.

Oil source

Refined soyabean oil obtained from USAID was used.

Determination of emulsion capacity

For determining emulsion capacity the method of Swift et al. (1961) was used with slight modifications. A desired amount of the groundnut flour or protein isolate was dispersed in 50 ml of distilled water in a Waring Blendor (Braun, West Germany). Oil was added and the mixture blended at 18,700 rpm for 1 min. A number of aliquots of such protein emulsions were prepared keeping the oil content as the only variable. The oil content between aliquots was varied by 5 ml increments till the emulsion broke. The emulsion breaks when the amount of oil exceeds EC of the protein. The breaking point was determined by measuring the viscosity of the emulsion which shows a sudden decrease when emulsion breaks. Visual examination also sufficed for the detection of emulsion inversion. The volume of oil added prior to the inversion point was recorded and the emulsification capacity was expressed as ml of oil emulsified per gram of protein isolate or flour.

Viscosity measurement

Viscosity of the emulsions, expressed as poise, was measured with a Ferranti Portable Viscometer (Model LV) using spindle VH and cylinder B at speed 3.

Emulsification capacity of groundnut protein isolate

The influence of different variables on the EC of the protein was studied as follows.

Effect of protein concentration. EC of the protein at different concentrations of neutralized protein (5–90 mg/ml) in 50 ml of distilled water was determined by viscosity measurements.

Effect of pH. The protein dispersion in distilled water was adjusted to the desired pH of 3–10 with either 3N HCl or 3N NaOH and EC was determined in each case.

Effect of sodium chloride at different pH range. EC at different pH ranges in presence of different molarities of NaCl (0.05–1.0M) was determined.

Effect of different drying methods. EC of freeze-dried, spray-dried and roller-dried protein isolates was determined at pH 6.8.

Effect of shear rate on viscosity. The nature of the protein emulsion was determined by applying various shear rates on the emulsion, prepared by using 2% protein isolate, at ambient temperature (27°C). A Brookfield Synchrolectic Viscometer Model LVT with spindle No. 1 was used.

Emulsification capacity of groundnut flour

The effect of concentration, particle size, and rate of mixing (13,000 rpm, 15,000 rpm and 18,700 rpm) on the EC of groundnut flour was studied.

Emulsification capacity of casein and soyabean protein

EC of casein and soyabean protein was determined at different pH values. Since the protein content of casein and soyabean protein were not identical to that of groundnut protein isolate, their weights were so adjusted that the protein concentration of the solution was 900 mg/50 ml.

Authors Ramanatham and Urs are with the Central Food Technological Research Institute, Protein Technology Discipline, Mysore 570013, India. Author Ran, formerly with the Central Food Technological Research Institute, is a doctorate student in the Food Science & Technolgy Dept., Iowa State University, Ames, IA 50013.

0022-1147/78/0004-1270$02.25/0
© 1978 Institute of Food Technologists

Table 1—Emulsification capacity and viscosity at different concentrations of groundnut flour[a]

Flour conc (g/50 ml)	Protein conc mg/ml	Emulsification capacity ml oil/g groundnut flour	Viscosity[b] (poise)
1	10	50	0.23
3	30	25	1.63
5	50	16	5.83
7	70	12.1	10.72
9	90	10.5	19.13

[a] Protein content of groundnut flour 50%. Emulsion was prepared using 50 ml distilled water at pH 6.8 and blending with refined soyabean oil for 1 min at 18,700 rpm. Viscosity of emulsion measured with Ferranti Portable viscometer (Model LV) using spindle VH and cylinder B at speed 3.
[b] Viscosity of emulsion prior to inversion point

Table 2—Effect of particle size on emulsification capacity of groundnut flour and viscosity of the emulsion

Groundnut flour (mesh)	Crude fiber (g/100g flour)	Emulsification capacity (ml oil/g of flour)	Viscosity[a] (poise)
60	3.83	16	5.83
100	3.33	18	9.33
200	3.0	21	10.86

[a] Viscosity of emulsion prior to inversion point. Groundnut flour was used at 5 g/50 ml conc in distilled water at pH 6.8. Other conditions were same as given in Table 1.

RESULTS & DISCUSSION

Effect of protein concentration

EC of protein isolate was highest at 5 mg/ml protein concentration. Thereafter the EC progressively decreased with increasing protein concentration (Fig. 1). Similar results of decrease in EC with protein concentration have been reported on meat proteins (Acton and Saffle, 1972; Tsia et al., 1972), sodium soy proteinate, potassium caseinate and nonfat dry milk (Pearson et al., 1965).

EC of groundnut flour was lower than that of protein isolate (Table 1 and Fig. 1). For instance, at 90 mg/ml protein concentration its EC was 10.5, whereas the EC of protein isolate at the same protein concentration was 19. This difference is perhaps due to the adverse influence of the high carbohydrate and fiber content in the groundnut flour. The effect of particle size (Table 2) on EC indicated that decrease in particle size increased EC appreciably. This effect can be correlated with the fiber content of groundnut flour which decreased as the mesh size of the flour increased. It has been reported by Yatsumatsu et al. (1972) that EC of soyabean products correlated positively with protein content and negatively with fiber content of the sample. The behavior of groundnut products follows the same pattern (Table 2).

Effect of pH

EC of the protein was affected markedly by pH (Fig. 2). The EC vs pH profile resembled a typical protein solubility curve suggesting that the emulsifying property was due to the dissolved protein. It is interesting to note that groundnut protein was more efficient in emulsifying the oil at the acidic pH of 3.0 than at alkaline pH of 8.0. However, at pH 10.0 it showed the highest EC. The EC vs pH profile of groundnut protein resembles that obtained with soy protein, cottonseed or nonfat dry milk (Crenwelge et al., 1974). However, it was much different from those obtained with water soluble protein extracted from meat and leaf protein concentrate of alfalfa (Swift and Sulzbacher, 1963; Wang and Kinsella, 1976).

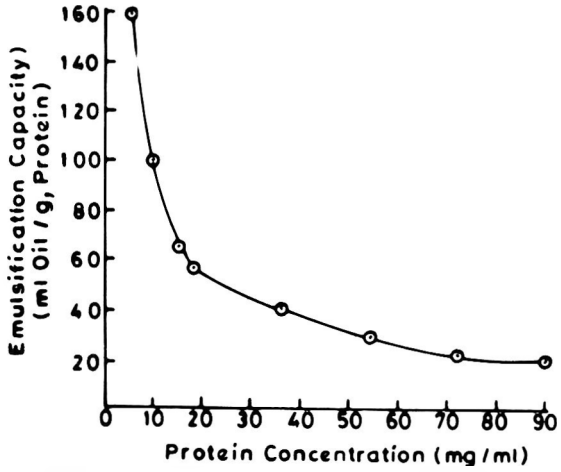


Fig. 1—Effect of protein concentration on emulsification capacity of groundnut protein isolate in distilled water, pH 6.8. Emulsion prepared by blending with refined soyabean oil for 1 min at 18,700 rpm.

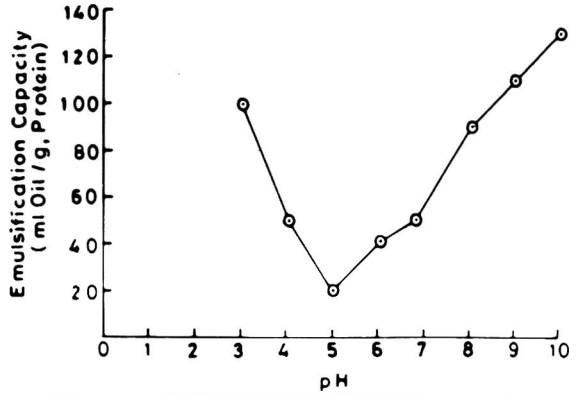


Fig. 2—Effect of pH on emulsification capacity of groundnut protein isolate in distilled water. Protein content of isolate = 90%. Other conditions were same as given in Fig. 1.

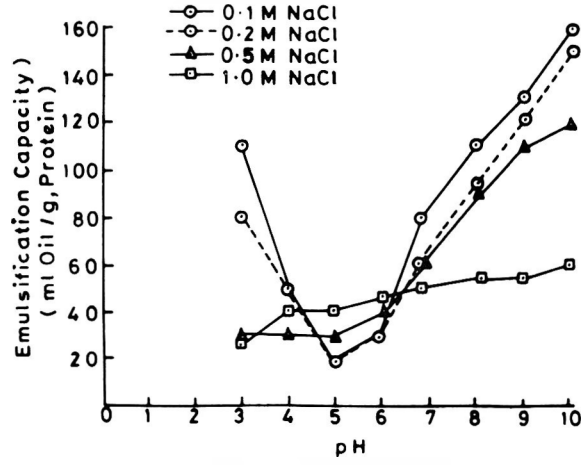


Fig. 3—Effect of sodium chloride on emulsification capacity of groundnut protein isolate. (Data obtained at 0.05M, 0.3M and 0.4M NaCl, not presented; appreciable change in EC was observed only at 0.1M, 0.2M, 0.5M and 1M NaCl conc).

Effect of NaCl

NaCl at low concentrations (0.05–0.2M) increased EC over that of the control in the pH range of 7–10 (Fig. 2 and 3). At

Table 3—Influence of the method of drying groundnut protein isolate on its emulsification capacity[a]

Method of drying	Emulsification capacity (ml of oil/g protein isolate)
Spray	55
Roller	50
Freeze	55

[a] Protein content of isolate = 90%; protein isolate was used at 1 g/50 ml conc in distilled water at pH 6.8. Other conditions of emulsification same as in Table 1.

Table 4—Effect of the rate of mixing on the emulsification capacity and viscosity of groundnut flour emulsions

Rate of mixing (rpm)	Emulsification capacity (ml oil/g flour)	Viscosity[a] (poise)
13,000	18	4.89
15,000	17	5.13
18,700	16	5.83

[a] Viscosity of emulsion prior to inversion point. Groundnut flour of 60 mesh was used at 5 g/50 ml conc. Other conditions were same as given in Table 1.

Table 5—Effect of protein concentration on the viscosity of groundnut protein isolate emulsions[a]

Protein conc (g/50 ml)	Quantity of oil emulsified	Viscosity[b] (poise)
0.9	55	0.12
1.8	80	1.4
2.7	84	4.2
3.6	85	6.67
4.5	95	9.33

[a] Emulsification conditions same as given in Table 1. Linear regression of viscosity (y) on protein concentration (x): $y = 2.63x - 2.76$. The slope of the line supports the hypothesis that viscosity increases uniformly with protein concentration. The t-test of significance for slope has been applied [$t_{0.05} = 3.18$, d.f. = 3].

[b] Viscosity of emulsion prior to inversion point.

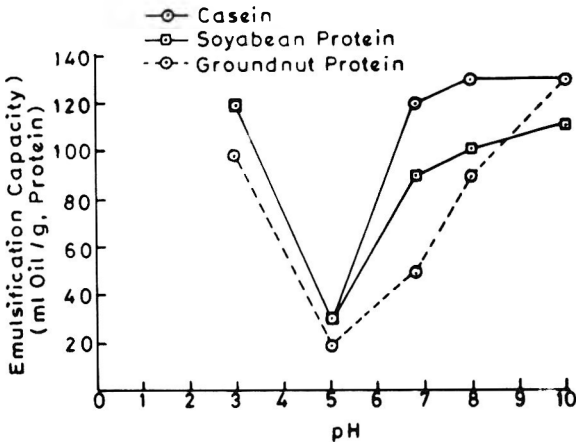


Fig. 4—Emulsification capacity of casein, soyabean protein and groundnut protein at different pH range. Protein concentration of the solutions 900 mg/50 ml of distilled water; blending time with refined soyabean oil for 1 min at 18,700 rpm.

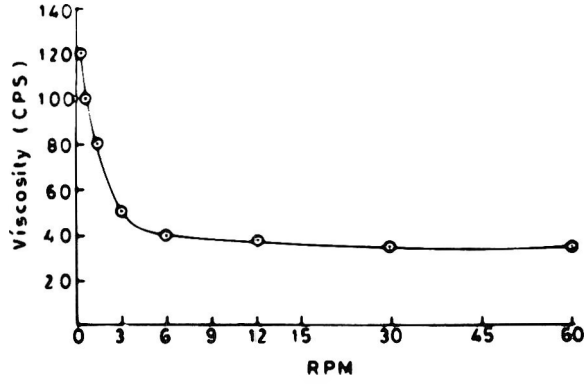


Fig. 5—Effect of shear rate on the viscosity of groundnut protein isolate emulsion. Emulsion prepared using 2% groundnut protein isolate (pH 6.8) at 27°C. Brookfield Synchrolectic Viscometer Model LVT with Spindle No. 1 was used for measuring viscosity of emulsion before inversion point.

acidic pH 3.0, the increase in EC was observed only at 0.1M NaCl concentration. The effect of increase in EC due to NaCl was also found in meat proteins (Carpenter and Saffle, 1965; Swift and Sulzbacher, 1963; Hegarty et al., 1963) and leaf protein concentrate (Wang and Kinsella, 1976). At higher NaCl concentration, EC decreases considerably, especially at low pH's. This is due to the precipitation of the protein and consequent decrease in the dissolved protein content. In the pH range 4–6, there was no appreciable change in EC at different NaCl concentrations (Fig. 2 and 3).

Effect of EC on drying of the protein

EC of roller dried protein isolate was slightly lower than that of spray-dried or freeze-dried material (Table 3). This may be due to the fact that in roller drying the protein is exposed to higher temperature and structural changes may occur.

Emulsification at different rates of mixing

EC appeared to decrease with the increase in speed of mixing (Table 4). Similar observations have been reported by many workers on meat proteins (Hegarty et al., 1963; Carpenter and Saffle, 1964; Swift et al., 1961; Ivey et al., 1970). Carpenter and Saffle (1964) have concluded that this is due to the fact that increased speeds produce greater shear rate which decreases the particle size of the oil droplet. There is thus an increase in the surface area of the oil to be emulsified by a limited amount of soluble protein. Ivey et al. (1970) observed that the diameter of the oil droplet decreased with the increase of speed of mixing.

EC of casein and soyabean protein

When compared to casein, groundnut protein isolate showed a lower EC at pH 6.8 (Fig. 4). However, at pH 10, EC of groundnut protein isolate was similar to that of casein. EC of casein was almost the same between pH 6.8 and 10, unlike the case of groundnut protein. Soyabean protein showed good emulsifying property at pH 6.8 in contrast to groundnut protein isolate. In the acidic pH 3.0, the soy protein had a higher EC than groundnut protein. At the isoelectric point, as expected, all three proteins had very poor emulsification property.

Viscosity

The viscosity very much depended on the shear rate (Fig. 5) suggesting the non-Newtonian nature of the emulsion. The emuslion was more specifically pseudoplastic since the viscosity decreased with the increase of shear rate.

As the protein concentration increased more oil was taken up (higher dispersed phase level) during emulsification, resulting in the increase of viscosity before the inversion point (Table 5). Viscosity of the emulsion increased with fineness of the flour (Table 2). It was notable that viscosity of the emulsion obtained with the flour of 200 mesh size was almost

double that of 60 mesh size. There was also a viscosity increase when the speed of mixing increased from 13,000 rpm to 18,700 rpm (Table 4).

CONCLUSION

GROUNDNUT PROTEINS are used in the preparation of a number of food products such as confections, bakery products, milk preparation, infant foods, ice-cream, coffee whitener and other special foods. In these formulations, use of synthetic emulsifiers can be avoided or reduced if the protein has high emulsifying property.

The present studies reveal that groundnut proteins have good emulsifying ability. The variables which affect EC of groundnut protein are its concentration in the solution, presence of NaCl, pH of the system, particle size and speed of mixing. The most important variable is pH of the solution. This is related to the solubility of the protein at different pH's. These results are in conformity with the observations made by other workers on meat proteins, casein and soyabean protein.

REFERENCES

Acton, J.C. and Saffle, R.L. 1970. Stability of oil in water Emulsions. 1 Effect of surface tension, level of oil, viscosity and type of protein. J. Food Sci. 35: 852.
Acton, J.C. and Saffle, R.L. 1971. Stability of oil-in-water emulsions. 2. Effects of oil phase volume, stability test, viscosity, type of oil and protein additive. J. Food Sci. 36: 1118.
Acton, J.C. and Saffle, R.L. 1972. Emulsifying capacity of muscle protein: phase volumes at emulsion collapse. J. Food Sci. 37: 904.
Bhatia, D.S., Parpia, H.A.B. and Baliga, B.P. 1966 Peanut Protein Isolate-Production and Properties. J. Food Sci. & Tech. 3: 2.
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.
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.
Carpenter, J.A. and Saffle, R.L. 1965. Some physical and chemical factors affecting the emulsifying capacity of meat protein extracts. Food Technol. 19(10): 111.
Crenwelge, D.D., Dill, C.W., Tybor, P.T. and Landmann, W.A. 1974. A comparison of the emulsifying capacity of some protein concentrates. J. Food Sci. 39: 175.
Hegarty, G.R., Bratzler, L.J. and Pearson, A.M. 1963. Studies on the emulsifying properties of some intracellular beef muscle proteins. J. Food Sci. 28: 663.
Inklaar, P.A. and Fortuin, J. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. Food Technol. 23: 103.
Ivey, F.J., Webb, N. and Jones, V.A. 1970. Effects of disperse phase, droplet size and interfacial film thickness on emulsifying capacity and stability of meat emulsions. Food Technol. 24: 1279.
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.
Saffle, R.L. 1968. Meat emulsions, Adv. Food Res. 16: 105.
Subrahmanyan, V., Rama Rao, G., Kuppuswamy, S., Narayana Rao, M. and Swaminathan, M. 1957. Standardization of conditions for the production of Indian multipurpose Food. Food Sci. 6: 76.
Swift, C.E., Lockett, C. and Fryar, A.J. 1961. Comminuted meat emulsions—The capacity of meats for emulsifying fat. Food Technol. 15: 468.
Swift, C.E. and Sulzbacher, W.C. 1963. Factors affecting meat protein as emulsion stabilizer. Food Technol. 17: 224.
Tsai, R., Cassens, R.G. and Briskey, E.J. 1972. The emulsifying properties of purified muscle proteins, J. Food Sci. 37: 286.
Wang, J.C. and Kinsella, J.E. 1976. Functional properties of novel proteins: Alfalfa leaf protein. J. Food Sci. 41: 286.
Yatsumatsu, K., Sawada, K., Moritaka, S., Toda, J. and Ishii, K. 1972. Whipping and emulsifying properties of soybean products. Agr. Biol. Chem. 36: 719.
Ms received 8/11/77; revised 12/9/77; accepted 12/14/77.

The authors gratefully acknowledge the help of Dr. M.S. Narasinga Rao, Project Coordinator, Protein Technology Discipline in critically going through the manuscript and for his helpful suggestions during the progress of the work.

GEL WATER-BINDING CAPACITY MEASUREMENT . . . From 1269

Bouton, P.E., Harris, P.V. and Shorthose, W.R. 1971. Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. J. Food Sci. 36: 435.
Clifford, J. and Child, T.F. 1971. Water in protein and polysaccharide gels. Proceedings of the First European Biophysics Congress, Baden, Austria, September. Ed. E. Broda and A. Locker, H. Springer-Lederer. Verlag der Wiener Medizinischen Akademie, Wien. 3: 461.
Ferry, J.D. 1948. Protein gels. Adv. Prot. Chem. 4: 1.
Grau, R. and Hamm, R. 1953. Eine einfache Methode zur Bestimmung der Wasserbindung in Muskel. Naturwissenschaften. 40: 29.
Grau, R. and Hamm, R. 1957. Uber das Wasserbindugsvermogen des Saugetiermuskels. 2. Mitt. Uber die Bestimmung der Wasserbindung des Muskels. Z. Lebensmittelunters. u-Forsch. 105: 446.
Grau, R., Hamm, R. and Baumann, A. 1953. Uber das Wasserbindungsvermogen des Saugetiermuskels. 1. Mitt. Der Einfluss des pH-Wertes auf die Wasserbindung von zerkleinertem Rindermuscle. Biochem. Z. 325: 1.
Hamm, R. 1958. Uber die Mineralstoffe des Saugetiermuskels. 1. Mitt. Magnesium, Calcium, Zink und Eisen und ihre Bedeutung fur die Muskelhydration. Z. Lebensmittelunters. u-Forsch. 107: 423.
Hoffman, K. 1975. Ein neues Gerat zur Bestimmung der Wasserbindung des Fleisches: Das "Kapillar-Volumeter." Fleischwirtschaft. 55(1): 25.
Izmailova, V.N., Ali, S.A. and Pchelin, V.A. 1966. Gelation of gelatin solutions. Fiztkhim. Mekhan. Dispersnykh. Struktur. Akad. Nauk SSR, Sb. Statel. 77.
Labuza, T.P. 1968. Sorption properties of foods. Food Technol. 22: 263.
Labuza, T.P. 1974. Sorption phenomena in foods: Theoretical and practical aspects. In "Theory, Determination and Control of Physical Properties of Food Materials," Ed. Rha. C. D. Reidel Publishing Co., Dordrecht, Holland.
Labuza, T.P. 1975. 1. The interpretation of sorption data in relation to the state of constituent water. 2. Oxidative changes in foods at low and intermediate moisture levels. In "Water Relations," Ed. Duckworth, R. Academic Press, London.
Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J. and McCall, W. 1976. Water activity determination: A collaborative study of different methods. J. Food Sci. 41: 910.
Labuza, T.P. and Rutman, M. 1968. The effect of surface active agents on sorption isotherms of a model food system. Can. J. Chem. Eng. 46: 364.
Liewicki, P.P., Busk, G.C. and Labuza, T.P. Measurement of gel water-binding capacity of gelatin, potato starch and carrageenan gels by suction pressure. J. Colloid & Interface Sci. (In press).
Marsh, B.B. 1952. Observations on rigor mortis in whale muscle. Biochim. Biophys. Acta. 9: 217.
Miller, W.O., Saffle, R.L. and Zirkle, S.B. 1968. Factors which influence the water-holding capacity of various types of meat. Food Technol. 22(9): 89.
Penny, I.F., Voyle, C.A. and Lawrie, R.A. 1963. A comparison of freeze-dried beef muscle of high or low ultimate pH. J. Sci. Food Agric. 14: 535.
Sollars, W.F. 1973. Fractionation and reconstitution techniques for studying water retention properties of wheat flours. Cereal Chem. 50: 708.
Vos, P. and Labuza, T.P. 1974. A technique for measurement of water activity in the high a_w range. J. Agr. Food Chem. 22: 326.
Wierbicki, E. and Deatherage, F.E. 1958. Determination of water-holding capacities of fresh meats. J. Agr. Food Chem. 6: 387.
Ms received 9/10/77; revised 1/21/78; accepted 1/30/78.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

This is paper No. 9878 from the University of Minnesota Agricultural Experiment Station. This study was supported in part by the University of Minnesota Agricultural Experiment Station Project No. 18-72 and Contract NAS 9-12560, NASA, Lyndon Johnson Space Center, Houston. TX.

ULTRASTRUCTURAL CHANGES IN DEFATTED SOY FLOUR INDUCED BY NONEXTRUSION TEXTURIZATION

M. V. TARANTO and K. C. RHEE

ABSTRACT

A Korean "textured" snack food press (Hand Press) was used to produce nonextrusion soy products. Constant processing conditions were plate temperature, top and bottom (190°C); pressure (21.1 kg/cm²); raw material moisture (30%) and premoistened sample mass (25g). The varying condition was retention time (2, 4, 6, 8 and 10 sec). The present study was initiated to investigate the sequence of cellular and sub-cellular changes taking place during the development of texture in defatted soy flour. Ultrastructural changes produced during nonextrusion texturization occurred in the following well-defined stages: two (2) sec stage, fusion of flour components; 2–8 sec stage, protein bodies deformed, ruptured and fused with carbohydrates forming a protein-carbohydrate matrix and final, 10 sec stage, fibrillation of the protein-carbohydrate matrix.

INTRODUCTION

IT HAS BEEN SHOWN by McAnelly (1964), Touba (1970), Sterner and Sterner (1976) and Taranto et al. (1978b) that defatted soy flour (SF) can be texturized by a nonextrusion process. McAnelly (1964) texturized premoistened SF by steaming and cooking under pressure, while Touba (1970), Sterner and Sterner (1976) and Taranto et al. (1978b) used compression between two heated plates. When the pressure is released, water is volatilized and the product expands becoming cellular or spongy in structure. The texture created by the nonextrusion process is comparable to the texture created by extrusion processing (Taranto et al., 1978b).

Taranto et al. (1978b) demonstrated that both extrusion and nonextrusion texturized SF (ETSF and NTSF, respectively) are composed of a protein matrix or sheet of varying uniformity in which the insoluble carbohydrates are dispersed. Smith and Crocco (1975) and Smith (1976) postulated that the protein-carbohydrate structure is formed by the rupture and fusion of the protein bodies of the soybean. Sippos and Witte (1961) demonstrated with soybean grits that the combined effect of heat and explosive vaporization caused destruction of the cytoplasmic network and the agglomeration of the protein bodies.

However, none of the previous works has studied the breakdown and fusion of cells and cellular components with respect to the process retention time (i.e., time that the premoistened flour is exposed to a controlled pressure and temperature). Therefore, the present study was initiated to investigate the sequence of cellular and sub-cellular changes taking place during the development of texture in NTSF.

MATERIALS & METHODS

Equipment and sample production

A Korean "textured" snack food press (Hand Press) described by Sterner and Sterner (1976) was used to produce nonextrusion products from premoistened SF. Constant processing conditions were plate temperature, top and bottom (190°C); pressure (21.1 kg/cm²); final flour moisture (30%) and premoistened sample mass (25g). The varying condition was retention time (2, 4, 6, 8 and 10 sec; $T_2 - T_{10}$).

Specimen preparation for transmitted light (TLM), transmission electron (TEM) and scanning electron microscopy (SEM)

Texturized products were prepared for TLM according to the procedures outlined by Cegla et al. (1978). Sections, 0.5–0.75 μm, were cut using a Porter-Blum MT-2 Ultra Microtome and heat fixed to glass slides, stained and examined.

Soybean cotyledons and texturized products were prepared for TEM according to Cegla et al. (1978). Sections, 600–800 Å, were cut using a LKB Ultrotome. Thin sections of soybean cotyledons were mounted on uncoated 300 mesh copper grids. Texturized SF thin sections were affixed to formvar coated, 200 mesh copper grids according to Galey and Nilsson (1966). Sections were stained with aqueous uranyl acetate (Watson, 1958), followed by lead (Sato, 1967) and examined with a Hitachi-HS-8 at 50 kv.

SF was prepared for SEM according to Lin et al. (1976). Specimens were examined with a JEOL-JSM-U3 at a 45° tilt and 15 kv.

RESULTS & DISCUSSION

THE PROTEINS of the soybean cotyledon are compartmentalized in storage vesicles called protein bodies or aleurone grains (Bils and Howell, 1963; Tombs, 1967) [Fig. 1 (1)]. Soybean oil is compartmentalized in storage structures called spherosomes (Bils and Howell, 1963; Saio and Watanabe, 1967) [Fig. 1 (1)].

SF is produced by flaking the dehulled soybean, extracting the oil with hexane, then desolventizing and grinding the extracted flakes into a flour. The SF used to produce the NTSF is composed of cell wall fragments, cell fragments (composed of cell walls, cytoplasm and protein bodies), isolated protein bodies and hull fragments [Fig. 1 (2).]. The components of the soy flour were characterized by their morphological similarity to seed and flour structures identified by Wolf and Baker (1975).

The present study confirms and substantially extends the work of Smith and Crocco (1975) and Smith (1976). The work has shown that the texture created by the nonextrusion process develops in a series of well defined stages. The first stage is the fusion of the cell wall fragments, isolated protein bodies (PB), and cell fragments [Fig. 1 (3, 4 and 5)]. This initial fusion of flour components takes place after a 2-sec retention time.

The second stage, occurring after a 4-sec retention time, is the continued deformation and fusion of PB [Fig. 2 (1, 2 and 4)] and cell fragments [Fig. 2 (1 and 4)]. Some of the PB, which appear to be aggregates of smaller PB, are beginning to breakdown [Fig. 2 (2 and 3)]. However, the membrane surrounding the PB can still be detected indicating that the bulk of the flour protein is not yet available for reaction [Fig. 2 (5)]. Note the large area of cell wall aggregation in the TLM micrograph [Fig. 2 (1)]. The area is composed mostly of cell walls, but cells in various states of preservation are also visible [Fig. 2 (6)]. Cellulose fibrils of the cell wall are shown in Figure 2 (7).

After a 6-sec retention time, fragmentation of the cell membrane [Fig. 3 (3)] and, probably, PB membrane occurs. The fragmentation of the latter frees the PB matrix proteins and the protein-carbohydrate matrix [Fig. 3 (1 and 2)], previously

Authors Taranto and Rhee are with the Food Protein R&D Center, F.E. Box 183, Texas A&M University, College Station, TX 77843.

0022-1147/78/0004-1274$02.25/0
© 1978 Institute of Food Technologists

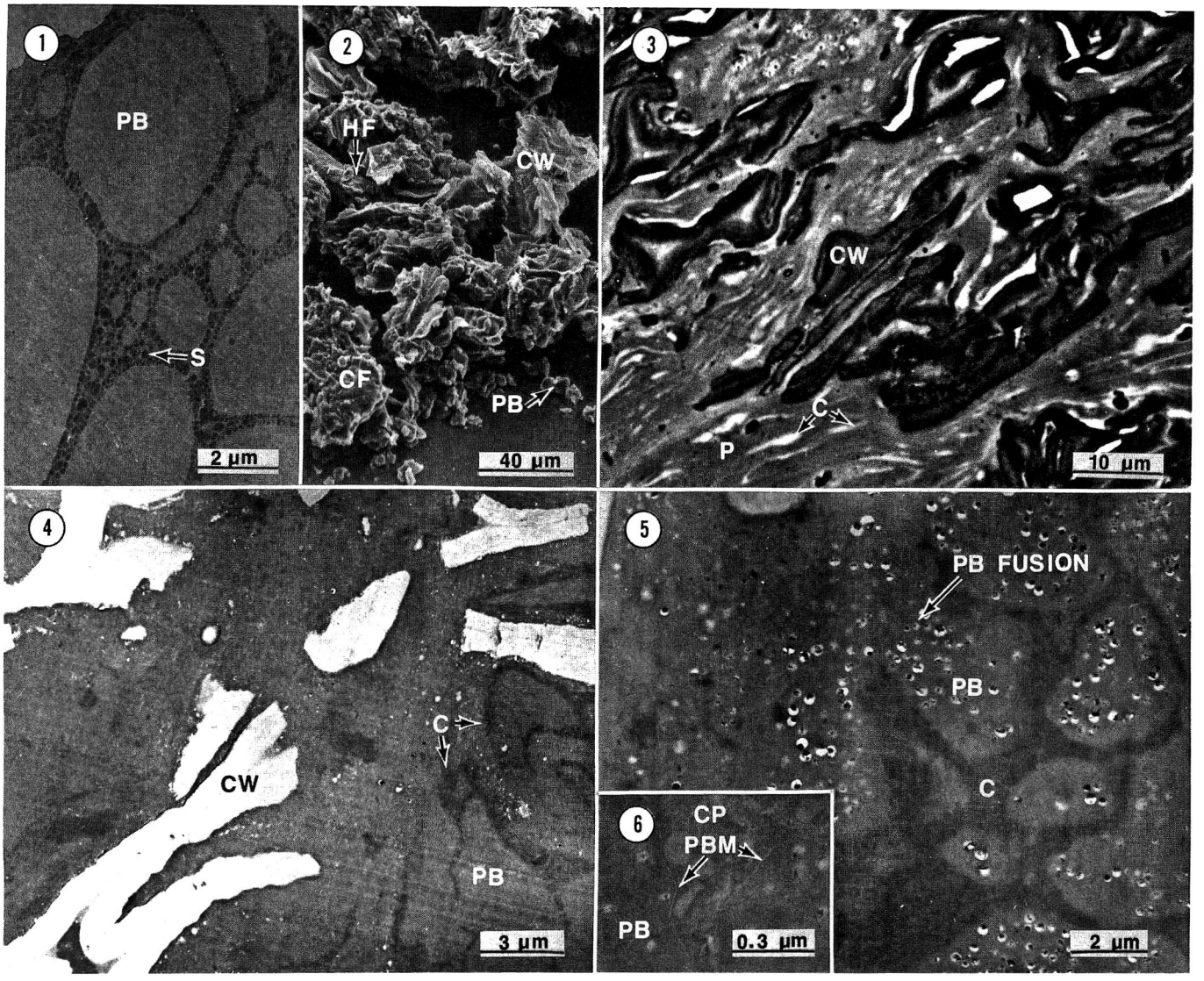


Fig. 1—Retention times T_0 and T_2. (1) is a TEM micrograph of a cross-section of a soybean cotyledon cell. The small electron dense particles labeled S are the spherosomes. These particles are ruptured and the oil extracted during the production of defatted soy flour. The less electron dense particles labeled PB are the protein bodies. (2) is a SEM micrograph of the defatted soy flour (Retention time—T_0). (3) is a TLM micrograph of the T_2-NTSF product: PAS (+) fractions are labeled CW—cell wall fragment and C—insoluble carbohydrate; Hg-Bromphenol Blue (+) fraction is labeled P—protein component. (4) and (5) are TEM micrographs showing the ultrastructural details of the fused flour components. The perforations of the PB in (5) are artifacts of fixation. (6) is a TEM micrograph of T_2 product showing the intact membrane surrounding the PB. C—insoluble carbohydrate; CF—cell fragment; CP—cytoplasmic remains; CW—cell wall fragment; HF—hull fragment; PB—protein body; PBM—protein body membrane.

described by Cegla et al. (1978) and Taranto et al. (1978a), begins to form. The protein and insoluble carbohydrate components of the protein-carbohydrate matrix were identified with the appropriate light microscope histochemistry. The periodic acid-Schiff's leucofuschin (PAS) test (McManus, 1948) was used to stain the insoluble carbohydrates. The mercuric (Hg)-bromphenol blue protein specific stain (Mazia et al., 1953) was used to localize proteins.

The next stage, occurring after an 8-sec retention time, is the continued development of the protein-carbohydrate matrix [Fig. 4 (2)]. Cell wall fragments can be distinguished, but no fibrillation of the protein-carbohydrate matrix can be detected. Large areas [Fig. 4 (1)] can be found in which the cell walls are intact, but the cell contents appear to have been reformed into protein packets surrounded by carbohydrates. Upon closer examination [Fig. 4 (3)], it is evident that the PB have been disrupted and the released protein has either fused or been encapsulated, probably by a PB membrane fragment, forming small protein spheres (PS). Large PS can be seen at the interface between the cell wall and cell interior [Fig. 4 (3 and 4)]. The PS at the cell wall-cell interior interface appear to have been formed by encapsulation of released protein by cell membrane fragments [Fig. 4 (4)].

The PS parallel the appearance of the protein structures reported to be PB in ETSF by Taranto et al. (1978a). It is quite likely that Taranto's et al. (1978a) PB are in fact PS formed by the membrane encapsulation of proteins released from ruptured PB.

The final stage in the development of texture occurs after a 10-sec retention time. Fusion of cellular fragments is extensive. The protein-carbohydrate matrix is well formed and fibrillation of the protein matrix can be detected [Fig. 5 (1, 2 and 3)]. Areas of incomplete PB fusion still can be found [Fig. 5 (4)], but it is evident that the fusion process is nearing completion by the severe deformation of the PB. Cell wall fragments are beginning to breakdown [Fig. 5 (5)]. The

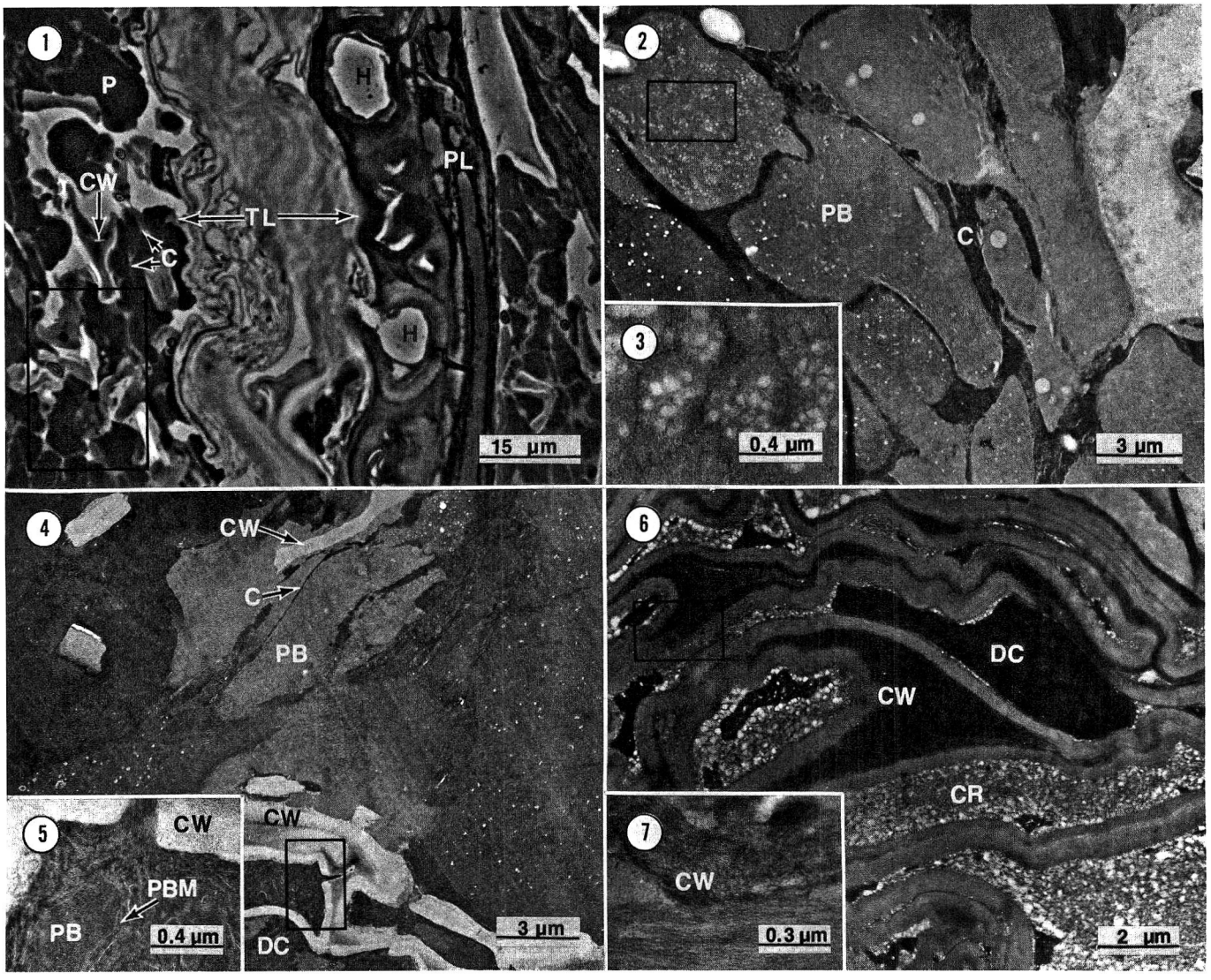


Fig. 2—Retention time T_4. (1) is a TLM micrograph showing the overall structural organization of fused flour components. The areas labeled P were Hg-Bromphenol Blue (+) indicating they are protein. Those areas labeled C were PAS (+) indicating they are insoluble carbohydrate. The structures in the center of the micrograph are remains of the seed coat (hull). The outer layers, palisade (labeled PL) and hourglass cells (labeled H) were PAS (+). The thick layer (layered TL) to the left of the hourglass layer was both PAS (+) and Hg-Bromphenol Blue (+) and is probably composed of the parenchyma, aleurone and compressed cells of the seed coat. (2) is a TEM micrograph of a similar area shown in box of (1). (3) is a blow-up of the area outlined by the box in (2) and shows the protein body breakdown in greater detail. (4) is a TEM micrograph showing the fusion process in greater detail. (5) is a blow-up of the boxed area of (4). (6) is a TEM micrograph showing the ultrastructural details of the TL layer seen in (1). (7) is a blow-up of an area similar to the boxed area of (6) showing the fibrillar nature of the cell wall material. C—insoluble carbohydrate; CW—cell wall; CR—cell remains; DC—deformed cell; P—protein; PB—protein body; PBM—protein body membrane.

breakdown products are fusing with the protein-carbohydrate matrix [Fig. 5 (5 and 6)].

Using SEM, Aguilera et al. (1976) were successful in documenting the gross morphological development of texture during the extrusion processing of SF. Their SEM technique, alone, did not elucidate the ultrastructural development of texture. The present study demonstrated that the use of SEM, in conjunction with TEM, TLM, histochemistry and the nonextrusion process was required to successfully describe in detail the sequential ultrastructural changes of texture development.

Taranto et al. (1978b) demonstrated that extrusion and nonextrusion processing produce comparable texturized products as shown by the similarities in surface morphology and ultrastructural details. Therefore, it seems reasonable to extend the present findings for the development of texture with the nonextrusion process to the extrusion process. However, it must be pointed out that Tarento et al. (1978a, b) demonstrated that there was no fibrillation of the protein-carbohydrate matrix in extrusion texturized products. A detailed explanation for the latter is given by Taranto et al. (1978b).

REFERENCES

Aguilera, J.M., Kosikowski, F.V. and Hood, L.F. 1976. Ultrastructural changes occurring during thermoplastic extrusion of soybean grits. J. Food Sci. 41(5): 1209.

Bils, R.F. and Howell, R.W. 1963. Biochemical and cytologica. changes in developing soybean cotyledons. Crop Sci. 3: 304.

Cegla, G.F., Taranto, M.V., Bell, K.R. and Rhee, K.C. 1978. Microscopic structure of textured cottonseed flour blends. J. Food Sci. 43: 775.

Galey, F.R. and Nilsson, S.E.G. 1966. A new method for transferring sections from the liquid surface of the trough through staining solutions to the supporting film of a grid. J. Ultrastruct. Res. 14: 405.

—Text continued on page 1278

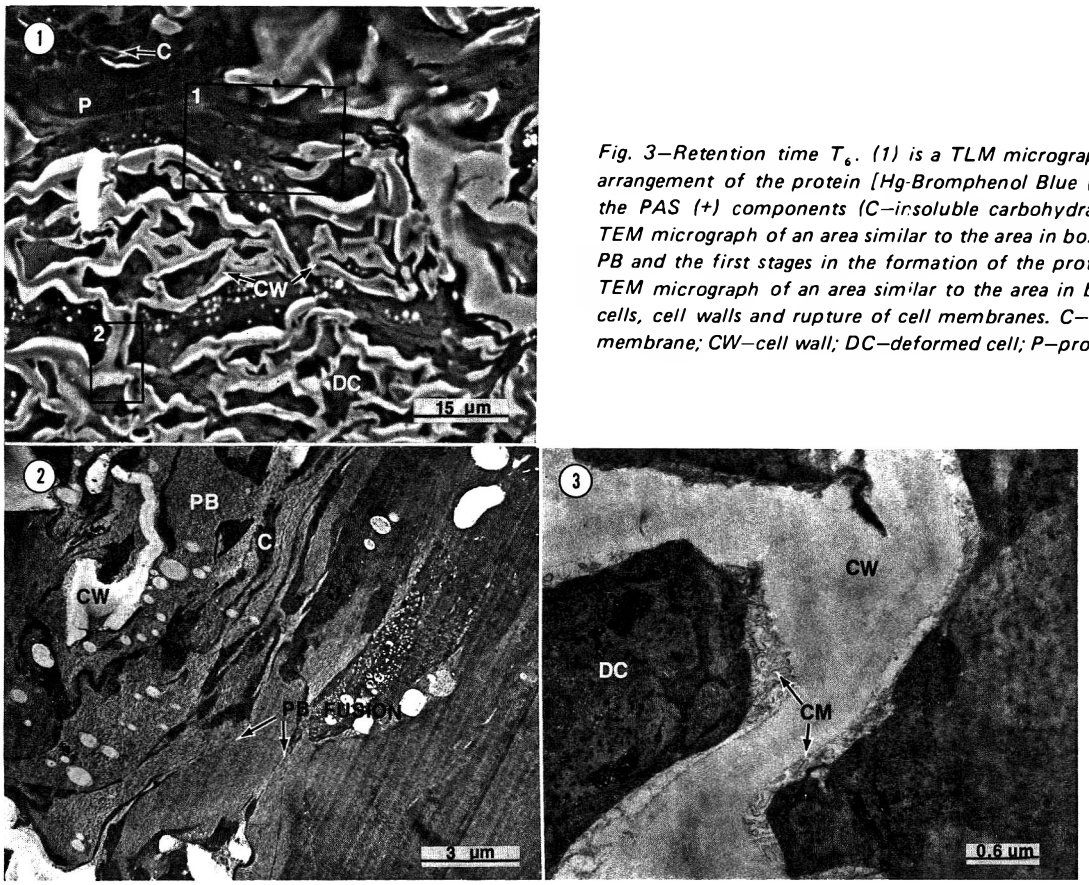


Fig. 3—Retention time T_6. (1) is a TLM micrograph showing the overall structural arrangement of the protein [Hg-Bromphenol Blue (+)] components (labeled P) and the PAS (+) components (C—insoluble carbohydrates and CW—cell wall). (2) is a TEM micrograph of an area similar to the area in box #1 of (1) showing the fusion of PB and the first stages in the formation of the protein-carbohydrate matrix. (3) is a TEM micrograph of an area similar to the area in box #2 of (1) showing deformed cells, cell walls and rupture of cell membranes. C—insoluble carbohydrate; CM—cell membrane; CW—cell wall; DC—deformed cell; P—protein; PB—protein body.

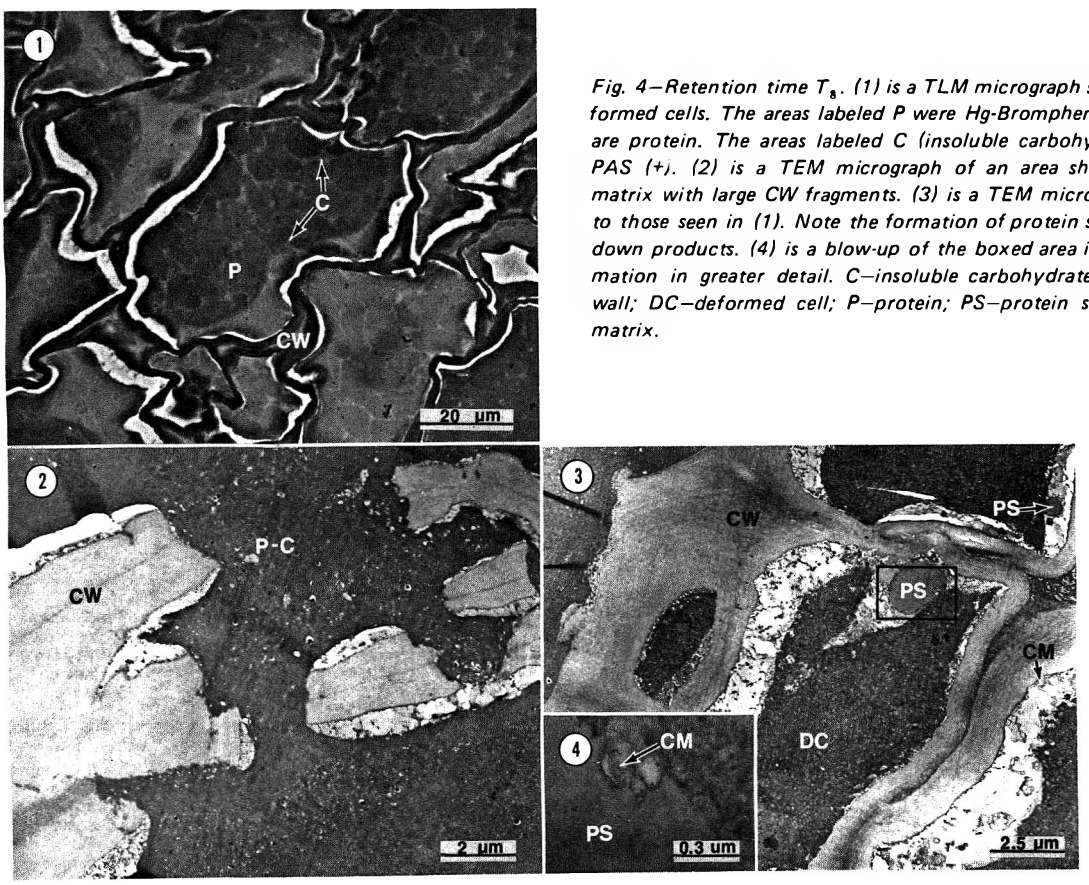


Fig. 4—Retention time T_8. (1) is a TLM micrograph showing an area of intact but deformed cells. The areas labeled P were Hg-Bromphenol Blue (+) indicating that they are protein. The areas labeled C (insoluble carbohydrate) and CW (cell wall) were PAS (+). (2) is a TEM micrograph of an area showing the protein-carbohydrate matrix with large CW fragments. (3) is a TEM micrograph of a deformed cell similar to those seen in (1). Note the formation of protein sacules from protein body breakdown products. (4) is a blow-up of the boxed area in (3) showing protein sacule formation in greater detail. C—insoluble carbohydrate; CM—cell membrane; CW—cell wall; DC—deformed cell; P—protein; PS—protein sacule; PC—protein-carbohydrate matrix.

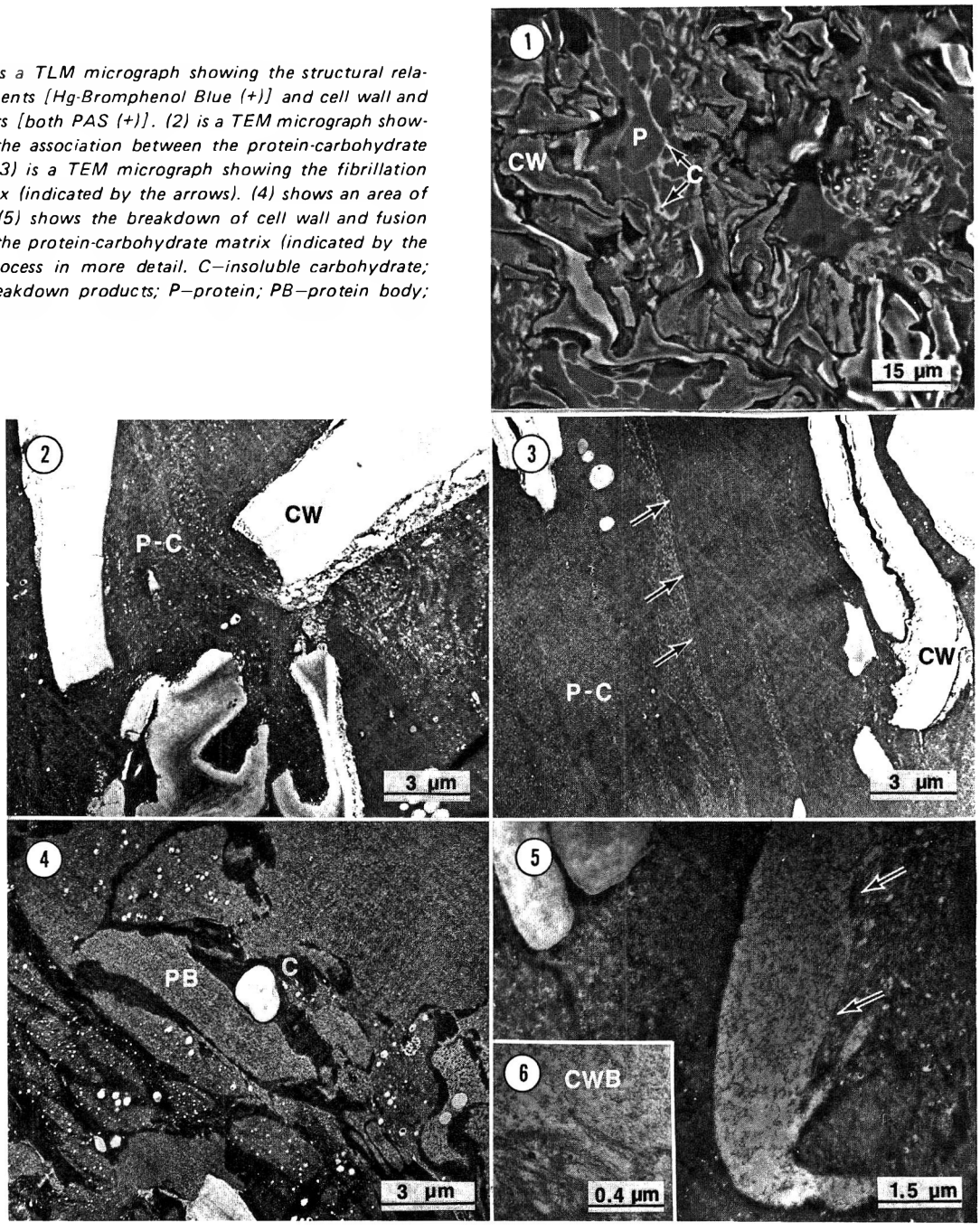


Fig. 5—Retention time T_{10}. (1) is a TLM micrograph showing the structural relationships between protein components [Hg-Bromphenol Blue (+)] and cell wall and insoluble carbohydrate components [both PAS (+)]. (2) is a TEM micrograph showing the ultrastructural details of the association between the protein-carbohydrate matrix and cell wall fragments. (3) is a TEM micrograph showing the fibrillation of the protein-carbohydrate matrix (indicated by the arrows). (4) shows an area of incomplete protein body fusion. (5) shows the breakdown of cell wall and fusion of the breakdown products with the protein-carbohydrate matrix (indicated by the arrows). (6) shows the fusion process in more detail. C—insoluble carbohydrate; CW—cell wall; CWB—cell wall breakdown products; P—protein; PB—protein body; PC—protein-carbohydrate matrix.

Lin, S.H.C., Bax, B.J., Yang, A. and Morr, C.V. 1976. Note on a light microscope technique to preview specimen mountings for scanning electron microscopy. Cereal Chem. 53(2): 153.

Mazia, D., Brewer, P.A. and Alfert, M. 1953. The cytochemical staining and measurement of protein with mercuric-bromphenol blue. Bio. Bull. 104: 57.

McAnelly, J.K. 1964. Bland, textured product from water-insoluble portion of defatted cooked soybean flour. U.S. Patent 3,142,571.

McManus, J.F.A. 1948. Histological and histochemical uses of periodic acid. Stain Technol. 23(3): 99.

Saio, K. and Watanabe, T. 1968. Observation of soybean foods under electron microscope. J. Food Sci. Technol. Jap. 15: 290.

Sato, T. 1967. A modified method for lead staining of thin sections. J. Electromicroscopy 16: 133.

Sippos, A. and Witte, N.H. 1961. The desolventizer-toaster process for soybean oil meal. J. Am. Oil Chem. Soc. 38(3): 11.

Smith, O.B. 1976. Textured vegetable proteins. In "World Soybean Research, Proceedings of the World Soybean Research Conference," Hill, L.D., Ed., p. 861. Interstate Printers and Publishers, Inc., Danville, IL.

Smith, O.B. and Crocco, S.C. 1975. Engineering "meat" analogs that look and taste like meat are a real challenge to engineered food's technology for affluent nations. Food Eng. 47(10): ef 22.

Sterner, M. and Sterner, H. 1976. Two techniques cut costs of vegetable protein. Food Eng. 3: 77.

Taranto, M.V., Cegla, G.F., Bell, K.R. and Rhee, K.C. 1978a. Texturized cottonseed and soy flours: A microscopic analysis. J. Food Sci. 43: 767.

Taranto, M.V., Cegla, G.F. and Rhee, K.C. 1978b. Morphological, Ultrastructural and Rheological Evaluation of Soy and Cottonseed Flours Texturized by Extrusion and Non-extrusion Processing. J. Food Sci. 43: 973.

Tombs, M.P. 1967. Protein bodies of the soybean. Plant Physiol. 42: 797.

Touba, A.R. 1970. Texturing of protein. U.S. Patent 3,843,816.

Watson, M.L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. and Biochem. Cytol. 4(4): 475.

Wolf, W.J. and Baker, F.L. 1975. Scanning electron microscopy of soybeans, soy flours, protein concentrates and protein isolates. Cereal Chem. 52(3): 387.

Ms received 10/20/77; revised 1/24/78; accepted 1/30/78.

This paper reports research funded by NSF grant #ENG 76-17269. The authors extend their appreciation to the Sterners from Meals for Millions, Santa Monica, CA for supplying the plans for the Hand Press. The authors extend special thanks to Dr. Gad Cegla for "planting" the seed of this work and to Ms. Cindy Archer for her technical assistance and encouragement.

CONTROL OF CHALKINESS IN SOYMILK

D. A. KUNTZ, A. I. NELSON, M. P. STEINBERG and L. S. WEI

ABSTRACT

Chalkiness is a defect used to describe a food which coats the mouth and throat with fine, grainy particles. This study evaluates the processing and formulation variables which affect chalkiness of Illinois process soymilk produced from dehulled soybeans. Alkalinity of the blanch solution, homogenization conditions, soymilk pH, and solids concentration had the most pronounced effects on chalkiness. Increasing blanch alkalinity decreased chalkiness; beverages adjusted to higher final pH were less chalky. Homogenization at higher temperatures or pressures also significantly reduced chalkiness. Chalkiness increased with soy solids concentration. Beverages produced using conditions to effect optimal quality were compared to low quality products. Centrifugal desludging reduced chalkiness to an imperceptible level. Particles retained by 150 mesh were primarily responsible for chalkiness. The process conditions leading to minimum chalkiness were: direct blanch of cotyledons in 0.25% $NaHCO_3$; homogenization at 180°F and 3500 psi; formulation with 6% soy solids; beverage pH of 7.5.

INTRODUCTION

IN RECENT YEARS soybean food products in general and soymilk in particular have received much attention in the Western world because they are economical sources of high quality protein. The whole soybean contains about 20% oil and 40% protein, dry basis, and the amino acid distribution of soy protein is close to that recommended by the FAO (1973).

The acceptance of soy beverages produced by the traditional water extraction process has been limited in non-Oriental countries because of their characteristic odor, described as beany, painty, bitter or rancid, which is caused by lipoxygenase (Wilkens et al., 1967; Nelson et al., 1976). Extraction of soybeans at temperatures above 80°C minimizes these enzyme-induced off-flavors, but has the drawback of substantial protein loss during filtration (Wilkens et al., 1967). Soymilk produced by the Illinois process (Nelson et al., 1975; 1976) is free of the off-flavor because lipoxygenase is heat-inactivated prior to cellular disruption. All the cotyledon solids except for 10–12% water solubles are included in the beverage, so essentially no protein is lost. This nonextracted product is free of anti-nutritional factors and has excellent suspension stability (Nelson et al., 1975; 1976).

Investigators have claimed that beverages produced by boiling-water extraction and filtration lack the chalky mouth feel that is characteristic of nonextracted products (Wilkens et al., 1967; Wilkens and Hackler, 1969). Chalkiness is the defect in a liquid food which coats the mouth and throat with fine, grainy particles. Most consumers dislike this tactual defect which is characteristic of many soymilks and is sometimes present in cow's milk. Several nonextraction processes (Hand et al., 1964; Lo, 1971; Miles, 1966; and Mustakas et al., 1971; 1972) started with cotyledons, and some of these applied high pressure homogenization (5000–8000 psi), colloid milling, and centrifugal clarification to improve this mouthfeel characteristic. The Illinois process soymilk made from whole soybeans was reported to have good mouthfeel when the soybeans were sufficiently softened in blanching and homogenization was done at 93.3°C and 3500 psi (Nelson et al., 1976).

This investigation reports on processing and formulation variables which affect chalkiness of Illinois process soymilk produced from cotyledons (hulls not included in beverage). The ultimate objective was to establish a process which would give minimum chalkiness and maximum smoothness.

1.	Whole soybeans	Bonus variety, 9–11% moisture
2.	Cotyledons	Prepare by heating whole soybeans at 99°C for 15 min.; dry dehull hot soybeans. Remove hulls and hypocotyls by air aspiration and size separation.
3.	Blanch	Drop cotyledons directly into boiling solution (5 lb soln/lb cotyledons) and blanch for 30 min at a rolling boil.
4.	Drain and rinse	Water rinse
5.	Add water	Dilute to 10% solids. Pass cotyledons with water through Rietz mill (model RP-6-K115) fitted with screen with 0.023 in. perforations. Two passes. Reserve some water to flush solids from the mill.
6.	Heat	To 85°C in steam jacketed kettle.
7.	Homogenize	At 3500 psi (first stage pressure) and 500 psi (second stage pressure) with a Gaulin model 15 M8TA.
8.	Add water	Mix with water to dilute to desired soy solids concentration.
9.	Adjust pH	By addition of 1.0N HCl or NaOH.
10.	Heat	To 85°C
11.	Homogenize	As above
12.	Bottle	
13.	Cool and store	At 1.1°C

Fig. 1—Process for preparation of soymilk.

MATERIALS & METHODS

Preparation of soymilk

Bonus variety commercial soybeans were dry cleaned to remove damaged and moldy seeds and all foreign material. Soybeans at 9–11% moisture were dry dehulled, and the cotyledons were processed into soymilk as outlined in Figure 1. All soy beverages were produced using these "standard process conditions," except as noted. Tap water was used throughout this work; this was a partially softened water containing 75–85 mg/L hardness as $CaCO_3$.

Organoleptic evaluation of chalkiness

In the initial studies, organoleptic evaluations of mouthfeel were performed by two expert judges. This facilitated screening of a large number of samples to identify the determinant processing and formulation variables. In subsequent studies, evaluations were made by seven trained panelists. Unflavored samples at room temperature were coded with three-digit random numbers and presented in random order. A maximum of four samples of 15 ml each were evaluated per session. Panelists were asked to swirl the samples before evaluating. Intensity of chalkiness was rated on a seven-point scale where: 1 = imperceptible, 4 = perceptible and 7 = very pronounced.

—Text continued on page 1280

Authors Nelson, Steinberg and Wei are with the Dept. of Food Science, University of Illinois, Urbana, IL 61801. Author Kuntz, formerly with the University of Illinois, is now with Quaker Oats Co., 617 W. Main St., Barrington, IL 60010.

0022-1147/78/0004-1279$02.25/0
© 1978 Institute of Food Technologists

Table 1—Chalkiness as affected by blanch solution, hydration, tenderization and pH adjustment during 30 min boiling blanch without prior soaking

Blanch solution	Hydration (g H_2O/g solid)	Maximum shear force (psi/50g solids)	Unadjusted soymilk pH	pH not adjusted	pH adjusted to 7.2	Overall mean score
				Mean score*		
0.06% HCl	1.434	102	6.6	4.8	3.9	4.4
Tap water	1.523	92	7.0	2.1	3.0	2.5
0.25% $NaHCO_3$	1.583	56	7.5	1.3	2.2	1.8
0.50% $NaHCO_3$	1.608	45	7.9	1.7	1.6	1.7
				Overall mean score		
				2.5	2.7	

Blanch solution, HCl vs all others and tap water vs $NaHCO_3$ significant at 0.5% level. Interaction significant at 5% level.

* 1 = imperceptible, 4 = perceptible, 7 = very pronounced

Objective evaluations

Particle size by filtration fractionation. Degree of tissue disruption was evaluated by determining particle size distribution. A weighed sample of soymilk of known solids concentration was placed between two tared wirecloth sections in a washing apparatus. Tap water was passed through the apparatus, first in one direction and then in the other. This was repeated several times to wash away solubles and particles smaller than the openings in the wirecloth. Then the wirecloths and retained particulate material were dried to constant weight and results expressed as percent of total beverage solids. This was repeated with wirecloths of 325, 200, 150 and 100 mesh; openings were, respectively, 49, 74, 105 and 149 microns square.

Water uptake by insoluble carbohydrate. Soymilk was centrifuged at 2200 × G for 30 min. The sludge was made to volume with a 30% KBr solution of density 1.25 g/ml and centrifuged at 2200 × G for 60 min. This sludge was made to volume with 39% NaBr solution of density 1.40 g/ml and centrifuged. Supernatant and sludge were separately washed several times in a similar manner with the NaBr solution to obtain the insoluble carbohydrate fraction free of other soy components. This was washed three times with water at 10°C to remove salt. The fraction was then dried by solvent exchange, first with methanol and then with pentanehexane. Water uptake by this dry carbohydrate was determined according to Baumann (1967).

Tenderness of blanched cotyledons. Tenderness of blanched cotyledons was determined with the LEE Kramer Shear Press (L.E.E., Inc. Washington, DC). A No. C274 test cell was used with a 3000 lb test ring using a 14.5 sec stroke. Sample size was held constant at 50g solids to account for differences in hydration of the cotyledons blanched in different solutions. The results are presented as the maximum shear force developed per 50g soy solids.

Viscosity. Viscosity was determined at 25°C with a Brookfield LVT Synchro-Lectric Viscometer (Brookfield Engineering Laboratories, Stoughton, MA).

Statistical analysis. The data were statistically analyzed according to Steel and Torrie (1960) for analysis of variance with F test; linear relationships were determined by calculating linear regression equations with correlation coefficients.

RESULTS & DISCUSSION

IN THE INITIAL STUDIES, the processing and formulation variables which had the most pronounced effects on chalkiness were: use of bicarbonate in the blanch solution, homogenization conditions, beverage pH and solids concentration. The effects of these variables on chalkiness were evaluated in subsequent studies as discussed below.

Blanch solution

Cotyledons were blanched in one acid, one neutral and two alkaline solutions as shown in Table 1. One set of soymilk samples was prepared from these cotyledons without pH adjustment (omit Step 9, Fig. 1) so that the final pH varied from 6.6–7.9 as shown in Table 1. A second set of samples was prepared with the pH standardized to 7.2. Chalkiness evaluations of both sets are summarized in Table 1.

Blanch solution had a significant (0.5%) effect on chalkiness; intensity decreased with increasing alkalinity of the solution. The beverages prepared from cotyledons blanched in 0.06% HCl were significantly (0.5%) more chalky than all other products. Also there was a significant (0.5%) difference between tap water and bicarbonate blanch samples. However, the difference between the two concentrations of $NaHCO_3$ was not significant. Although pH adjustment per se was not significant, there was a significant (5%) interaction indicating that the effect of pH adjustment depended on the blanch treatment. Based on this study, it was concluded that the mechanism causing chalkiness is strongly influenced by the blanch solution.

The effect of blanch solution on hydration and tenderness of blanched cotyledons is summarized in Table 1. Increasing the alkalinity of the blanch solution resulted in greater hydration and tenderization of the cotyledon tissue. Comparison of these results and chalkiness scores in Table 1 indicates that the tactual quality of the beverages was improved by greater tissue hydration and tenderization during blanching.

Soymilk pH

The objective was to further evaluate the effect of alkalinity during blanching and to investigate the possibility of an interaction between alkalinity of blanch solution and adjusted soymilk pH. Cotyledons were blanched (Step 3, Fig. 1) in tap water containing 0.0, 0.25 or 0.50% $NaHCO_3$. At Step 9 (Fig. 1), each batch was subdivided and adjusted to four pH levels by addition of 1.0N NaOH or HCl. After 2 days to allow equilibration, the pH was determined at 25°C as shown in Table 2.

Mean chalkiness scores in Table 2 indicate the soymilk pH had a significant effect (0.5%) on chalkiness; regardless of blanch treatment, intensity of chalkiness was consistently lower at higher soymilk pH. Single degree of freedom comparisons of overall treatment mean scores for soymilk pH showed that the difference between pH 6.4 samples and all others and the difference between pH 6.8 products and the beverages at pH 7.2 or 7.7 were significant (0.5%). Mean scores for pH 7.2 and 7.7 products were not significantly different. These results show that alkaline conditions in post-blanch processing resulted in improved tactual quality. It was postulated that heating (Step 10, Fig. 1) in a more alkaline medium could effect greater hydration of the tissue fragments and more extensive tissue disruption during final homogenization.

As in the preceding study, blanch solution had a significant (5%) effect on chalkiness. Single degree of freedom comparisons showed that the water blanch samples were significantly (1%) more chalky than those produced by bicarbonate blanch treatments; the mean scores were 3.3 and 2.5, respectively. The difference between the two concentrations of $NaHCO_3$

Table 2—Chalkiness as affected by blanch solution, pH adjustment of soymilks and relation between blanch solution and soymilk solids

Blanch solution	Soymilk pH				Overall mean score	Soy solids (%)				Overall mean score
	6.4	6.8	7.2	7.7		5	6	7	8	
	Mean score*					Mean score*				
Tap water	4.4	3.1	2.9	2.9	3.3	3.1	3.4	3.7	3.7	3.5
0.25% $NaHCO_3$	2.8	2.4	2.1	1.9	2.3	1.5	1.6	2.0	2.2	1.8
0.50% $NaHCO_3$	4.6	2.8	1.6	1.5	2.6	1.6	2.3	1.8	2.5	2.1
	Overall mean score					Overall mean score				
	3.9	2.7	2.2	2.1		2.1	2.5	2.5	2.8	
	Soymilk pH, pH 6.4 vs all others, pH 6.8 vs pH 7.2–7.7 and interactions significant at 0.5% level. Blanch solution significant at 5% level and tap water vs $NaHCO_3$ significant at 1% level.					Soy solids concentration, 5% vs 6–7–8%, blanch solution and tap water vs $NaHCO_3$ solution significant at 0.5% level. 6–7% vs 8% significant at 5% level.				

* 1 = imperceptible, 4 = perceptible, 7 = very pronounced

was not significant. There was a significant (0.5%) interaction of effects of blanch solution and soymilk pH. While chalkiness decreased with increasing beverage pH, the magnitude of the decrease differed for the various blanch treatments.

Soy solids concentration

The effect of soy solids concentration was evaluated in the range of 5–8% solids (2.5–4.0% protein). Cotyledons were blanched with three concentrations of bicarbonate, 0, 0.25 and 0.5%, as above. Each batch was subdivided after Step 7 (Fig. 1), and different amounts of water were added to obtain the desired solids levels. Each sample was adjusted to pH 7.5 at Step 9.

The treatment mean chalkiness scores are presented in Table 2. The variation in degree of chalkiness of the beverages over the range of 5–8% solids was small, but significant (0.5%). Chalkiness increased with increasing solids concentration; this was generally consistent for each blanch treatment, indicating a simple concentration effect. Single degree of freedom comparisons showed that at 5% solids, samples were significantly (0.5%) less chalky than the other three while the 8% samples were significantly (5%) more chalky than the 6 and 7% soymilks. There was no significant difference between the 6 and 7% samples.

The concentration of bicarbonate in the blanch solution also had a significant (0.5%) effect on chalkiness. The water blanch samples were significantly (0.5%) more chalky than those produced using bicarbonate blanch, but there was no significant difference between the 0.25 and 0.50% samples.

Viscosity could affect chalkiness, either in the way chalkiness is perceived during organoleptic evaluation or by affecting efficiency of particle size reduction during homogenization. Therefore, viscosity of samples represented in Table 2 was determined and presented in Figure 2. Three samples at the same viscosity, about 40 cp, were compared; namely, the 8% solids, water blanch; 6% solids, 0.25% $NaHCO_3$ blanch; and 5% solids, 0.50% $NaHCO_3$ blanch. Their chalkiness scores were 3.7, 1.6 and 1.6, respectively, indicating that viscosity per se had no effect on perception of chalkiness. It was concluded that the difference in chalkiness among these three samples was primarily due to differences in bicarbonate concentration of blanch solution. Bicarbonate concentration probably affected tenderization of the tissue (Nelson et al., 1976) and thereby affected particle size reduction during subsequent homogenization.

Homogenization temperature

Temperature of the liquid as it enters the homogenizer was studied because it could affect the resulting particle size which

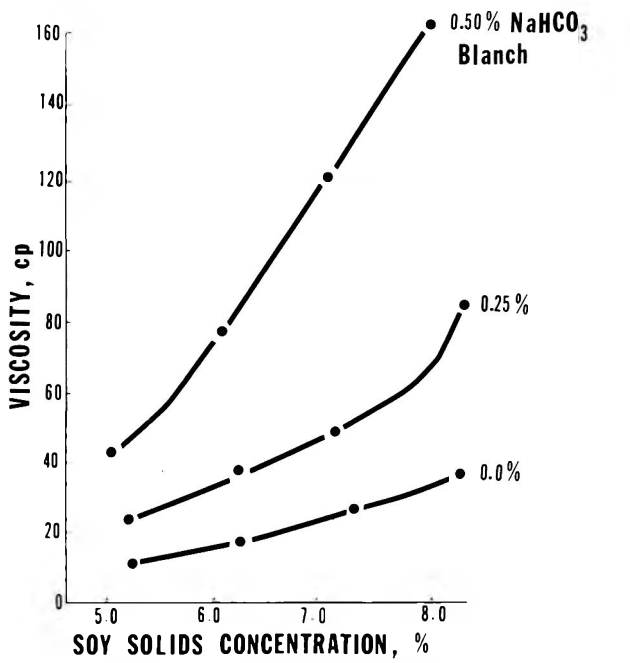


Fig. 2—Beverage viscosity as affected by concentration of bicarbonate blanch solution and soy solids concentration.

may in turn, affect chalkiness. Bicarbonate blanch was the second factor studied.

Step 3 (Fig. 1) was done with tap water and with 0.25% bicarbonate in tap water. Both Step 6 and Step 10 (Fig. 1) were done at the same temperature: 15.6, 48.9 or 82.2°C. The slurries which had been homogenized (3500 psi) at 15.6 and 48.9°C a second time were, prior to Step 12, pasteurized by heating to 85°C and, to break clumps formed by heating, passed through the homogenizer a third time with no pressure on the first stage and 500 psi on the second. In all cases, dilution in Step 5 was to 7% solids. The pH of the water blanch samples was adjusted to 7.5 to equal that of the bicarbonate blanched samples.

Table 3 contains the mean treatment scores. Homogenization temperature had a significant (0.5%) effect on chalkiness; the lower the product temperature during first and second homogenization, the more pronounced the chalkiness. The difference in chalkiness between 82.2°C and the two lower temperatures was significant (0.5%). Also, the 15.6°C beverages were significantly (5%) more chalky than those homogenized

Table 3—Chalkiness as affected by blanch solution, homogenization temperature and homogenization pressure

Blanch solution	Homogenization temperature (°C)			Overall mean score	Homogenization pressure (psi)			Overall mean score
	15.6	48.9	82.2		2000	3500	5000	
	Mean score*				Mean score*			
Tap water	3.6	2.9	2.1	2.9	5.4	3.5	2.4	3.7
0.25% $NaHCO_3$	2.8	2.2	1.9	2.3	2.8	2.0	1.6	2.1
	Overall mean score				Overall mean score			
	3.2	2.5	2.0		4.1	2.8	2.0	
	Homogenization temperature, 15.6° and 48.9° vs 82.2°C and blanch solution significant at 0.5% level; 15.6 vs 48.9°C significant at 5% level.				Homogenization pressure, 2000 vs 3500–5000 psi, 3500 vs 5000 psi and blanch solution significant at 0.5% level. Interaction significant at 5% level.			

* 1 = imperceptible, 4 = perceptible, 7 = very pronounced

at 48.9°C. The benefit of this high temperature was thought to be due to more extensive hydration and tenderization of the tissue during heating which, in turn, results in greater disruption of tissue particles during homogenization.

The effect of bicarbonate blanch was again significant (0.5%). The water blanch products were more chalky than bicarbonate blanch beverages at each temperature. In case of both blanch treatments, mouthfeel improved with increasing temperature. Although this improvement was greater for the water blanch, the interaction of blanch treatment and temperature was not significant.

Homogenization pressure

Operating pressure on the homogenizer was studied for its effect on chalkiness. Again, bicarbonate blanch was the second factor studied. Steps 7 and 11 (Fig. 1) were done at 2000, 3500 or 5000 psi and Step 3 was done with tap water or 0.25% bicarbonate in tap water. The pH of all beverages was adjusted to about 7.5. Mean chalkiness scores in Table 3 show that use of higher homogenization pressure significantly (0.5%) reduced chalkiness. Single degree of freedom comparisons of mean scores for 2000 vs 3500 and 5000 psi samples and 3500 vs 5000 psi samples found both differences to be significant (0.5%). These results indicated that use of higher homogenization pressure caused greater reduction in particle size of the material responsible for chalkiness of soy beverage. As in the other studies, use of bicarbonate blanch resulted in beverages with significantly (0.5%) less chalkiness. The significant (5%) interaction between homogenization pressure and blanch solution indicates that the improvement of mouthfeel with increasing homogenization pressure was greater with the water blanch beverages. However, the advantages of using a bicarbonate blanch with lower pressure are lower power requirements, greater capacity, and less wear on the homogenizer.

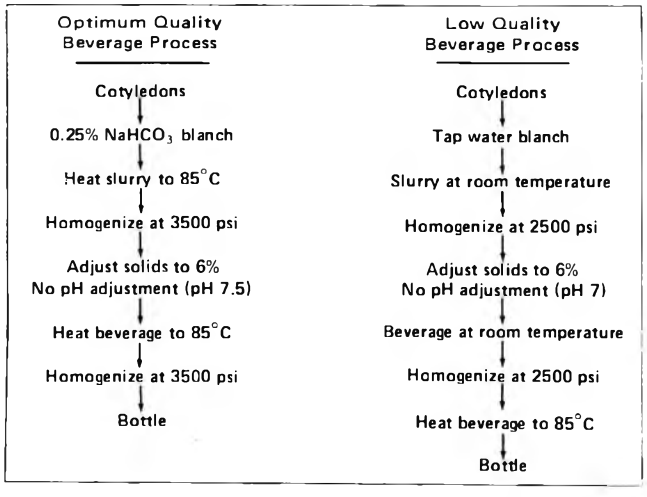


Fig. 3—Comparison of optimum and low quality beverage processes.

Characterization of optimum and low quality beverages

A series of single factor comparisons further evaluated the effects of processing variables. The organoleptic evaluations generally substantiated the results discussed above. Furthermore, filtration fractionation of beverages using 325 mesh screens indicated that tissue disruption was less extensive in chalkier samples. Based on these comparisons, two processes were selected; one used conditions that had resulted in optimum tactual quality and the other used detrimental conditions. These are shown in Figure 3. Finished products were evaluated by organoleptic and objective methods.

Portions of the beverages were centrifuged for 30 min at 2200 × G (4°C); complete removal of particles was confirmed by microscopic examination. As shown in Table 4, this desludging had a significant (0.5%) and very pronounced favorable effect on chalkiness. Removal of the particulate material reduced intensity of chalkiness so the defect was rated as imperceptible (mean score of 1.0) in both optimum and low quality soymilks. These results confirmed that the particulate fraction is responsible for chalkiness. As expected, optimum quality, nondesludged beverages were significantly (0.5%) less chalky than the low quality ones. A significant (0.5%) interaction reflected the much greater reduction in chalkiness intensity resulting from desludging the very chalky, low quality beverages.

There were pronounced differences in distribution of solids and protein between supernatant and sludge from the optimum and low quality beverages, Table 4. For the optimum quality beverages, 66% of the solids and 72% of the protein were in the supernatant while for the low quality beverages only 30% of the solids and 24% of the protein were in the supernatant. These results supported the concept that particle size reduction was much more extensive for the beverages produced using bicarbonate blanch and homogenization at high temperature and 3500 psi.

Filtration fractionation of optimum and low quality beverages showed that, at each screen size, solids retention was less from samples produced using the optimum quality process (Table 5). The differences were significant (0.5%) in case of 325, 200 and 150 mesh. This confirmed that disruption of

Table 4—Chalkiness as affected by desludging and soy solids and protein distribution of soy milks produced by beverage processes (Fig. 4)

Beverage process	Desludged: No	Desludged: Yes	Overall mean score	Solids distribution: Supern.	Solids distribution: Sludge	Protein distribution: Supern.	Protein distribution: Sludge
	Mean score*			% of total		% of total	
Optimum quality	1.6	1.0	1.3	65.6	34.4	72.4	27.6
Low quality	5.3	1.0	3.1	30.0	70.0	24.4	75.6
	Overall mean score						
	3.4	1.0					

Beverage process, desludging and interaction significant at 0.5% level

* 1 = imperceptible, 4 = perceptible, 7 = very pronounced

particulate material was more complete in the optimum quality sample. As expected, after the two beverages had been desludged by centrifugation, solids retention on the 325 mesh screen was negligibly low in both cases (Table 5).

Mean chalkiness scores and the results of filtration fractionation for two replicates of each type of soymilk are in Table 6. Linear regression analyses showed high correlations (r) between organoleptic chalkiness scores and percent solids retention by 325, 200 and 150 mesh. According to the linear regression equation for 150 mesh results, the score when solids retention is 0 would be 1.16. This is close to the minimum intensity of 1.0 (equivalent to imperceptible chalkiness). Although based on a small number of comparisons, these analyses indicated that chalkiness is caused by particles retained on 150 mesh.

Hydration of insoluble carbohydrate from the two beverages was evaluated. The optimum and low quality beverages were desludged and the sludge samples were fractionated to obtain insoluble carbohydrate as described above. One pair of fractionated and washed samples was soaked in water with shaking for 12 hr at 60°C prior to dehydration.

The initial sludge solids from optimum and low quality beverages showed protein concentrations of 40 and 75%, respectively. In both cases, fractionation of the sludge reduced the protein content of the fractionated solids to only 20%. Microscopic examination showed this material to be discrete cell wall or fiber fragments. The effect of beverage process on water uptake by fractionated beverage solids was significant (0.5%). Sludge solids from beverages produced using the optimum quality process took up more water than sludge solids from low quality beverages (23.1 and 18.1 ml/g, respectively). There was no systematic variation in water uptake with variation in protein content. Therefore, it was concluded that differences in water uptake truly reflected a significant difference in hydration of this beverage fraction. This conclusion was substantiated by the effects of long-term soaking of the density fractionated sludge samples at 60°C. After drying, water uptake by the sludge solids from the low quality, water blanch beverage increased to a level comparable to that for the bicarbonate blanch sample (23.1 and 23.8 ml/g, respectively). Evidently, the basic role of the bicarbonate blanch is to promote hydration of the cell wall material and this in turn promotes cell disruption and particle size reduction.

Table 5—Solids retention from soymilks by 325, 200, 150 and 100 mesh screens as affected by process and desludging

Mesh	Beverage process: Optimum quality	Beverage process: Low quality	Significance between treatment means
	Mean solids retained (%)		
325	1.26	3.25	0.5%
200	0.36	1.64	0.5%
150	0.09	0.99	0.5%
100	0.01	0.07	NS
	Desludged soymilk		
325	0.02	0.08	NS

Table 6—Evaluation of linear relationship between chalkiness of soymilk and particle size by filtration fractionation

Beverage process	Rep.	Chalkiness mean score*	Mesh: 325	200	150	100
			Solids retained (%)			
Optimum quality	1	1.6	1.30	0.37	0.12	0.02
	2	1.6	1.22	0.38	0.10	0.01
Low quality	1	5.4	3.68	1.83	1.05	0.12
	2	5.1	2.98	1.49	1.01	0.04
			Correlation coefficient, r			
			0.984	0.992	0.999	0.788
			Score extrapolated to 0% solids retained			
			−0.45	0.60	1.16	1.84

* 1 = imperceptible, 4 = perceptible, 7 = very pronounced

REFERENCES

Baumann, H. 1967. Apparatur nach Baumann zur Bestimmung der Flussigkeitsaufnahme von pulvrigen Substanzen. G.I.T. Fachzeitschrift fur das Laboratorium, 6: 540 (In German).

FAO. 1973. Energy requirements and protein requirements. WHO Tech. Rep. Ser. No. 522, p. 63.

Green, J.W. 1963. Drying and reactivity of cellulose. In "Methods in Carbohydrate Chemistry," Vol. 3. "Cellulose," Ed. Whistler, R.L. Academic Press Inc., New York, NY.

Hand, D.B., Steinkraus, K.H., Van Buren, J.P., Hackler, L.R., El Rawi, I. and Pallesen, H.R. 1964. Pilot-plant studies on soymilk. Food Technol. 18: 139.

Lo, K.S. 1971. Process for preparing a soybean beverage. U.S. Patent 3,563,762.

Miles, C.P. 1966. Process of producing milk from soybeans. U.S. Patent 3,288,614.

Mustakas, G.C., Albrecht, W.J., Bookwalter, G.N., Sohns, V.E. and Griffin, E.L. Jr. 1971. New process for a low cost high protein beverage base. Food Technol. 25: 534.

Mustakas, G.C., Albrecht, W.J. and Bookwalter, G.N. 1972. Production of vegetable protein beverage base. U.S. Patent 3,639,129.

Nelson, A.I., Steinberg, M.P. and Wei, L.S. 1975. Soybean beverage base. U.S. Patent 3,901,978.

Nelson, A.I., Steinberg, M.P. and Wei, L.S. 1976. Illinois process for preparation of soymilk. J. Food Sci. 41: 57.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., New York.

Wilkens, W.F. and Hackler, L.R. 1969. Effect of processing conditions on the composition of soy milk. Cereal Chem. 46: 391.

Wilkens, W.F., Mattick, L.R. and Hand, D.B. 1967. Effects of processing method on oxidative off-flavors of soybean milk. Food Technol. 21: 1630.

Ms received 8/15/77; revised 12/22/77; accepted 12/30/77.

EFFECT OF WATER ACTIVITY ON NUCLEASE PRODUCTION BY Staphylococcus aureus

J. F. KAMMAN, S. R. TATINI and T. P. LABUZA

ABSTRACT

The effect of a_w and three polyol humectants, glycerol, propylene glycol and butylene glycol on the production of heat-stable staphylococcal nuclease by *S. aureus* 196E was examined. Nuclease enzyme activity as detected by the DNA-agar-diffusion technique was related to the growth pattern in various test conditions. Of the conditions tested, 20% glycerol (0.95 a_w), 10% proplyene glycol (0.97 a_w), and 10% butylene glycol (0.98 a_w) were shown to result in a reduced rate of nuclease production per unit cell, when compared to conditions of higher water activity.

INTRODUCTION

THE VIABILITY of *Staphylococcus aureus* at intermediate water activities is of concern to the food industry, particularly in view of increased development and usage of intermediate moisture foods (IMF). The minimum water activity (a_w) of about 0.86 for growth of *S. aureus* has been reported and confirmed by several authors (Scott, 1957; Troller, 1973; Plitman et al., 1973).

The causative factor in staphylococcal food poisoning is the enterotoxin produced by the microorganism. Studies by Lachica et al. (1972), Tatini et al. (1975, 1976), and Cords and Tatini (1973) have reported on the usefulness of an extracellular deoxyribonuclease (DNase) of *S. aureus* as an indicator and screening procedure for the assessment of staphylococcal growth and the likely presence of enterotoxins. Staphylococcal nuclease (E.C. No. 3.1.4.7) is relatively thermostable and is active at high pH. Anfinsen et al. (1971) have described many of the chemical and catalytic properties of the enzyme.

Using the nuclease assay procedure of Lachica et al. (1971) under optimal conditions reported by Kamman and Tatini (1977), the present study reports on the production of heat-stable staphylococcal nuclease as a function of a_w. Three polyol humectants, glycerol, propylene glycol and butylene glycol, were utilized to control a_w and were also tested for their specific effect on the production of DNase.

EXPERIMENTAL

Test systems

The test medium of Brain Heart Infusion (BHI) broth (BBL) was prepared using 36.5g dry BHI/1,000 ml (pH 7.5) of solution. Each solution was made with different concentrations of glycerol (Mallinckrodt), propylene glycol (1,2-propanediol, American Mineral Spirits Company), or butylene glycol (1,3 butanediol, Celanese Corporation) according to percent weight. Duplicate 100 ml volumes of each solution were sterilized. Single samples of each test solution were inoculated with *S. aureus* 196E and were incubated under static conditions at 25°C. The entire study was performed twice. The initial concentration of bacteria was $4-6 \times 10^4$ and $2-4 \times 10^3$ organisms/ml, respectively, for each trial.

Viable count determination

At appropriate intervals, a 0.1 ml aliquot of the appropriate dilution was immediately plated in duplicate by the surface spread technique on trypticase soy agar (TSA, BBL) and incubated at 37°C for 24 hr before enumeration.

Heat-stable nuclease assay

At appropriate intervals, a 1.0 ml aliquot was removed from the test solutions and boiled for 15 min followed by centrifugation for 10 min at 10,000 rpm (Sorvall RC-2). The Toluidine Blue O-DNA-Agar (TDA) diffusion technique of Lachica et al. (1971) was employed using the methods and optimum conditions for assay detailed by Kamman and Tatini (1977). The extracted enzyme was incubated in TDA plates for 2 hr at 50°C and the resultant zone diameter was measured with calipers and recorded. All enzyme assays were performed with duplicate wells and two measurements per well per reading.

Measurement of a_w

Matching sterilized test solutions which had not been inoculated were measured for a_w by a Sina-Scope (Sine, Zurich, Switzerland) which had been calibrated with salt solutions of known a_w and by the vapor pressure manometer (VPM) technique. Both of the aforementioned techniques have been described by Labuza et al. (1976).

Test for polyol interference on enzyme activity

Extracted heat-stable nuclease from stationary phase *S. aureus* 196E grown in BHI was diluted 1:5 with sterile BHI and polyol to a final polyol concentration of 0%, 20% glycerol, 10% propylene glycol, or 10% butylene glycol, by weight. At 0, 24 and 48 hr (after dilution with polyol), 5 μl aliquots were removed and activity was measured in TDA plates.

Curve fitting

Regression programs for lines of best fit were run on a Control Data Cyber 74 computer housed at the University of Minnesota computer center.

Table 1—Depression of a_w in test systems by polyols

Polyol	% Weight in BHI	a_w
Glycerol	10	0.99
	20	0.95
	30	0.90
	40	0.87
Propylene glycol	5	0.98
	10	0.97
	15	0.96
	20	0.94
Butylene glycol	5	0.99
	10	0.98
	15	0.96
	20	0.94

RESULTS & DISCUSSION

TABLE 1 shows the depression of a_w by different test humectants according to percent weight. The selected test solutions were based on results of Plitman et al. (1973) which described the bacteriostatic and bactericidal effects of glycerol, propylene glycol, and butylene glycol on the growth of *S. aureus* in BHI broth.

Results of the effect of glycerol on the growth of *S. aureus* are shown in Figure 1A. Similar growth curves for propylene glycol and butylene gylcol systems are presented in Figures 2A and 3A, respectively. While data points for one run are presented, it should be noted that similar growth curves and effects were seen in both experimental runs. The concentra-

Authors Kamman, Tatini and Labuza are with the Dept. of Food Science & Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

0022-1147/78/0004-1284$02.25/0
© 1978 Institute of Food Technologists

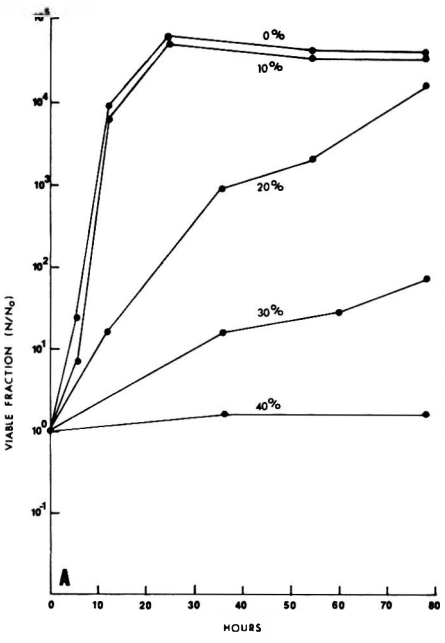


Fig. 1A—Effect of glycerol concentration (% wt) on growth of S. aureus 196E in BHI.

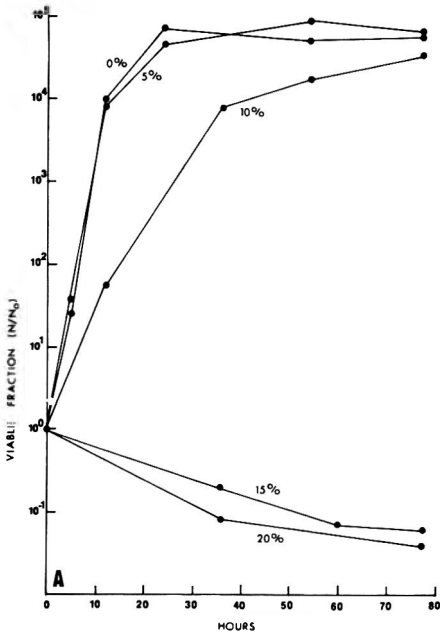


Fig. 2A—Effect of propylene glycol concentration (% wt) on growth of S. aureus 196E in BHI.

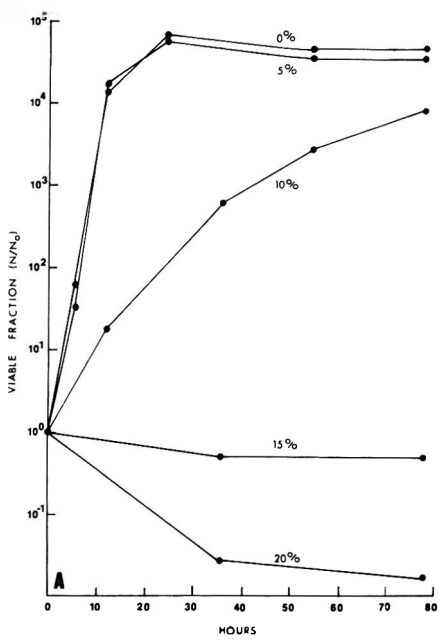


Fig. 3A—Effect of butylene glycol concentration (% wt) on growth of S. aureus 196E in BHI.

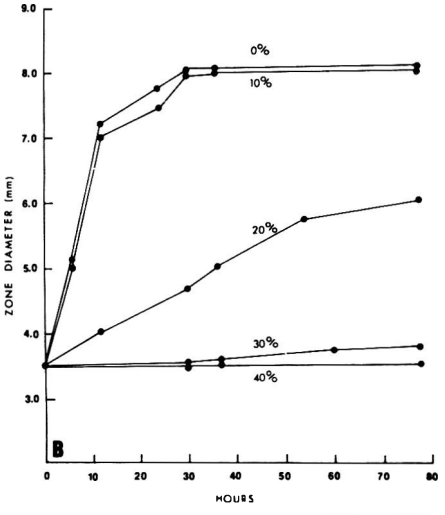


Fig. 1B—Effect of glycerol concentration (% wt) on the production of heat-stable DNase by S. aureus 196E in BHI.

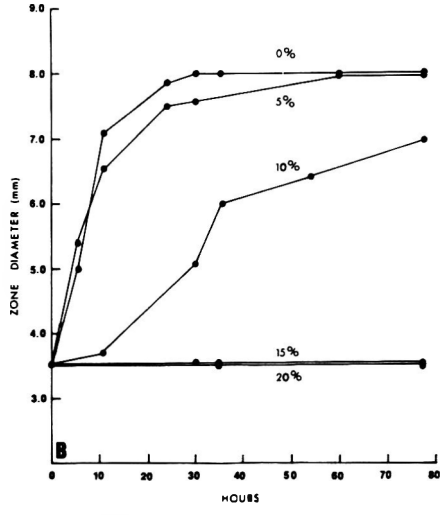


Fig. 2B—Effect of propylene glycol concentration (% wt) on the production of heat-stable DNase by S. aureus 196E in BHI.

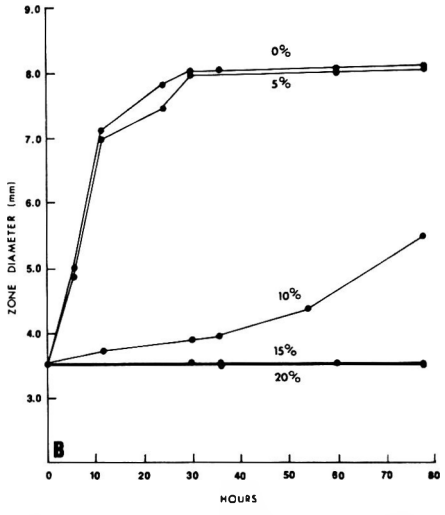


Fig. 3B—Effect of butylene glycol concentration (% wt) on the production of heat-stable DNase by S. aureus 196E in BHI.

tions of polyol found to be inhibitory (repressive) to growth were 40% glycerol ($0.87\ a_w$), 15% propylene glycol ($0.96\ a_w$), and 15% butylene glycol ($0.96\ a_w$). These values are quite similar to those reported by Plitman et al. (1973).

Figures 1B, 2B and 3B show the production of heat-stable nuclease as reflected by enzyme activity in each test system with time. Cords and Tatini (1973) have demonstrated that zone diameter in the TDA system is linearly related to the log of enzyme concentrations. In those systems of uninhibited staphylococcal growth (e.g. 10% glycerol) the activity of heat-stable nuclease as measured by zone size followed the growth rate pattern of the microorganism in which stationary phase was reached within 24 hr. At inhibitory levels of growth (e.g. 15% propylene glycol), there is no change in zone diameter with time, as expected. However, at intermediate growth levels, some effect of a_w on the production of heat-stable nuclease was noticeable in terms of zone diameter. In those systems, 20% glycerol, 10% propylene glycol, and 10% butylene glycol (a_w 0.95, 0.97, 0.98 respectively), the growth of *S. aureus* 196E reached near stationary phase by 78 hr. These systems were monitored up to 144 hr with no further increase in zone diameter (nuclease activity) detected.

A possible explanation for this effect may be due to inactivation of produced heat-stable nuclease by the humectants. To test this, nuclease was introduced into appropriate systems for measurement of enzyme inactivation as indicated by zone size (Table 2). After 48 hr there was no evidence of enzyme inactivation in any system.

Figures 4, 5 and 6 present cumulative data for each polyol in which growth (log of viable count) is plotted as a function of zone diameter. The resultant linear functions were determined by regression. The correlation coefficients for each

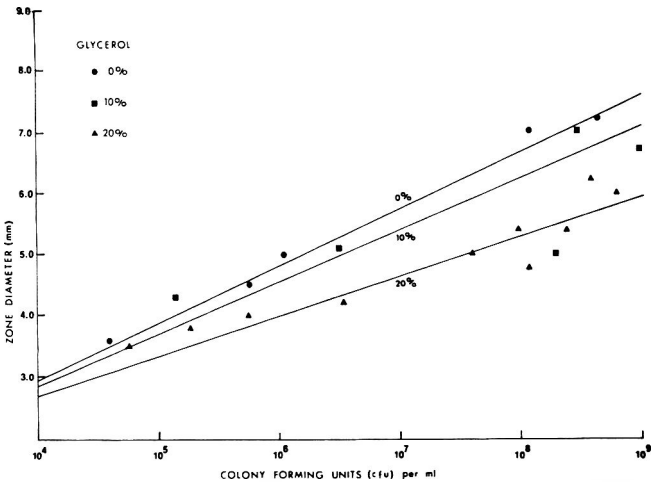


Fig. 4—Correlation of enzyme activity as measured by zone diameter in TDA system to growth of S. aureus 196E in glycerol test systems.

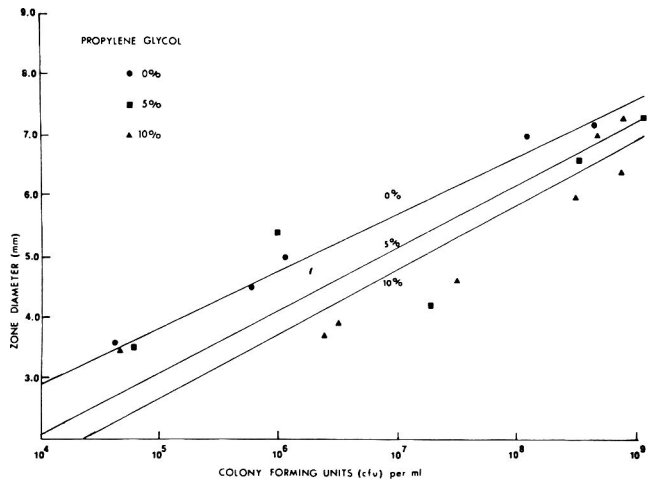


Fig. 5—Correlation of enzyme activity as measured by zone diameter in TDA system to growth of S. aureus 196E in propylene glycol test systems.

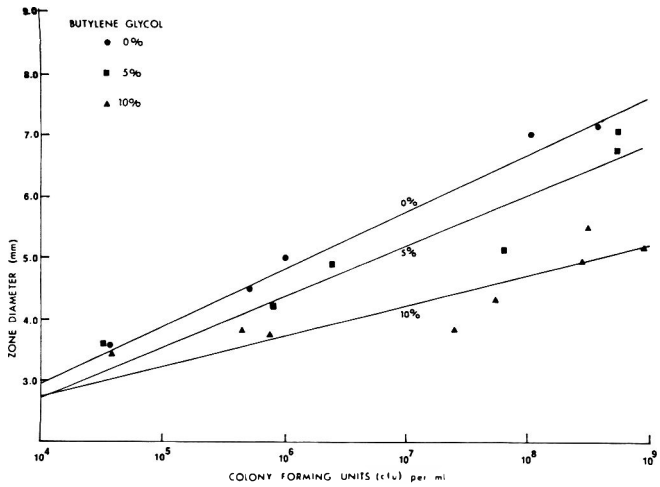


Fig. 6—Correlation of enzyme activity as measured by zone diameter in TDA system to growth of S. aureus 196E in butylene glycol test systems.

Table 2—Effect of polyols on DNase activity

	Hours		
	0	24	48
0% Polyol	5.2[a]	5.4	5.3
20% Glycerol	5.5	5.4	5.4
10% Propylene glycol	5.3	5.3	5.3
10% Butylene glycol	5.2	5.3	5.4

[a] Zone diameter in mm, 1:5 dilution of 196E DNase

Table 3—Comparison of test conditions at 10^7 cfu and regression statistics

Test condition (% humectant)	Zone diameter (mm) at 10^7 cfu	Regression correlation coefficient	F-Ratio of regression
0%	5.7	0.99	350.6**
10% Glycerol	5.3	0.88	14.4*
20% Glycerol	4.6	0.95	74.9**
5% Propylene glycol	5.1	0.80	6.9[a]
10% Propylene glycol	4.8	0.93	33.6**
5% Butylene glycol	5.1	0.95	36.6**
10% Butylene glycol	4.2	0.88	2[illegible].3**

[a] Significant at 0.10
* Significant at 0.05
** Significant at 0.01

system are given in Table 3. As seen in all cases but one, the F test ratio showed significance at the 0.05 level or better. In all cases, those conditions of reduced growth rate (e.g. 10% butylene glycol) resulted in a linear relationship which has a smaller zone diameter for any given viable count than for uninhibited growth.

A comparison between test conditions at 10^7 colony forming units is also given in Table 3. At this population, there is a considerable difference in enzyme activity between 0% polyol and the reduced growth rate conditions of 20% glycerol, 10% propylene glycol and 10% butylene glycol. These data also demonstrate the bactericidal differences between humectants. For example, 10% glycerol (a_w 0.99) has a zone diameter of 5.3 mm at 10^7 colony forming units whereas 10% butylene glycol (a_w 0.98) has a zone diameter of only 4.2 mm.

This work suggests that under conditions of stress, there is reduced production of heat-stable nuclease by viable staphylococci. These conditions of stress (lower a_w, bactericidal humectants) may function to inhibit somewhat the production or release of such extracellular enzymes as nuclease. The correlation of this inhibition to the presence or synthesis of toxin is unknown at this time. However, studies by Troller (1971, 1972) and Troller and Stinson (1975) have demonstrated significant inhibition of enterotoxin production due to decreases in a_w. The effect of a_w on the presence of nuclease appears to be quite similar to the reported effect of a_w on the presence of enterotoxin. Further studies should examine the effects of other stress variables on the rate of DNase production and, more importantly, the correlation of DNase to enterotoxin under conditions of stress.

REFERENCES

Anfinsen, C.B., Cuatrecasas, P. and Taniuchi, H. 1971. Staphylococcal nuclease, chemical properties and catalysis. In "The Enzymes" Vol. 4, p. 177. Academic Press, New York.

Cords, B.R. and Tatini, S.R. 1973. Applicability of heat-stable deoxyribonuclease assay for assessment of staphylococcal growth and the likely presence of enterotoxin in cheese. J. Dairy Sci. 56: 1512.

Kamman, J.F. and Tatini, S.R. 1977. Optimal conditions for assay of staphylococcal nuclease. J. Food Sci. 24: 421.

Labuza, T.P., Acotts, K., Tatini, S.R. and Lee, R.Y. 1976. Water activity determination: A collaborative study of different methods. J. Food Sci. 41: 910.

—Continued on page 1292

GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF LENTHIONINE IN Shiitake MUSHROOM (Lentinus edodes) WITH SPECIAL REFERENCE TO THE RELATION BETWEEN CARBON DISULFIDE AND LENTHIONINE

YOSHIO ITO, MASATAKE TOYODA, HIDEYO SUZUKI and MASAHIRO IWAIDA

ABSTRACT

A simple gas chromatographic procedure for the determination of lenthionine, a flavor component in *shiitake* mushroom, was developed. The method involves the extraction of lenthionine with n-hexane from the soaked mushrooms and soak solution and subsequent determination by flame photometric detector gas chromatography. Lenthionine was formed by soaking dried mushroom in water and warming at neutral or alkaline pH, but a little amount of lenthionine was produced at acidic pH. The relation coefficient between the formation of lenthionine and the evolution of carbon disulfide in 20 samples was 0.507. It is assumed that lenthionine is formed by immersing the dried mushroom in water and gradually decomposes to carbon disulfide.

INTRODUCTION

IN A PRECEDING PAPER, (Toyoda et al., 1978), a sensitive procedure for determination of carbon disulfide in *shiitake* mushroom (*Lentinus edodes*) was established, being able to detect a few ppm level of carbon disulfide in mushroom.

Since the amount of carbon disulfide increased by soaking dried mushroom, it was suggested that a considerable part of the carbon disulfide might have been derived from some constituents of the mushroom. Accordingly, the precursor substance in mushrooms was pursued in this report. It is widely known that there is a flavor substance, lenthionine, in mushroom (Morita and Kobayashi, 1966). Lenthionine—a sulfur-containing compound—was reported to be enzymatically produced from lenthinic acid in dry mushrooms (Yasumoto et al., 1971a, b), and that it was heat-decomposed to produce hydrogen sulfide (Wada et al., 1967). In this paper we shall attempt to clarify whether lenthionine is responsible for the endogenous formation of carbon disulfide in the *shiitake* mushroom. In order to determine the interrelationship between carbon disulfide and lenthionine, an accurate procedure for determining lenthionine in mushrooms had to be established. Accordingly, solvent extraction of lenthionine from *shiitake* mushroom and subsequent determination by gas liquid chromatography (GLC) with flame photometric detecting system (FPD) was developed.

MATERIALS & METHODS

Lenthionine

Lenthionine, a gift of Takeda Chemical Industries Ltd, had the following physical data: mp. 61.0°C; Anal. Calc. for $C_2H_4S_5$: C, 12.75; H, 2.14; S, 85.11; Found: C, 12.74; H, 2.15; S, 85.14. Mass spectra (direct method, 70 eV): m/e 188 (M^+). The infra-red and nuclear magnetic resonance spectra of lenthionine used agreed with these data (Morita and Kobayashi, 1966).

The standard solutions were prepared by dissolving 10g of crystallized lenthionine in dioxane followed by further dilution to 0.5–2 μg/ml with n-hexane.

Buffer solutions

pH 2–4, 0.2M phosphoric acid–0.2M KH_2PO_4 buffer; pH 5–7, 0.2M KH_2PO_4–0.2M K_2HPO_4 buffer; and pH 8–12, 0.2M K_2HPO_4 - 0.2M NaOH buffer.

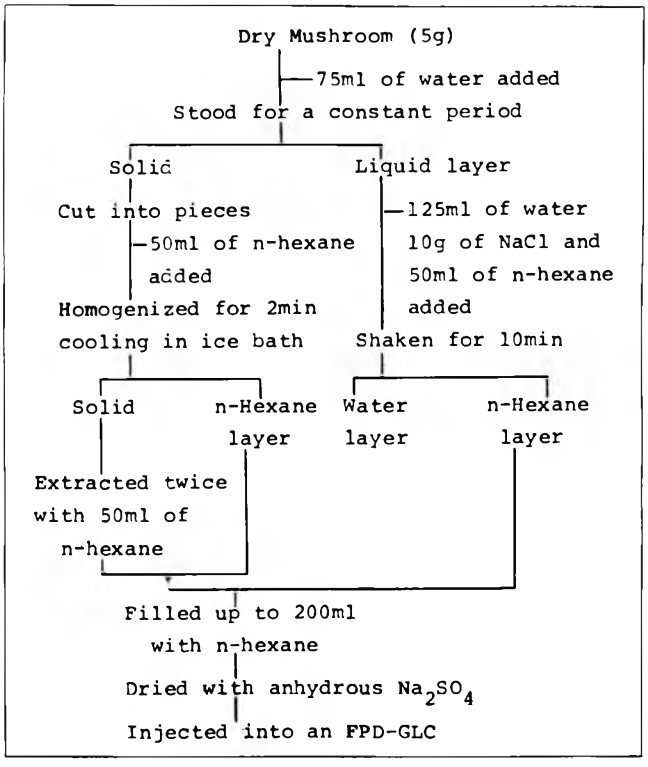


Fig. 1—Scheme of procedure for extraction of lenthionine from soaked mushroom (Lentinus edodes).

Gas chromatography

Yanaco G-80 FPD-gas chromatograph (Yanagimoto Mfg. Co. Ltd., Kyoto) equipped with glass tubes 75 cm in length × 0.3 cm i.d. packed with 0.3% OV-17 on Neosorb NK (porous glass beads) 40/60 was used. Flow rate of carrier gas (N_2) was 25 ml/min. Air and hydrogen flow rates: 250 and 60 ml/min, respectively. Temperature of injection port and detector: 200°C. Column temperature: 170°C. Injection volume was 5 μl.

Determination procedure for CS_2

Determination of carbon disulfide was carried out according to the method described in a previous report (Toyoda et al., 1978).

Determination procedure for lenthionine

Dried mushroom is immersed in water for a specific time. The solid material (fruiting body) is taken out, put into a blender cup containing n-hexane, and subjected to simultaneous extraction and homogenization with n-hexane. The residual soak water, after dilution with water, is extracted with n-hexane through vigorous shaking. The two n-hexane layers are combined and dried with anhydrous sodium sulfate. An aliquot of the n-hexane layer is injected into an FPD-gas chromatograph to determine lenthionine content (See Fig. 1).

RESULTS

Determination of lenthionine by FPD-GC

FPD-gas chromatogram of lenthionine showed a single peak

All authors are with the National Institute of Hygienic Sciences, Osaka Branch: No. 6, Hoenzaka-cho, Higashi-ku, Osaka, 540 Japan.

0022-1147/78/0004-1287$02.25/0
© 1978 Institute of Food Technologists

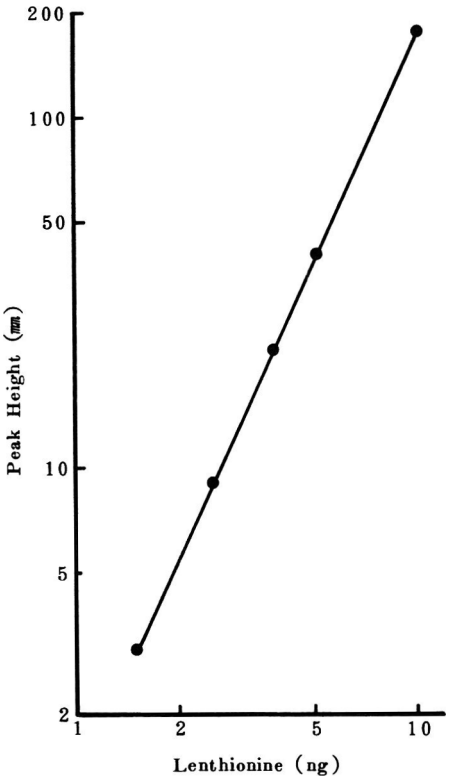


Fig. 2—Standard curve for lenthionine by flame photometric gas-liquid chromatography. Chromatographic conditions: column 75 cm X 0.3 cm i.d. glass column packed with 0.3% OV-17/Neosorb NK (40–60 mesh); column temperature 170°C; injection temperature 200°C; carrier N_2 flow rate 25 ml/min (co-ordinates in logarithmic scale).

Table 1—Recoveries of lenthionine from Shiitake mushroom (Lentinus edodes)[a]

Lenthionine		Recovery
Added (μg)	Found (μg)	(%)
0	11.5 ± 0.6	—
50	58.0 ± 3.4	93.0 ± 7.3
100	105.4 ± 5.3	93.9 ± 5.6
250	246.8 ± 7.5	94.1 ± 3.2
500	477.0 ±11.6	93.1 ± 2.5
1000	953.5 ±11.3	94.2 ± 1.2

[a] Mean value of five trials

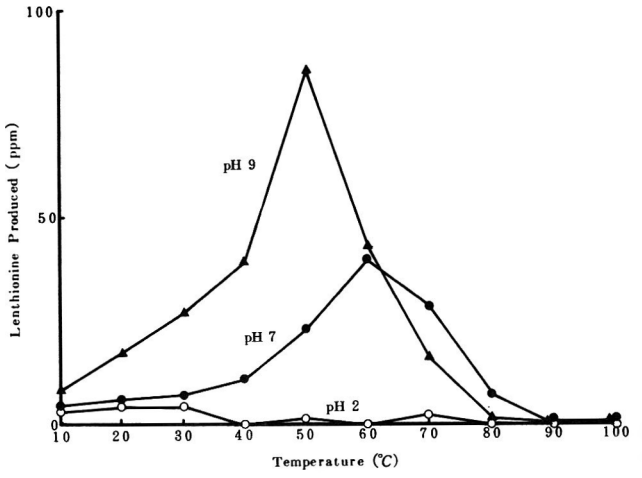


Fig. 3—Relation between amounts of lenthionine produced and incubation temperature (Incubated for 1 hr). Five grams of dried mushroom were immersed in each 75 ml of buffer solution at pH 2, 7 and 9 for 1 hr at temperatures ranging from 10 to 100°C and then subjected to lenthionine determination.

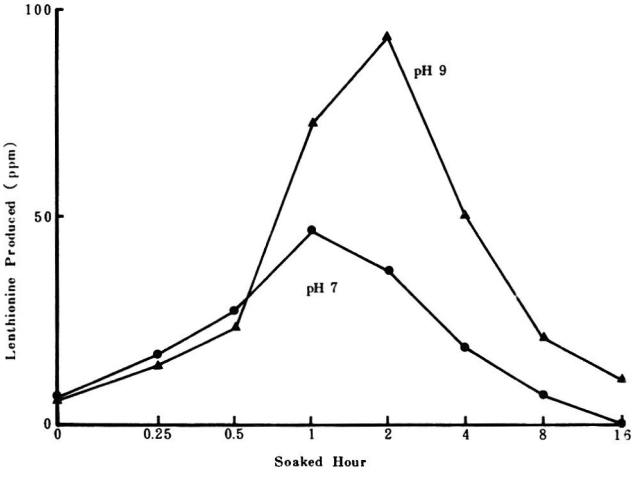


Fig. 4—Time course of lenthionine formation at pH 7 and 9. Five grams of dried mushroom were immersed in each 75 ml of buffer solution of pH 7 and 9 for 16 hr at 22°C. Lenthionine content was analyzed at intervals.

at the retention time of 2 min. A calibration curve prepared by plotting peak height (mm) against concentration of lenthionine in logarithmic scale is shown in Figure 2. A linear gas chromatographic response was obtained within 1.5–10 ng of lenthionine. Repeatability at the 5 ng level of lenthionine was ± 2.4% in CV (n = 10).

Extraction of lenthionine and recovery study

The procedure for the extraction of lenthionine from mushroom is presented schematically in Figure 1. The lower limit of detection was 0.1 ppm. Dried mushroom samples were spiked with various amounts of lenthionine as indicated in Table 1. Percent recovery was about 93.0%.

Optimum conditions for formation of lenthionine from dried mushrooms

Figure 3 shows the formation of lenthionine was at its maximum at temperatures of 60°C and 50°C at pH 7 and 9, respectively. In the next phase, the time course of lenthionine formation was pursued. As illustrated in Figure 4, formation of lenthionine was at its maximum after 1 hr at pH 9, and 2 hr at pH 7, followed by a rapid decrease due to decomposition of lenthionine produced.

Evolution of CS_2 from lenthionine

A water solution of either lenthionine or *shiitake* homogenate spiked with lenthionine was subjected to CS_2 determination after distillation as described previously (Toyoda et al., 1978). Results are shown in Table 2. When both homogenization and distillation were carried out at pH 2, carbon disulfide was not detected at all. When these procedures were carried out at pH 7, carbon disulfide was formed. Spikage of 500 μg of lenthionine to dry mushroom resulted in the formation of 47.5 μg carbon disulfide. The yield was calculated to be 9.5%.

Relation between carbon disulfide and lenthionine

Taking into account the previously known optimum conditions with respect to the lenthionine formation and the usual conditions for cooking, the relationship between production of lenthionine and evolution of carbon disulfide was plotted in Figure 5. The relative coefficient was calculated as 0.507. A weak positive relationship was found between them.

DISCUSSION

MORITA AND KOBAYASHI (1966) immersed fruiting body of dried mushrooms in water and after removal of residue by centrifugation, extracted lenthionine from the sediment by methylene chloride. From our preliminary experiment it became clear that methylene chloride was not an excellent extractant since it will not easily separate from the mushroom homogenate after shaking. n-Hexane was suitable as an extractant with satisfactory recovery of lenthionine and good separation. Since the characteristic flavor was detected from the soaked solution, too, we tried to extract the lenthionine present both in the solid material and soak solution. The average distribution of lenthionine present in the soak solution (of 20 samples of mushroom) was found to be 18.2%. Hence, it becomes evident that, in order to calculate the total production of lenthionine in *shiitake* mushroom it is necessary to take into account the lenthionine present in the soak solution (cf. Fig. 2).

Lenthionine was formed by soaking dried mushroom in water and warming at neutral or alkaline pH; only small amount of lenthionine was produced at acidic pH. These results were consistent with analytical data that optimum pH of enzymes related to the formation of lenthionine from lenthinic acid ranged from 7–9 (Iwami et al., 1975), and that these enzymes do not act at pH 2.

Based on these results, it is quite safe to conclude that lenthionine is formed enzymatically during soaking dried mushrooms in water under neutral pH. As described in a previous report, the determined amount of carbon disulfide in dried mushroom was much influenced by the pH of the prepared homogenate. This might be due to the presence of some precursors that produce carbon disulfide. Lenthionine was selected as one of the precursors from its chemical structure. Solutions of lenthionine and *shiitake* homogenate spiked with lenthionine was examined. Carbon disulfide was not produced at acidic pH but was produced at neutral pH, consistent with results of our previous report (Toyoda et al., 1978).

The large difference in carbon disulfide production from the spiked water solution (2.35 μg) and from the spiked dried mushroom solution (54.0 μg) might be attributed to the difference in solubility of lenthionine in water and mushroom homogenate. The presence of saccharides, peptides etc. in mushroom may have contributed to the increase in the solubility of lenthionine. When lenthionine was dissolved in Tween-80 solution, the carbon disulfide produced was 8.9%. From these facts, it is presumed that lenthionine was one of the precursors that produced carbon disulfide. The participation of lenthionine in carbon disulfide evolution was also supported by the result that an increase in the amount of lenthionine in mushrooms tends to increase the evolution of carbon disulfide as shown in Figure 6.

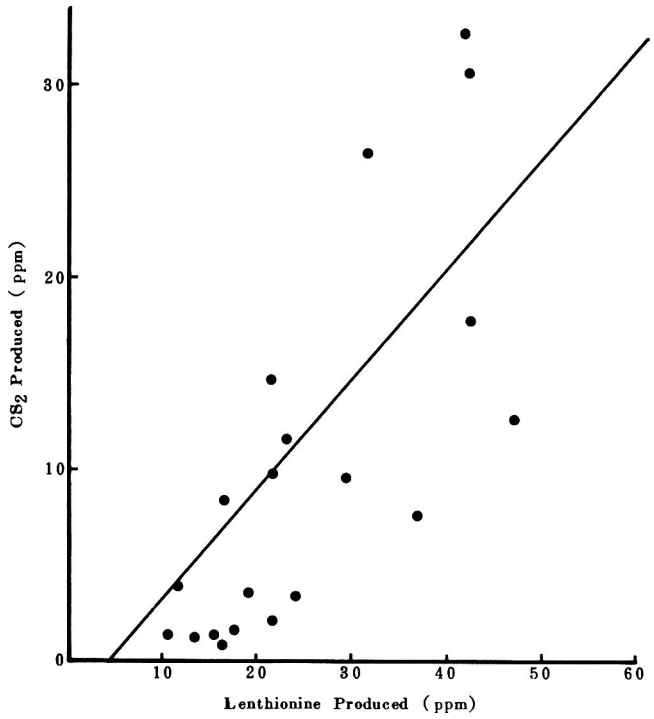


Fig. 5—Interrelationship between production of CS_2 and lenthionine. $y = 0.5721x + 2.4246$; $r = 0.507$ (significant at 5% level). Five grams of dried mushroom were immersed in 75 ml of water at pH 7 and warmed at 60°C for 1 hr, followed by determination of lenthionine and carbon disulfide. In the latter case, dried mushroom was homogenized and distilled at pH 7.

Table 2—Effect of pH and lenthionine on the production of carbon disulfide[a]

Sample	pH of H/D[b]	Lenthionine added (μg)	CS_2 found (μg)	CS_2 produced / Lenthionine added (%)
Water	2	500	N.D.	0
	7	500	2.35	0.47
	2	0	1.55	–
Dry mushroom		500	1.55	0
	7	0	7.30	–
		500	54.8	9.50

[a] Mean value of three trials; 5-g samples were used. N.D. < 0.05 μg
[b] Homogenization/Distillation

REFERENCES

Iwami, K., Yasumoto, K., Nakamura, K. and Mitsuda, H. 1975. Properties of α-glutamyltransferase from Lentinus edodes. Agr. Biol. Chem. 39: 1933.

Iwami, K., Yasumoto, K., Nakamura, K. and Mitsuda, H. 1975. Reactivity of Lentinus α-glutamyltransferase with lentinic acid as the principal endogenous substrate. Agr. Biol. Chem. 39: 1941.

Iwami, K., Yasumoto, K., Nakamura, K. and Mitsuda, H. 1975. Enzymatic cleavage of cysteine sulfoxide in Lentinus edodes. Agr. Biol. Chem. 39: 1947.

Morita, K. and Kobayashi, S. 1966. Isolation and synthesis of lenthionine, and odorous substance of shiitake, an edible mushroom. Tetrahedron Letters 6: 573.

Toyoda, M., Suzuki, H., Ito, Y. and Iwaida, M. 1978. Gas liquid chromatographic determination of carbon disulfide in shiitake mushroom (Lentinus edodes). J. Food Sci. 43: 0000.

Wada, S., Nakatari, H., Fujinawa, S., Kimura, H. and Hagaya, M. 1967. Studies on lenthionine, a new aroma-bearing substance from shiitake. Eiyō-to-Shokuryō (J. Jap. Soc. Food & Nutr.) 20: 355.

Yasumoto, K., Iwami, K. and Mitsuda, H. 1971a. A new sulfur-containing peptide from Lentinus edodes acting as a precursor for lenthionine. Agr. Biol. Chem. 35: 2059.

Yasumoto, K., Iwami, K. and Mitsuda, H. 1971b. Enzyme-catalized evolution of lenthionine from lentinic acid. Agr. Biol. Chem. 35: 2070.

Ms received 8/21/77; revised 2/10/78; accepted 2/15/78.

GAS-LIQUID CHROMATOGRAPHIC DETERMINATION OF CARBON DISULFIDE IN SHIITAKE MUSHROOM (Lentinus edodes)

MASATAKE TOYODA, HIDEYO SUZUKI, YOSHIO ITO and MASAHIRO IWAIDA

ABSTRACT

A simple, rapid chromatographic procedure for the determination of carbon disulfide in *shiitake* mushrooms was developed. The method involves homogenization of dried mushroom, followed by distillation of the homogenate in the presence of acetone. Carbon disulfide in acetone is determined by GLC with a flame photometric detecting system. The carbon disulfide content in dried mushroom was affected considerably by the pH of homogenation and distillation; 0.02–3.4 ppm of carbon disulfide in dried mushrooms under acidic condition increased to 0.98–72.5 ppm under neutral conditions. The increased carbon disulfide under neutral conditions is purported to be derived from a mushroom component. Therefore, residual carbon disulfide in dried *shiitake* mushrooms must be determined under acidic conditions of homogenation and distillation. The lower detection limit was 0.025 ppm.

INTRODUCTION

CARBON DISULFIDE has been recognized to be a poisonous substance. Poisoning occurs not only from inhalation but also by ingestion and skin absorption, accompanied by such physiological disorders as stimulation of intestinal mucosa, peripheral neuritis, injuries of acaustic and visual nerve, etc. (Cesaro et al., 1952; Paulus and Prokop, 1953). Besides, carbon disulfide is often used as a fumigant to destroy insects in foods.

Toward the end of 1976, empty ampules which might have contained carbon disulfide were found by food inspectors in imported cargos of mushrooms in harbors located in the western part of Japan.

In the present work, an attempt was made to develop a method for determining residual carbon disulfide in *shiitake* mushroom. Considerable work has been reported on the determination of carbon disulfide by use of gas-liquid chromatography (GLC) (Berk, 1965; Grant and Vaugham, 1965; Bielorai and Alumot, 1966; Heuser and Scudamore, 1968, 1969; McLeod and McCully, 1969; Berk et al., 1970), with flame photometric detector (FPD) being found most sensitive and suitable for this purpose. Methods previously employed for fumigant extraction from foods include direct solvent extraction (Goodwin et al., 1961; Thornton and Anderson, 1965) and steam distillation in the presence of toluene (Berk et al., 1970).

Because of the high volatility of carbon disulfide, it was difficult to successfully carry out a clean-up procedure after extraction, with high recovery yield. Accordingly, distillation was adopted in this report. Samples were distilled in the presence of acetone, the distillate trapped into cold acetone, and an aliquot injected into a gas chromatograph. Nanogram quantities of carbon disulfide could be determined by FPD-GC. From the results it became clear that the determined values were affected considerably by pH during the analytical procedure. Moreover, it became evident that the presence of carbon disulfide in *shiitake* mushroom did not indicate merely the presence of residual fumigant but that carbon disulfide evolved from the constituents of the *shiitake* mushroom itself

All authors are with the National Institute of Hygienic Sciences, Osaka Branch: No. 6, Hoenzaka-cho, Higashi-ku, Osaka 540, Japan.

0022-1147/78/0004-1290$02.25/0
© 1978 Institute of Food Technologists

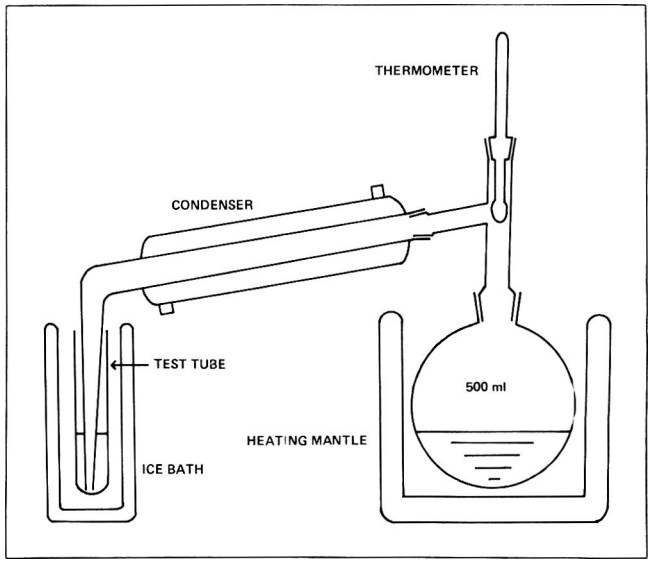


Fig. 1—Apparatus for distillation of carbon disulfide.

MATERIALS & METHODS

Samples

All of the imported dried mushrooms tested were the product of South Korea. Domestic raw and dried mushrooms from Tokushima (Shikoku Island), Hiroshima, Hyogo and Shiga (the latter three belonging to the Japanese mainland) were confirmed not to have been fumigated with carbon disulfide.

Moisture determination

Moisture in *shiitake* mushrooms was determined according to procedures outlined in *Standard Methods of Analysis for Hygienic Chemists* (1973).

Homogenization of *shiitake* mushroom

Five grams of fruiting bodies of dried *shiitake* mushroom, cut into pieces, were added to 75 ml of water, with or without 5 ml of 25% phosphoric acid, homogenized for 3 min, and cooled in an ice-water bath. The homogenate was then washed into a distillation flask with the aid of 25 ml of water.

Distillation procedure

The distillation apparatus used is shown in Figure 1. After the addition of 5 ml acetone and two drops of antifoaming agent (silicone oil), the distillation flask was placed on a mantle heater. The collection tube was previously filled with 5 ml cold acetone. Distillation was carried out up to a temperature of 80°C; under this condition, acetone was distilled out while water was not. The distillate was made up to 10 ml with acetone and, after drying with 2g of anhydrous sodium sulfate, 2 μl were injected into a gas chromatograph.

Gas-liquid chromatography

Yanaco G-80 gas chromatograph (Yanagimoto Mfg. Co., Ltd., Kyoto) equipped with FPD, and a 1.5m × 0.3 cm i.d. glass tube packed with 20% TCEP [1,2,3,-tris-(2-cyanoethoxy) propane] on Chromosorb W 30/60 was used. Nitrogen, air and hydrogen flow rates were 10 ml, 250 ml and 60 ml/min, respectively. The injection port and detector

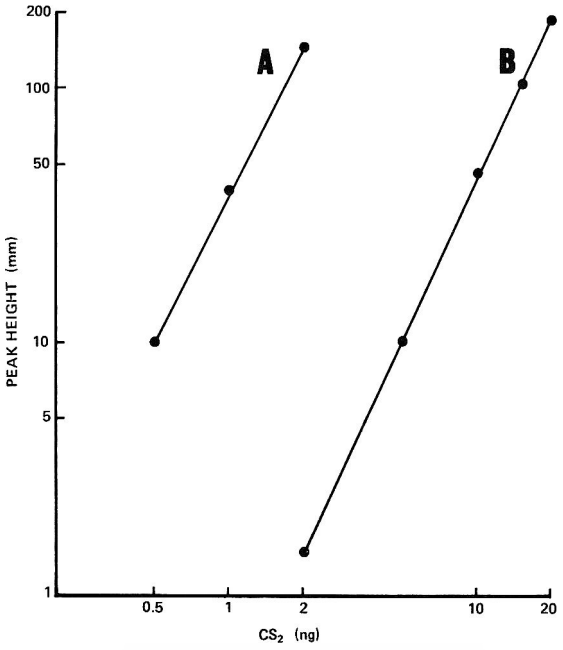


Fig. 2—Calibration curve for carbon disulfide by FPD-GLC. Gas-liquid chromatographic conditions: column 20% TCEP/Chromosorb W (30–60 mesh); column temperature 70°C; injection temperature 110°C; carrier N_2 flow rate 10 ml/min; column length 1.5m × 0.3 cm i.d. The co-ordinates are plotted in logarithmic scale: (A) Sensitivity 1/10 × 1/256; (B) Sensitivity 1/10 × 1/1024. [TCEP = 1,2,3,-tris(2-cyanoethoxy)propane]

temperatures were adjusted to 100°C, while the column temperature was held at 70°C.

Gas-liquid chromatography–Mass spectrometry

Shimadzu Model LKB-9000B gas chromatograph-mass spectrometer equipped with a 2m × 0.3 cm i.d. glass tube packed with 20% TCEP on Chromosorb W 30/60 was used. Carrier gas flow rate was 30 ml/min; the column temperature was held at 70°C and the injection port temperature at 120°C. Conditions for mass spectral scans were: electron energy 70 eV, emission current 120 μA.

Carbon disulfide standard solutions

Standard solutions (0.25–10.0 μg/ml) were prepared by dissolving redistilled carbon disulfide into acetone.

RESULTS

THE CALIBRATION CURVE for carbon disulfide (Fig. 2) was prepared from measurements of peak heights (mm) on gas chromatograms. A linear response was obtained within 0.5–2 ng and 2–20 ng of carbon disulfide. The detection limit was 0.025 ng corresponding to a concentration of 0.025 ppm carbon disulfide in the mushroom sample. The retention time of the sample peak coincided with that of the carbon disulfide standard. Mass spectrum of the sample agreed with that of the carbon disulfide standard, and indicated the molecular weight to be 76 (parent peak, 100%).

The effect of pH of homogenization and/or distillation procedure on the determination value of carbon disulfide was pursued. Imported, commercial and domestic dried *shiitake* mushroom samples were subjected to the determination with or without phosphoric acid. These results are shown in Table 1. The carbon disulfide content in *shiitake* mushroom was dependent upon the pH of homogenization or distillation solutions, showing remarkable increases under neutral conditions. Under acidic conditions, the average value of carbon disulfide content in imported mushroom was 1.42 ppm (dry basis), versus 1.22 ppm in the commercial samples investigated. A similar amount of carbon disulfide was detected from

Table 1—Effect of pH on the determination of carbon disulfide (ppm) in imported, commercial and domestic dried "Shiitake" mushrooms

		Homogenization/Distillation[a]		
		pH 7/pH 7	pH 7/pH 2	pH 2/pH 2
Imported sample	1	64.2 (72.5)	12.4 (14.0)	0.25(0.29)
no.	2	42.3 (46.5)	5.0 (5.5)	1.7 (1.9)
	3	32.8 (36.1)	13.2 (14.5)	0.02(0.02)
	4	12.6 (13.2)	1.4 (1.5)	1.7 (1.8)
	5	11.4 (12.9)	6.7 (7.6)	3.0 (3.4)
	6	11.3 (12.4)	1.4 (1.5)	0.31(0.34)
	7	9.5 (10.8)	9.6 (10.9)	3.4 (3.9)
	8	8.3 (9.3)		0.76(0.95)
	9	1.7 (1.9)		2.6 (3.4)
	10	32.4 (36.6)		1.2 (1.4)
	11	9.4 (11.4)		0.95(1.1)
	12	7.6 (8.5)		0.72(0.81)
	13	1.5 (1.7)		0.35(0.40)
	14	1.5 (1.6)		0.29(0.31)
Average		17.6 (19.7)	7.10 (7.93)	1.23(1.42)
Commercial	1	14.6 (16.5)		2.5 (2.8)
no.	2	9.8 (11.1)		1.1 (1.2)
	3	4.0 (4.5)		0.25(0.28)
	4	3.5 (3.9)		0.81(0.90)
	5	3.3 (3.8)		1.3 (1.5)
	6	2.2 (2.5)		1.1 (1.2)
	7	0.87(0.98)		0.56(0.63)
Average		5.47(6.18)		1.09(1.22)
Domestic	1	30.5 (33.7)		1.7 (1.9)
no.	2	1.08(1.24)		0.52(0.60)

[a] Values expressed on whole basis; values in parenthesis expressed on a dry basis.

Table 2—Recoveries of carbon disulfide from mushroom (Lentinus edodes)[a]

Added (ppm)	pH of homogenization and distillation: 7 Found (ppm)	7 Recovery (%)	2 Found (ppm)	2 Recovery (%)
0	1.5 ± 0.3	–	0.25 ± 0.01	–
0.1	–	–	0.33 ± 0.02	80.0 ± 6.1
1	2.4 ± 0.7	90.7 ± 29.2	1.21 ± 0.04	96.0 ± 3.3
10	10.8 ± 1.1	93.0 ± 11.0	10.20 ± 0.09	99.5 ± 0.9
50	48.8 ± 2.5	94.6 ± 4.9	–	–
100	100.3 ± 5.6	98.8 ± 5.5	–	–

[a] Mean value of five trials; 5-g samples were used.

domestic *shiitake* mushrooms (from Hyogo and Shiga) which had been confirmed not to have been carbon disulfide fumigated, suggesting that the carbon disulfide determined under neutral conditions was not residual fumigant but was derived from some other component(s) present in the *shiitake* mushroom itself.

These results confirm that residual or natural-occurring carbon disulfide in dried *shiitake* mushroom should be determined under acidic conditions of homogenization and distillation to prevent development of carbon disulfide from the constituents of *shiitake* mushroom during the analytical process.

Recoveries of carbon disulfide under acidic or neutral conditions were calculated at various amounts of spike levels of carbon disulfide to dried *shiitake* mushrooms. Results are shown in Table 2. The average recovery at the 1 ppm level was found to be 96.0 and 90.7%, respectively.

DISCUSSION

THE CARBON DISULFIDE in dried *shiitake* mushroom was expelled by steam distillation and the distillate received in

acetone, since it had been confirmed that the response of carbon disulfide was highest in FPD-GC when acetone was used as the solvent and carbon disulfide was diluted with acetone. In direct steam distillation of *shiitake* mushrooms, it was impossible to obtain a constant rate of carbon disulfide recovery. Accordingly, when 5 ml of acetone were added to dried *shiitake* mushroom, followed by steam distillation, a good recovery of carbon disulfide was obtained (see Table 2).

When the homogenization and/or distillation procedure was carried out under neutral pH (see Table 1), the amount of carbon disulfide in *shiitake* mushroom showed a remarkable increase. About the same amounts of carbon disulfide from domestic dried *shiitake* mushrooms were detected from domestic raw ones (from Tokushima and Hiroshima) on a dry basis. It is not clear whether the carbon disulfide detected is residual fumigant or a natural-occurring component in the *shiitake* mushroom itself. Accordingly, this should be borne in mind in the inspection of imported carbon disulfide-containing *shiitake* mushrooms as concerns condemnation of the product.

In Japan, the *shiitake* mushroom is a most commonly used food. It is customarily eaten after immersion in warm water and cooking in boiling water with other foods. Therefore, it is necessary to know the effect of cooking on the development of carbon disulfide from *shiitake* mushroom. The carbon disulfide content of dried mushrooms increased markedly by presoaking them in water. It can be assumed that the amount of carbon disulfide (0.98–72.5 ppm) in *shiitake* mushroom tested under neutral conditions (Table 1) corresponds to that developed from mushrooms during cooking. Thus the amount of carbon disulfide is of significance as previously described.

From another experiment it was observed that the larger the diameter of the fruit body of *shiitake* mushroom, the higher the content of carbon disulfide. While Japanese prefer the smaller *shiitake* mushrooms, Chinese restaurant managers generally prefer the larger.

REFERENCES

Berk, B. 1965. Determination of fumigant gases by gas chromatography. J. Agr. Food Chem. 13: 373.

Berk, B., Westlake, W.E. and Gunther, F.A. 1970. Microdetermination of phosphine by gas-liquid chromatography with microcoulometric, thermionic, and flame photometric detection. J. Agr. Food Chem. 18: 143.

Bielorai, R. and Alumot, E. 1966. Determination of residues of a fumigant mixture in cereal grain by electron-capture gas chromatography. J. Agr. Food Chem. 14: 622.

Cesáro, A.N., Sipala, E. and Zunini, E. 1952. Carbon disulfide poisoning. Folia Med. 35: 661.

Goodwin, E.S., Goulden, R. and Reynolds, J.G. 1961. Rapid identification and determination of residues of chlorinated pesticides in crops by gas-liquid chromatography. Analyst 86: 697.

Grant, D.W. and Vaugham, G.A. 1965. Determination of p.p.m. concentrations of carbon disulphide in benzene by electron capture gas chromatography. Nature 208: 75.

Heuser, G.S. and Scudamore, K.A. 1968. Fumigant residues in wheat and flour: solvent extraction and gas-chromatographic determination of free methyl bromide and ethylene oxide. Analyst 93: 252.

Heuser, G.S. and Scudamore, K.A. 1969. Determination of fumigant residues in cereals and other foods. Multidetection scheme for gas chromatography of solvent extracts. Analyst 20: 566.

McLeod, H.A. and McCully, K.A. 1969. Head space gas procedure for screening food samples for dithiocarbamate pesticide residues. J. AOAC 52: 1226.

Paulus, W. and Prokop, O. 1953. Acute carbon disulfide poisoning. Vergiftungsfällen 14: 232.

Standard Methods of Analysis for Hygienic Chemists—With Commentary. 1973. Authorized by the Pharmaceutical Society of Japan, p. 53.

Thornton, J.S. and Anderson, C.A. 1965. Electron capture gas chromatographic determination of residues of chemagro 2635 in crops and soil. J. Agr. Food Chem. 13: 509.

Ms received 8/21/77; revised 2/10/78; accepted 2/15/78.

EFFECT OF a_w ON NUCLEASE PRODUCTION . . . From 1286

Lachica, R.V.F., Genigeorgis, C. and Hoeprich, P.D. 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. 21: 585.

Lachica, R.V.F., Hoeprich, P.D. and Genigeorgis, C. 1972. Metachromatic agar-diffusion microslide technique for detecting nuclease in foods. Appl. Microbiol. 23: 168.

Plitman, M., Park, Y., Gomez, R. and Sinskey, A.J. 1973. Viability of Staphylococcus aureus in intermediate moisture meats. J. Food Sci. 38: 1004.

Scott, W.J. 1957. Water relations of food spoilage microorganisms. In "Advances in Food Research" Vol. 7, p. 83. Academic Press, New York.

Tatini, S.R., Soo, H.M., Cords, B.R. and Bennett, R.W. 1975. Heat-stable nuclease for assessment of staphylococcal growth and likely presence of enterotoxins in foods. J. Food Sci. 40: 352.

Tatini, S.R., Cords, B.R. and Gramoli, J. 1976. Screening for staphylococcal enterotoxins in food. Food Technol. 30(4): 64.

Troller, J.A. 1971. Effect of water activity on enterotoxin B production and growth of Staphylococcus aureus. Appl. Microbiol. 21: 435.

Troller, J.A. 1972. Effect of water activity on enterotoxin A production and growth of Staphylococcus aureus. Appl. Microbiol. 24: 440.

Troller, J.A. 1973. The water relations of food-borne bacterial pathogens: A review. J. Milk & Food Technol. 36: 276.

Troller, J.A. and Stinson, J.V. 1975. Influence of water activity on growth and enterotoxin formation by Staphylococcus aureus in foods. J. Food Sci. 40: 802.

Ms received 11/17/77; revised 1/21/78; accepted 1/30/78.

This paper is scientific series paper no. 10,123 from the University of Minnesota Agricultural Experiment Station supported in part by the University of Minnesota Agricultural Experiment Station project 18-72 and contract NAS 9-12560, Lyndon Johnson Manned Space Center, Houston, TX.

The assistance of Dr. David Thompson, Dept. of Food Science & Nutrition, University of Minnesota, in computer analysis is gratefully acknowledged.

BLOOD SERUM LIPID PATTERNS OF ADOLESCENT BOYS FED CORN OIL, PEANUT OIL, OR SAFFLOWER OIL

C. KIES, M. E. LOPEZ-RICO, H. M. FOX, K. M. STANEK and A. CHAN

ABSTRACT

The objective of the present study was to compare the effects of feeding low-fat diets to adolescent boys based on safflower oil, corn oil, or peanut oil on the fatty acid composition of several blood lipid fractions. Boys (aged 13–16 yr) were divided into three groups of 4–5 subjects each and fed weighed controlled diets containing 20% of calories from the test fats for 22 days. Fasting blood samples were drawn the morning of the first day of the study and the morning following the last day of controlled feeding. Blood serum was fractionated by column chromatography into phospholipid, cholesterol ester, triglyceride, and free fatty acid fractions. These were methylated and fatty acid composition determined by gas chromatography. In general in all fractions, feeding of corn oil or safflower oil resulted in increases in linoleic ($C^{2=}_{18}$) acid content and increases in oleic ($C^{2=}_{18}$) acid content. Peanut oil feeding resulted in decrease or little change in linoleic acid content and increase in oleic acid content. Results of this study suggest that peanut oil would be better classified with such animal fats as butter oil and beef tallow in human metabolic response than with such vegetable oils as corn oil or safflower oil.

INTRODUCTION

IT HAS BEEN REPORTED that relationships exist between dietary fat and body lipids, particularly lipid composition of liver, kidney, heart, muscle, adipose tissue, brain and blood (American Heart Association 1973; Inter-Society Commission for Heart Disease Resources, 1970.) Whereas the effects of dietary fat on total plasma lipid content and plasma lipid class composition have been reported frequently, less information is available on changes in the fatty acid patterns of specific blood plasma lipid classes resulting from feeding particular dietary fats (Hansen et al., 1964; Calvin et al., 1971; Pikaar and Fernandes, 1966; Olegard and Svennerholm, 1966; Dole et al., 1959; Roel et al., 1959).

Earlier studies from this laboratory have demonstrated that blood lipid class composition and fatty acid patterns of these classes from small groups of adults and adolescent boys can be quickly altered by selective qualitative and/or quantitative changes of dietary fat in controlled, experimental diets (Kies et al., 1972; Lin, 1973; Kies et al., 1967). Alterations in blood lipid class composition observed included changes in levels of serum total cholesterol, triglycerides, phospholipids and free fatty acids. Furthermore, changes were also defined in the fatty acid composition of these lipid fractions as a result of change in dietary fat.

In these previous studies, comparative effects of the following pairs of dietary fat were investigated: corn oil versus butter oil, safflower oil versus beef tallow. Peanut oil in studies with rabbits has been found to be more atherogenic than corn oil, a factor attributed to the arachedonic and behenic acid content of peanut oil and to the triglyceride structure of the fat on the basis of further investigations (Kritchevsky et al., 1973). The objective of the current study was to compare blood lipid patterns of adolescent boys fed diets containing safflower oil, peanut oil or corn oil as the primary fat sources at the 20% dietary calorie level. Thus, a three-way comparison of three fats high in polyunsaturated fatty acids was made.

All authors are with the Dept. of Food & Nutrition, University of Nebraska, Lincoln, NE 68583.

0022-1147/78/0004-1293$02.25/0
© 1978 Institute of Food Technologists

Table 1—Component fatty acids of dietary test fats in terms of percent of total fatty acids

Fatty acid	Safflower oil (%)	Peanut oil (%)	Corn oil (%)
Lauric acid: C_{12}	0.48	trace	–
Myristic acid: C_{14}	0.24	trace	–
Palmitic acid: C_{16}	6.87	17.05	21.78
Palmitoleic acid: $C^{1=}_{16}$	–	–	–
Stearic acid: C_{18}	0.15	2.09	2.07
Oleic acid: $C^{1=}_{18}$	10.03	44.87	25.54
Linoleic acid: $C^{2=}_{18}$	82.21	35.98	50.59

EXPERIMENTAL

THE OBJECTIVE of the study was to compare the effect of variation in source of dietary fat on serum lipid pattern of adolescent boys. The vegetable oils chosen for the study differ in the content of polyunsaturated fatty acids with safflower oil having the largest amount of linoleic acid (82.2%), corn oil being intermediate (50.6%) and peanut oil having the lowest value (35.9%). Table 1 lists the fatty acid composition of the dietary oils used in this study.

Plans

The study consisted of a 22-day study divided into a 5-day introductory adjustment period and four experimental periods of 4 days each. Objectives, procedures, data and conclusions of the protein and nitrogen aspects of this study were given in an earlier report by Chan et al. (1973). Subjects were divided into three groups. Throughout the study each group received one test fat as the near sole source of dietary fat. The test fat used (safflower oil, corn oil, and peanut oil) are described in Table 1. Fat calories accounted for 20% to total energy. The fat source was incorporated into a starch bread product during the protein depletion period and into oat breakfast cereal bread during remaining periods. The basal diet was composed of starch or test bread, fat, instant coffee or tea, nonprotein bouillion and some low protein fruits and vegetables. This basal diet provided approximately 0.8g total nitrogen per day. Test protein sources were four different industrially processed, oat-based, dry breakfast cereal products. During the study, vitamins and mineral supplements were provided to meet the daily requirements. Energy intake was adjusted to meet the individual needs of each subject.

Subjects

Adolescent boys aged from 13–16 yr of age were volunteer subjects for the study. All 14 subjects were of normal health as determined by each boy's family physician. During the course of the study subjects lived in their own homes but reported to the metabolism laboratory of the Department of Food & Nutrition for consumption of meals. At the beginning of the study average body weight of subjects was 148.4 lb. At the end of the study, their average body weight was 147.2 lb. Body weight was recorded for each subject every day.

Samples and analysis

Fasting blood samples were collected on the morning of day 1 and day 23 of the study. Blood samples were allowed to clot for approxi-

Table 2—Blood serum phospholipid, cholesterol and triglyceride values of subjects when consuming self-selected and test fat diets based on corn oil, safflower oil, or peanut oil

Diet	Blood serum lipid component		
	Cholesterol (mg/100 ml)	Phospholipid (mg/100 ml)	Triglyceride (mg/100 ml)
Group I			
Self-selected	165	182	82.4
Safflower Oil	112	162	65.4
Group II			
Self-selected	158	180	58.6
Corn Oil	130	166	66.2
Group III			
Self-selected	163	210	63.0
Peanut Oil	127	171	76.0

mately 6 hr at 30°C before spun in a clinical centrifuge to separate the serum from the clot. Serum samples were frozen for later analysis.

Lipids were extracted from serum basically by a modification of the method described by Folch et al. (1957). Three ml of blood serum were added drop by drop to 60 ml of chloroform-methanol (2:1 v/v) and allowed to stand for 30 min at room temperature with occasional shaking. The mixture was filtered and 40 ml 2% (v/v) monobasic potassium phosphate was added. The mixture was transferred to a 250 ml separatory funnel and after vigorous shaking stored overnight at 4°C. The chloroform layer was drawn off and the remaining aqueous phase was dried with anhydrous sodium sulfate. Lipids were extracted with 10 ml chloroform. The extraction was repeated twice and the solvent evaporated to dryness in a 40°C water bath. Lipids were transferred to a pre-weighed 25 ml Erlenmeyer flask in 10 ml petroleum ether and evaporated to dryness under nitrogen in a desiccator and reweighed. The difference between the two weighings is total lipids. The sample was then transferred to a graduated centrifuge tube with 10 ml petroleum ether and dried under a water vacuum to 0.3 ml for fractionation.

Fractionation of lipid classes was carried out by column chromatography method over silicic acid as described by Lis et al. (1961). The only modifications made were in the construction of the columns with a glass jacket to allow for refrigeration with cold water to prevent evaporation of the solvents at room temperature. The eluants containing the four different lipid fractions were evaporated under nitrogen at 40°C. Erlenmeyer flasks were reweighed to calculate the amount of each fraction.

Methylation of lipid fractions was accomplished by reaction of individual lipid fractions with boron trifluoride (BF_3)-methanol reagent prepared as described by Metcalf and Schmitz (1961). Reagents and times for preparation of methyl esters are shown in Table 3. Screw cap closed centrifuge tubes were placed in a mineral oil bath at 100°C for the times indicated. After cooling esters were extracted by adding 2 volumes of pentane, then 1 volume of water, shaking briefly, and centrifuging until both layers were clear (5 min at 3000 rpm). The upper layer was removed in a graduated centrifuge tube. The extraction was repeated two times using 2 ml of pentane. Anhydrous sodium sulfate was added to remove any water from the pentane ester containing fraction. The dried sample was transferred to a centrifuge tube and solvent evaporated under water vacuum.

Determination of fatty acids was done by gas chromatography, using an Aerograph, Model 90 P-3 instrument equipped with thermal conductivity detector. A 180 X 0.65 cm stainless steel column packed with diethylene glycol succinate on Chromosorb P, HMDS (hexamethyl disilazane) treated mesh size 60/80, was conditioned at 190°C for 24 hr. Helium carrier gas flow rate was adjusted to 60 ml/min. Temperatures of the injector and detector were maintained at 238° and 212°C respectively. Each sample was injected at a 182°C column oven temperature. Calculation of the peak area was based on an integrator count.

Statistical analysis of data by analysis of variance was done by the Statistical Laboratory, Institute of Agriculture and Natural Resources, University of Nebraska. F student test was used to determine the treatment effect.

RESULTS & DISCUSSION

COMPARISONS of mean phospholipid, cholesterol and triglyceride levels of subjects while receiving safflower oil, corn oil, or peanut oil as the near sole source of dietary fat are shown in Table 2. Regardless of source of dietary oil, blood serum cholesterol and phosopholipid levels were decreased. This may well have been associated with the low fat content of the basal test diet, the relatively high fiber content, or the low cholesterol content. All of these factors have been identified as having blood cholesterol lowering effects. Phospholipid levels tend to directionally follow blood cholesterol levels. Triglyceride levels showed no statistically significant changes in comparison to pre-experimental values.

Comparisons of fatty acid patterns of serum lipid fractions of adolescent boys, while on three experimental diets in which corn oil, safflower oil and peanut oil were the sources of dietary fats, were made in this study.

Mean values for all subjects on fatty acid composition of individual lipid fractions (cholesterol ester, triglyceride, free fatty acid, and phospholipid) and additive mean values for all fatty acids are shown on Tables 3–6. Data presented include mean pre-values and post-values.

In this experiment palmitic (C_{16}), oleic ($C_{18}^{1=}$) and linoleic ($C_{18}^{2=}$) showed statistically significant differences in the fatty acid patterns of subjects' blood serum lipid fractions as a result of feeding the experimental diets. Although other changes indicative of possible important biological trends were noted, discussion will be centered on changes occurring in proportional amounts of these three fatty acids.

Data on fatty acid patterns for all fractions are expressed in terms of percent of total fatty acid in that lipid fraction. Thus, total values for all fatty acids on each fraction approach 100%. Because values for fatty acids below C_{14} and above $C_{18}^{2=}$ were not included and because of unknowns, values do not exactly total 100%. Therefore changes in values are actually in terms of: (1) changes in percent composition figures; and (2) changes in percentage of percent composition figures.

Changes in fatty acid patterns of the cholesterol ester fraction are shown in Table 3.

Linoleic acid ($C_{18}^{2=}$) was the fatty acid found in largest amounts in the cholesterol ester fraction regardless of diet fed. A positive change was affected in the corn oil and safflower oil groups. Safflower oil, containing 82.2% linoleic acid, resulted in greater change (+10.8%) compared to the change produced by corn oil feeding (+3.2%). Corn oil linoleic acid content is 50.5%. Peanut oil (35.9% linoleic) feeding resulted in a decrease in the level of linoleic acid in the cholesterol ester fraction (−19.5%).

Previous reports (Pikaar and Fernandes, (1966) have indicated that the cholesterol ester fraction is very sensitive to change in fatty acid composition of dietary fat especially oleic and linoleic acid. Since rank order of change in serum cholesterol ester composition for these two fatty acids is the same as the rank order of composition for those two fatty acids in the test fats, this study supports these earlier drawn conclusions.

Changes in fatty acid patterns of the triglyceride fraction of blood serum of subjects are shown in Table 4. In this fraction high levels of oleic acid and fairly large amounts of palmitic acid were found. The linoleic acid content was lower than in the cholesterol ester fraction. Stearic and palmitoleic acid contents were low.

Greatest changes were observed in the oleic and linoleic acid content of this fraction with feeding of corn oil and safflower oil. Oleic and linoleic acid values varied reciprocally. Oleic acid decreased by 4.7% for the corn oil group and by 22.4% for the safflower oil group. On the other hand, linoleic acid increased by 169.1% in the corn oil group and by 50.1% in the safflower oil group. Peanut oil feeding resulted in slight increase of both oleic and linoleic acids in the triglyceride fraction. However, the linoleic acid content was lower in this group compared to the corn oil and safflower groups.

Whereas changes observed in the palmitic acid content of the triglyceride fraction were positive for safflower and peanut

Table 3—Cholesterol ester fraction: Fatty acid composition

Fatty acid	Safflower oil group[a] Pre-test (%)	Safflower oil group[a] Post-test (%)	Corn oil group[a] Pre-test (%)	Corn oil group[a] Post-test (%)	Peanut oil group[a] Pre-test (%)	Peanut oil group[a] Post-test (%)
C_{14}	1.0	1.4	0.9	0.8	1.7	1.4
C_{16}	15.2	17.1	15.1	13.7	14.9	22.4
$C_{16}^{1=}$	3.7	1.1	2.3	3.6	4.9	5.0
C_{18}	0.8	3.0	2.1	3.7	3.3	3.6
$C_{18}^{1=}$	23.9	16.0	20.1	16.0	27.2	28.9
$C_{18}^{2=}$	55.4	61.4	57.9	59.7	47.9	38.6

[a] Mean; Fatty acid values for pre-test and post-test samples expressed as percent fatty acid in total for each sample

Table 4—Triglyceride fraction: Fatty acid composition

Fatty acid	Safflower oil group[a] Pre-test (%)	Safflower oil group[a] Post-test (%)	Corn oil group[a] Pre-test (%)	Corn oil group[a] Post-test (%)	Peanut oil group[a] Pre-test (%)	Peanut oil group[a] Post-test (%)
C_{14}	2.8	2.2	3.8	1.4	2.2	1.7
C_{16}	24.1	27.4	36.9	33.2	29.7	33.4
$C_{16}^{1=}$	4.5	4.5	5.1	2.1	5.1	3.8
C_{18}	6.8	4.8	2.4	7.6	9.0	2.5
$C_{18}^{1=}$	43.5	33.8	33.1	31.5	43.1	45.3
$C_{18}^{2=}$	17.6	26.4	6.9	18.5	10.8	11.8

[a] Mean; Fatty acid values for pre-test and post-test samples expressed as percent fatty acid in total for each sample

oil groups, the opposite effect occurred in corn oil group of subjects. These changes do not correlate directly with dietary palmitic acid. However, the low content of palmitic acid for the safflower oil group might reflect the low palmitic acid (6.8%) content of this dietary oil. Myristic, palmitoleic and stearic acid levels showed minor changes. Particularly interesting is the increase in palmitoleic acid for the corn oil and peanut oil groups, since none of the dietary fats had any detectable palmitoleic acid. This increased level could be the result of the conversion of dietary palmitic acid to palmitoleic acid of blood serum triglyceride.

Changes in fatty acid patterns in blood serum free fatty acids of subjects are shown in Table 5. Palmitic and oleic acids were found in highest amounts in this fraction. In this fraction both unsaturated (oleic and linoleic) and saturated fatty acids (palmitic and stearic) showed significant changes as a result of feeding experimental diets. This contrasts with the several reports (Pikaar and Fernandes, 1966) on the relatively little influence of dietary fat on saturated fatty acid patterns of serum lipid fractions.

Linoleic acid content of the free fatty acid fraction showed a significant increase when corn and safflower oils were the dietary fats. This increase probably reflects the high linoleic acid content of safflower oil (82.2%) and corn oil (50.5%). Peanut oil feeding resulted in slight increase in linoleic acid value. Linoleic acid content of peanut oil is lower (35.9%) compared to content of safflower and corn oils. Hence, the effect of dietary fat composition of linoleic acid is seemingly quickly reflected in changes in free fatty acid fractions of subjects consuming the fat.

Changes in oleic acid in the free fatty acid fraction were not as great as for the linoleic acid in the same fraction. Positive change in oleic acid value was observed after feeding diets based on safflower oil and peanut oil. A decrease in the oleic acid value was found in the corn oil group. Considering the oleic acid content of the dietary fats, 10.0%, 25.5% and 44.8% for safflower oil, corn oil and peanut oil respectively, there is little correlation between level of this fatty acid in the diet and the level found in the free fatty acid fraction of serum lipids. Pre-test and post-test levels of stearic acid (C_{18}) of free fatty acid fraction of all subjects were found to be higher than those of the cholesterol or triglyceride fractions. A consistent decrease in stearic acid value of free fatty acid fraction was observed for all three dietary fats. Peanut oil diet resulted in a significant decrease of stearic acid (−46.7%). Changes of the same fatty acid in the safflower oil and corn oil groups were −24.3% and −24.9%, respectively. The decrease in stearic acid is probably due to the very low content of this acid in the dietary fats. Palmitic acid (C_{16}) level of free fatty acid fraction remained unchanged when peanut oil was the test fat. On the other hand, both safflower oil and corn oil depressed the pamitic acid value. This effect is in agreement with the low palmitic acid content of safflower oil (6.8%). The fact that corn oil feeding (21.7% palmitic acid) whereas peanut oil (17.0% palmitic) resulted in no change, points out that there is no simple correlation between dietary palmitic acid value and free fatty acid fraction content of the same fatty acid.

In the phospholipid fraction, palmitic (C_{16}) was the fatty acid found in largest amounts regardless of diet fed. Appreciable amounts of linoleic ($C_{18}^{2=}$), stearic (C_{18}) and oleic ($C_{18}^{1=}$) were also present. Corn oil or safflower oil feeding resulted in no significant changes in subjects' serum phospholipid contents of palmitic acid (C_{16}); however, peanut oil feeding resulted in a 31.4% increase.

As in other fractions, feeding of safflower oil or corn oil resulted in decreases in oleic ($C_{18}^{1=}$) acid content of the serum phospholipid fraction and in increases in the linoleic acid ($C_{18}^{2=}$) content. Peanut oil feeding had the reverse effect re-

Table 5—Free fatty acid fraction: Fatty acid composition

Fatty acid	Safflower oil group[a] Pre-test (%)	Safflower oil group[a] Post-test (%)	Corn oil group[a] Pre-test (%)	Corn oil group[a] Post-test (%)	Peanut oil group[a] Pre-test (%)	Peanut oil group[a] Post-test (%)
C_{14}	3.9	2.1	2.9	1.7	1.7	3.8
C_{16}	41.2	29.6	36.4	31.7	37.7	38.1
$C_{16}^{1=}$	4.4	4.6	4.2	4.0	4.2	4.4
C_{18}	17.3	13.1	16.1	12.1	24.3	13.0
$C_{18}^{1=}$	25.3	28.6	32.3	27.9	26.3	33.3
$C_{18}^{2=}$	6.0	20.9	7.7	19.3	5.2	6.7

[a] Mean; Fatty acid values for pre-test and post-test samples expressed as percent fatty acid in total for each sample

Table 6—Phospholipid fraction: Fatty acid composition

Fatty acid	Safflower oil group[a] Pre-test (%)	Safflower oil group[a] Post-test (%)	Corn oil group[a] Pre-test (%)	Corn oil group[a] Post-test (%)	Peanut oil group[a] Pre-test (%)	Peanut oil group[a] Post-test (%)
C_{14}	0.2	0.6	0.3	0.8	0.2	0.1
C_{16}	36.1	36.1	36.3	36.4	31.9	41.9
$C_{16}^{1=}$	0.8	0.1	0.5	0.8	0.7	0.7
C_{18}	21.3	21.8	16.9	18.7	19.8	19.0
$C_{18}^{1=}$	18.2	11.1	17.0	14.7	16.2	16.9
$C_{18}^{2=}$	23.3	30.2	29.1	30.6	30.4	21.4

[a] Mean; Fatty acid values for pre-test and post-test samples expressed as percent fatty acid in total for each sample

sulting in a slight increase in the oleic acid ($C_{18}^{1=}$) content (4.19%) and a pronounced decrease in the linoleic acid ($C_{18}^{2=}$) content (−29.66%).

CONCLUSIONS

PEANUT OIL has not been widely studied in human metabolism studies, although it is commonly used edible fat in many parts of the world. Peanut production and production of peanut oil for industrial purposes is substantial indicating the possibilities of its increased usage in American diets. However, studies with small animals by Kristchevsky et al. (1973) indicate that peanut oil is quite atherogenic.

Although peanut oil contains a considerable amount of linoleic acid ($C_{18}^{2=}$), the content is much lower than that of corn oil and safflower oil. Response of subjects to feeding of corn oil, safflower and peanut oil on fatty acid composition of blood serum lipid fractions in study was rapid with statistically significant changes occurring within the 22-day controlled feeding period. In general, directional responses of corn oil and safflower oil fed subjects were similar in fatty acid composition of all lipid fractions. However, peanut oil feeding resulted in opposite effects. In fact, patterns resulting from the feeding of peanut oil were closely similar to those found in earlier studies with adolescent boys when butter oil or beef tallow were fed (Kies et al., 1972). Oleic acid ($C_{18}^{1=}$) content tended to decrease in all fractions when safflower oil or corn oil were fed and to increase when peanut oil was dietary fat. The reverse effect was found for linoleic acid ($C_{18}^{2=}$) content; that is, corn oil and safflower oil fed subjects increased linoleic acid content of all serum lipid fractions while peanut oil feeding resulted in decreases or little change in content of this fatty acid.

REFERENCES

American Heart Association, 1973. Diet and coronary heart disease: An official statement.

Calvin, W.W., Bailey, M.C., Davis, J.T., Rogers, N. and Coniglio, J.G. 1971. Serum lipids in breast-fed infants and in infants fed evaporated milk. Am. J. Clin. Nutr. 14: 83.

Chan, A., Kies, C. and Fox, H.M. 1973. Protein value of oat-based breakfast cereal for growing humans. University of Nebraska, Lincoln, Unpublished Paper.

Dole, J.P., James, A.T., Webb, J.B.W., Rijack, M.A. and Sturman, M.F. 1959. The fatty acid patterns of plasma lipids during alimentary lipemia. J. Clin. Invest. 38: 1544.

Folch, J., Lees, M. and Sloane, S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497.

Hansen, A.E., Wiese, H.F., Adam, D.J.D., Boelsche, A.N., Haggard, M.F., Davis, H., Newson, W.T. and Pesut, L. 1964. Influence of diet on blood serum lipids in pregnant women and newborn infants. Am. J. Clin. Nutr. 15:11.

Inter-Society Commission for Heart Disease Resources: Primary Prevention of the Atherosclerotic Disease, 1970. Circulation 42:A-55-A-9S. (Revised, April, 1972).

Kies, C., Korslund, M. and Fox, H. 1972. Influence of dietary fat on serum lipid patterns of adolescent boys. American Home Economics Association. Annual Meeting, Detroit, MI, June, 25-30.

Kies, C., Senel, S.H., Fox, H.M. and William, E.R. 1967. Serum lipid variation of human adults on selected plant protein—Low fat intake as influenced by fat source. 1. General methods and effect of total nitrogen intake. Dept. of Food & Nutrition, University of Nebraska. Unpublished data.

Kritchevsky, D., Tepper, S.A., Vesselinovitch, D. and Wissler, R.W. 1973. Cholesterol vehicle in experimental atherosclerosis 13. Randomized peanut oil atherosclerosis 17: 225.

Lin, L.S. 1973. The influence of different types of dietary fat on the fatty acid pattern of some serum lipid fractions in adolescent boys. M.S. thesis, University of Nebraska.

Lis, E.W., Tinoco, J. and Okey, R. 1961. A micromethod for fractionation of lipids by silicic acid chromatography. Anal. Biochem. 2: 100.

Metcalf, L.D. and Schmitz, A.A. 1961. The rapid preparation of fatty acid ester for gas chromatographic analysis. Anal. Chem., 33: 363.

Olegard, R. and Svennerholm, L. 1971. Effects of diet on fatty acid composition of plasma and red cell phosphoglycerides in three-month-old infants. Acta Paed. Scand. 60: 505.

Pikaar, N.A. and Fernandes, J. 1966. Influence of different types of dietary fat on the fatty acid composition of some serum lipid fractions in infants and children. Am. J. Clin. Nutr. 19: 194.

Roels, O.A., Leurquin, P. and Trout, M. 1959. Serum polyunsaturated fatty acids in groups of Africans with low and high fat intake. J. Nutr. 69: 195.

Roels, O.A., Roesl-Broadhurst, D.M. and Trout, M. 1963. Serum lipids and diet: A comparison between three population groups with low, medium and high fat intake. J. Nutr. 79: 211.

Ms received 6/21/77; revised 12/13/77; accepted 12/20/77.

Supported by and a contribution to USDA, CSRS Regional Research Project NC-95. Published as Nebraska Agricultural Experiment Station Journal Series article No. 5359.

A HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINING THE STABILITY OF FREE METHIONINE IN METHIONINE-FORTIFIED FOOD SYSTEMS

L. S. O'KEEFE and J. J. WARTHESEN

ABSTRACT

A method based on high pressure liquid chromatography was developed to monitor the stability of free methionine in methionine-fortified food systems. Free amino acids were extracted from a model food system with 10% methanol and were subsequently reacted with dansyl chloride. Reverse phase chromatography was used to separate and quantify the dansyl derivatives of methionine, methionine sulfoxide, and methionine sulfone. In a soy protein-cellulose model system, the stability of added methionine was influenced by heat, added glucose, and moisture. Conversion of methionine to methionine sulfoxide or sulfone did not take place while heating with exposure to air, with added iron, or under conditions that favored Maillard browning and methionine destruction. Extrusion processing of fortified soy flour caused a 12% loss in free methionine. Methionine oxidation occurred under oxidizing conditions imposed by hydrogen peroxide.

INTRODUCTION

TOTAL SULFUR AMINO ACID content is a factor which limits optimum protein utilization in a number of plant products that are currently or potentially important sources of protein for human consumption (Kies, 1972). Fortification of certain protein sources with free methionine has been shown to increase the protein efficiency ratio (PER) in laboratory animals or the level of nitrogen retention in humans. The addition of DL- or L-methionine to diets based on potatoes, oats, and textured vegetable protein results in a significant improvement in nitrogen balance in human adult subjects (Kies and Fox, 1972; Kies et al., 1975; Kies and Fox, 1971). Methionine fortification has also been used to raise the PER of soybean-based blends (Bookwalter et al., 1975), cowpea powder (Onayemi and Potter, 1976), pinto beans (Dunlap et al., 1974) and other legumes (Kawatra et al., 1970).

The benefit of methionine fortification can be obtained only if the level of methionine in the product at the point of consumption is carefully controlled. The effectiveness of fortification could be reduced by the loss of free methionine during processing and storage. Conversely, overfortification with DL-methionine can lead to deleterious effects resulting from amino acid imbalance or the toxic effects of excesses of methionine (Sauberlich, 1961; Benevenga and Harper, 1967).

Numerous investigators have demonstrated losses of protein-bound methionine resulting from reaction with oxidants and carbohydrates during various processing procedures (Tannenbaum et al., 1969; Cuq et al., 1973; Shemer et al., 1973). In addition to these reactions, free methionine, with an available amino group, would likely be subject to reactions with carbonyl compounds and eventual destruction via Maillard browning reactions (Dunlap et al., 1974; Horn et al., 1968). Of the various methionine-related compounds produced by these degradative interactions, only methionine sulfoxide may be nutritionally available as methionine to humans (Njaa, 1962; Miller and Samuel, 1970), so the measurement of methionine sulfoxide in foods is needed for nutritional purposes and to provide a better understanding of the mechanism of methionine destruction.

The procedures currently available for evaluating methionine stability in foods are either slow, inaccurate, incapable of differentiating added methionine from protein methionine, or incapable of measuring both methionine and methionine sulfoxide. Amino acid analysis by ion exchange chromatography is slow and not well suited to the handling of a large number of samples. Variability and lack of sensitivity have been reported with gas chromatographic and microbiological methods for methionine and methionine sulfoxide (Tannenbaum et al., 1968; Conkerton, 1974; Miller et al., 1965). Procedures involving color formation with nitroprusside or the cyanogen bromide reaction do not measure methionine sulfoxide (McCarthy and Sullivan, 1941; Finlayson and Mackenzie, 1976). The objectives of this research were to develop a method which was capable of rapidly and accurately measuring the nutritionally available forms of free methionine and to determine the influence of formulation and heat treatment on the stability of free methionine in model food systems.

MATERIALS & METHODS

Extraction procedure

Two grams of ground, dry sample were added to 35 ml of 10% methanol in water (v/v) and the mixture was stirred for 20 min with a magnetic stirrer For samples consisting of 10% solids and 90% water, 3.5 ml of methanol and 13 ml of distilled water were added to 20g of sample prior to stirring. Ten ml of 40% trichloroacetic acid (TCA) were then mixed with the sample. The sample was filtered through a 10–20 μm pore size sintered glass vacuum filter. The residue was washed with 25 ml of distilled water. The filtrate was quantitatively transferred to a 100 ml volumetric flask, and the pH was adjusted to approximately 9.5 with 5N NaOH. The samples were brought to volume with distilled water and 0.5 ml aliquots were taken for methionine determination.

Methionine determination

The reaction conditions for amino acids and dansyl chloride (1-dimethylaminonaphthalene-5-sulfochloride, Sigma Chemical Company, St. Louis, MO) were adapted from the procedures of Gray (1967) and Bayer et al. (1976). One-half ml of 10mM dansyl chloride in acetone, 0.5 ml of 0.1M borate buffer pH 9.5, and 0.5 ml of sample were mixed in a 5 ml sample vial and reacted at 40°C for 1 hr. Ten μl were used for chromatographic analysis.

High pressure liquid chromatography (HPLC) was performed on a Waters Associates high pressure liquid chromatograph (Milford MA, Model U6K injector, 6000A solvent delivery system, 440 absorbance detector). The detector output was recorded and integrated on a Hewlett-Packard 3330A recorder-integrator. Separation of dansyl methionine and related derivatives was accomplished using a μBondapak C_{18} column (30 cm, 4 mm i.d., Waters Associates). The mobile phase consisted of acetonitrile and 0.01M Na_2HPO_4 buffer (pH 7.9), 1:4, v/v. The flow rate was 2 ml/min. The absorbance detector monitored absorbance at 254 nm. A single analysis run required approximately 10 min to complete.

DL-methionine (Sigma Chemical Co.) reacted with dansyl chloride gave a peak which cochromatographed with standard dansyl methionine

Authors O'Keefe and Warthesen are with the Dept. of Food Science & Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

0022-1147/78/0004-1297$02.25/0
© 1978 Institute of Food Technologists

(Sigma Chemical Co.) and a linear response in peak area was obtained when various concentrations of methionine were used. Dansyl methionine sulfoxide and sulfone standards were prepared by reaction with dansyl chloride. The quantification of dansyl derivatives of methionine and related compounds was accomplished by comparison of peak areas from sample data to those of external standards prepared each day by reacting water solutions of 0.1 mg/ml DL-methionine, DL-methionine sulfoxide (Calbiochem, Los Angeles, CA), or DL-methionine sulfone (Calbiochem) in the same manner as sample solutions.

The nitroprusside colorimetric determination of methionine was carried out according to the method of McCarthy and Sullivan (1941) as modified by Ferrel et al., (1969). A standard curve was used to determine the quantity of methionine in the sample.

Sample preparation

The model system used for methionine stability studies consisted of 40% isolated soy protein (Central Soya, Chicago, IL), 0 or 4% D-glucose, 0.5% DL-methionine, and sufficient micro-crystalline cellulose (FMC Corp., Newark, DE) to make 100%. To insure even distribution of the free methionine throughout the model system, the ingredients were slurried in a minimum of distilled water and mixed for 1 min in a blender. This mixture was frozen rapidly in shallow pans to prevent settling of insoluble solids and freeze dried. The dry samples were ground to a fine powder in a blender. The moisture content of the material was 8.2% as determined according to Horwitz (1975). Samples with a slightly higher moisture content (11.1%) were prepared by hydrating the dry ground model system in an evacuated desiccator over a saturated $CuCl_2$ solution for 4 days. Three-gram portions of the model system were placed in retortable foil pouches and vacuum sealed. The pouches were heated in a water bath at 80°C or 100°C for periods up to 1 hr. Immediately after heating, the pouches were immersed in an ice water bath until cooled.

Fortified soy flour was prepared by slurrying 99.5g of soy flour (Cargill, Minneapolis, MN) and 0.5g DL-methionine in distilled water and blending for 1 min. This mixture was also frozen in shallow pans, freeze dried, ground to a fine powder, packaged, and heated in the same manner as the model system.

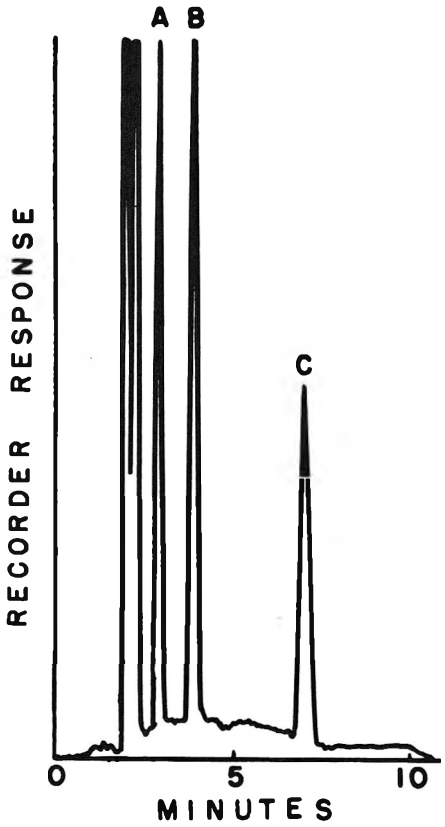


Fig. 1—High pressure liquid chromatogram of the dansyl derivatives of methionine and related compounds: (A) dansyl methionine sulfoxide; (B) dansyl methionine sulfone; (C) dansyl methionine. Solvent flow rate was 2.0 ml/min.

Liquid samples of 10% solids were prepared by slurrying 25g of the freeze-dried model system in 225g of distilled water and mixing for 1 min in a blender. Twenty gram portions of the liquid were weighed in 100 ml beakers and were heated open to the air in a shaking water bath (100 cycles/min) at 100°C for periods up to 1 hr. After heating, the samples were cooled in an ice water bath. Extraction of all liquid samples was carried out immediately after cooling and in the same beaker used for the heat treatment.

To evaluate the effect of added iron, 1g of $FeCl_3$ was added to 20g glucose-free liquid samples prepared as above. These were heated in a shaking water bath at 100°C for 1 and 3 hr, cooled, and extracted immediately.

Two levels of hydrogen peroxide, representing an amount equimolar to the free methionine present in the sample and an amount in a 13 molar excess, were added to 20g glucose-free liquid samples. These samples were not heated, but were held at room temperature for 17 hr prior to analysis.

For the extrusion treatment, a 6-kg quantity of soy flour, fortified to 0.5% with DL-methionine, was prepared by mixing 5970g of soy flour and 30g of DL-methionine in a twin shell dry blender (Patterson and Kelley Co., Inc.) for 2 hr. The fortified flour was mixed with approximately 2L of distilled water in a Blakeslee industrial mixer until crumbly (about 30 min). The mixture was then processed using a high temperature/pressure extruder (Brabender, Model 2003). The three individually controlled heated barrel zones were set at 58°C, 158°C and 200°C. Total residence time of the soy flour in the heated barrel was about 10 sec. The extruded product was allowed to air dry overnight and was then ground to a powder in a blender prior to methionine and moisture analysis.

All samples used in any single experiment were prepared in one batch to insure consistent methionine fortification in all samples in that experiment. All treated samples were heated at one time and analyzed at one time. Duplicate samples were prepared for each individual experimental treatment and duplicate analyses were performed on each sample. Untreated samples were used as controls and the loss of methionine due to treatments was represented as a percent of the methionine in the untreated control. To test for statistical significance, heating curves from the methionine stability experiments were plotted logarithmically and the slopes of the plotted lines were tested for significant difference at $p = 0.05$.

RESULTS & DISCUSSION

DANSYL CHLORIDE reacts with primary amino groups to form derivatives which absorb strongly at 254 nm. Methionine was detectable by HPLC at levels as low as 10 ng per injection, which is equivalent to a sample containing 0.015% free methionine when analyzed according to the conditions described. The reaction between dansyl chloride and methionine gave a consistently linear response between peak area of dansyl methionine and the amount of methionine for a range representing 10 ng to 30μg of methionine injected.

Dansyl methionine and dansyl methionine sulfoxide were totally separated from one another and from reagent peaks under the HPLC conditions described. In addition, dansyl methionine sulfone was separated from other compounds under these chromatographic conditions, and the formation of the sulfone in model systems undergoing oxidation could be followed. Peak areas for methionine sulfoxide and methionine sulfone gave linear responses to varied concentrations of the respective amino acids added to control samples. A typical chromatogram is shown in Figure 1. No interfering peaks were noted in unfortified samples.

The efficiency in extracting 0.5% methionine from the soy model system was 89.2 ± 4.2% (mean ± standard deviation). Ten percent methanol was used as the extraction solvent for free methionine because it gave higher recoveries of methionine compared to extraction with water or 20% methanol in water. The extraction efficiencies of methionine sulfoxide and methionine sulfone from the model system were similar to the recovery for methionine. The addition of TCA to the sample allowed separation of the protein by filtration.

An estimate of variability of the formation and quantification of dansyl methionine for the dansyl chloride reaction was

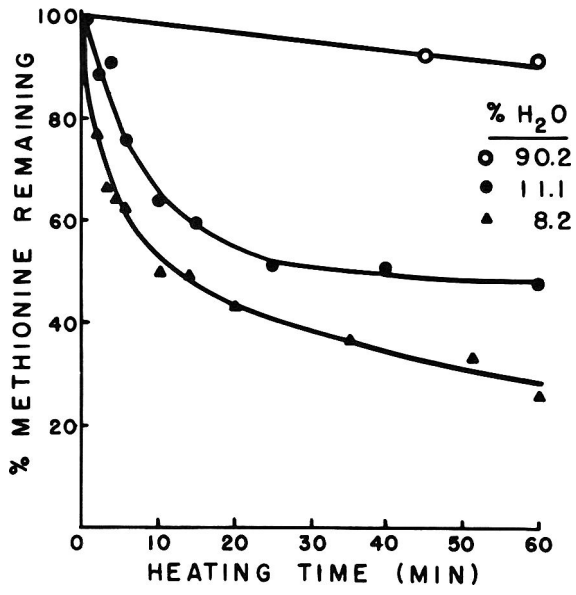


Fig. 2—Effect of moisture content and heating time at 100°C on the stability of free methionine in a glucose-containing model system.

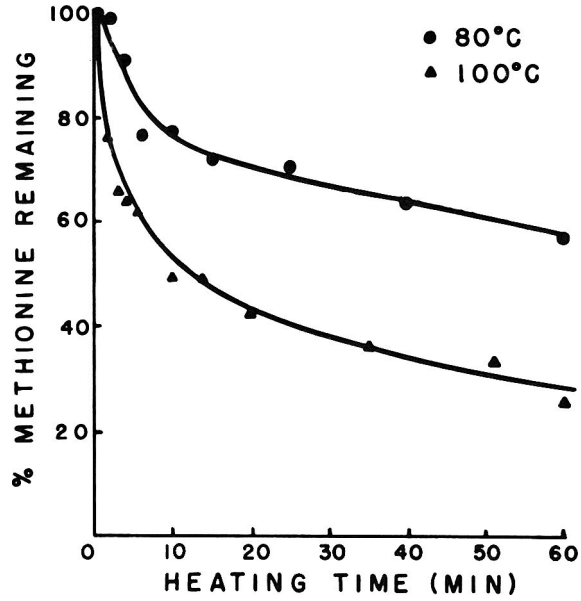


Fig. 3—Effect of temperature and heating time on the stability of free methionine in glucose-containing model systems at 8.2% moisture.

obtained by calculating the coefficient of variation. Using 56 reaction pairs generated during the first heating experiment, the coefficient of variation was calculated to be 3.8%.

The free methionine values from the HPLC method and the nitroprusside colorimetric method were compared using a paired t test. Because browning pigments can interfere with the colorimetric method, twenty heat treated liquid samples that did not contain appreciable levels of browning pigments were chosen for the comparison. There were no significant differences in the free methionine values generated by the two methods ($p = 0.5$).

The HPLC method developed was used to study the stability of free methionine under various processing conditions. The effect of moisture content on the stability of free methionine in glucose-containing model food systems heated at 100°C is shown in Figure 2. The rate of loss of free methionine is significantly greater at the 8% moisture level than at the 11% mositure level ($p = 0.05$). In the 8% moisture samples, 50.5% of the free methionine was lost after 10 min and 74.5% was destroyed after 60 min. In the 11% moisture samples, 36.4% of the free methionine was destroyed in 10 min and 52.7% after 60 min at 100°C. Evidence of browning was noted in these low moisture samples after heating, and the loss of free methionine appeared to parallel the development of browning pigments. Increasing the moisture content to 90% greatly decreased the rate of loss of free methionine, with only 9.1% loss of free methionine noted after 60 min of heating.

The effect of temperature on free methionine stability in the 8% moisture model system is shown in Figure 3. The rate of loss of free methionine is significantly greater at 100°C than at 80°C ($p = 0.05$). A progressive increase in browning was observed with increased heating time, and the samples heated at 80°C showed less browning than the samples heated at 100°C. When glucose was not present in the 8% moisture model system, no loss of free methionine was noted after 1 hr of heating at 100°C. These results provide an indication that Maillard browning is the destructive mechanism for free methionine in this system. When glucose was not present in the system, heating induced neither brown color development nor free methionine loss. Addition of glucose to the heated system led to both the loss of free methionine and the formation of brown color.

Heating of methionine-fortified soy flour for 1 hr at 100°C also showed no significant loss of free methionine. Brown color was not observed in soy flour samples heated under these conditions. The flour contained 6% moisture, and was comparable to the low moisture glucose-containing model system where loss of free methionine was demonstrated with heating. Because soy flour contains few reducing carbohydrates, there was probably little opportunity for the interactions which would lead to non-enzymatic browning and the consequent loss of free methionine. Significant formation of methionine sulfoxide or methionine sulfone was not observed in any of the samples of soy flour or the model system heated under dry conditions.

The effect of oxidizing conditions on the stability of free methionine in food systems was examined. The liquid model food containing 90% moisture and no glucose was heated at 100°C under conditions which provided contact of the sample with air. No significant loss of free methionine was noted, nor was any methionine sulfoxide or sulfone detected. The combination of heat and air appeared insufficient to cause oxidation of free methionine to methionine sulfoxide or sulfone detected.

Iron is frequently included in the formulations of soy milks sold in the United States. Although the ferrous iron is the more nutritionally available form, inter-conversion between ferrous and ferric iron may occur in a food system. The reduction of ferric to ferrous ion in a heated system, where air is present, could possibly induce the oxidation of free methionine. This possiblility was examined during the heating of glucose-free liquid model food samples to which $FeCl_3$ had been added. Even after 3 hr at 100°C, no loss of free methionine or oxidation to sulfoxide or sulfone was observed. The combination of iron, air, and heat appeared to be insufficient to cause oxidation of free methionine in a fortified liquid system. The addition of iron to methionine-fortified soy milk products should have little effect on the oxidative stability of free methionine.

The addition of hydrogen peroxide to the glucose-free liquid model food as an oxidant had a significant effect on the stability of free methionine. When hydrogen peroxide was added in an equimolar amount to the free methionine present, 13% of the free methionine was oxidized to methionine sulfoxide and 89% was recovered as free methionine after 17 hr at 22°C. When a 13 molar excess of H_2O_2 was added, 8.7% of

the free methionine originally present was recovered, 47.6% was oxidized to methionine sulfoxide, 11.8% was oxidized to methionine sulfone, and 31.9% was destroyed after 17 hr at 22°C. Both hydrogen peroxide and decomposing lipid peroxides have been shown to promote the oxidation of protein bound methionine to methionine sulfoxide (Cuq et al., 1973; Tannenbaum et al., 1969). The oxidative action of hydrogen peroxide indicates that decomposing lipid peroxides may promote the oxidation of free methionine as well.

Despite the demonstrated stability of free methionine in dry soy flour heated at 100°C for 1 hr, the feasibility of methionine fortification in extruded soy products remained uncertain due to the browning which can occur under moist, high temperature conditions. Methionine-fortified soy flour containing 26% moisture was processed in a high temperature/pressure extruder. Of the methionine originally added to the system, 12% was destroyed by the treatment, but no methionine was recovered as the sulfoxide or sulfone. The soy flour was visually darker after the extrusion treatment. Although few reducing carbohydrates are present in soy flour, it has been postulated that very high temperatures may cause hydrolysis of some of the carbohydrates to increase the number of reducing groups available for Maillard browning (Evans and Butts, 1949).

SUMMARY

A METHOD based on high pressure liquid chromatography has been developed which accurately and rapidly quantifies free methionine, free methionine sulfoxide, and free methionine sulfone in methionine-fortified soy based products. Applying this method to study the stability of free methionine in food systems, it appears that free methionine is destroyed rapidly in food systems undergoing Maillard browning. Increasing the temperature of processing significantly increased the rate of loss. Decreasing the moisture content appeared to increase the rate of free methionine loss for the moisture levels studied (8, 11, 90%). No loss of free methionine was observed in the 8% moisture model system, when glucose was not added to the system. The application of extruder conditions to a methionine-fortified soy flour system resulted in a slight loss of free methionine, possibly via Maillard browning following hydrolysis of the carbohydrates by the high temperatures.

Methionine was not oxidized by heating liquid systems expose to air or in the presence of iron. Addition of hydrogen peroxide to methionine-fortified food systems resulted in oxidation of the methionine to both methionine sulfoxide and methionine sulfone at room temperature. These results should be considered when formulating and processing methionine fortified food products.

REFERENCES

Bayer, E., Grom, E., Kaltenegger, B. and Uhmann, R. 1976. Separation of amino acids by high performance liquid chromatography. Anal. Chem. 48: 1106.

Benevenga, N.J. and Harper, A.E. 1967. Alleviation of methionine and homocysteine toxicity in the rat. J. Nutr. 93: 44.

Bookwalter, G.N., Warner, K., Anderson, R.A., Mustakas, G.C. and Griffin, E.L. 1975. Fortification of dry soybean-based foods with DL-methionine. J. Food Sci. 40: 266.

Conkerton, E. 1974. Gas chromatographic analysis of amino acids in oilseed meals. J. Agr. and Food Chem. 22: 1046.

Cuq, J.L., Provansal, M., Guilleux, F. and Cheftel, C. 1973. Oxidation of methionine residues of casein by hydrogen peroxide. Effects on in vitro digestibility. J. Food Sci. 38: 11.

Dunlap, C.J., Guadagni, D.G., Miers, J.C. and Wagner, J.R. 1974. Methionine supplement alters flavor, PER of pinto beans canned in tomato sauce. Food Prod. Devel. 8: 88.

Evans, R.J. and Butts, H.A. 1949. Studies on the heat inactivation of methionine in soybean oil meal. J. Biol. Chem. 178: 543.

Ferrel, R.E., Fellers, D.A. and Shepherd, A.D. 1969.Determination of free lysine and methionine in amino acid-fortified wheat. Cereal Chem. 46: 614.

Finlayson, A.J. and Mackenzie, S.L. 1976. A rapid method for methionine determination in plant materials. Anal. Biochem. 70: 397.

Gray, W.R. 1967. Dansyl chloride procedure. In "Methods in Enzymology," Vol. 11, p. 139, Ed. Hirs, C.W. Academic Press, New York, NY.

Horn, M.J., Lichtenstein, H. and Womack, M. 1968. Availability of amino acids. A methionine-fructose compound and its availability to microorganisms and rats. J. Agr. Food Chem. 16: 741.

Horwitz, W. (Ed.) 1975. "Official Methods of Analysis of the Association of Official Analytical Chemists," 12th ed, #14.076. Washington, D.C.

Kawatra, B.L., Garcha, J.L. and Wagle, D.S. 1970. Effect of treatment and addition of methionine and lysine on the nutritive value of guar dhal and its supplemental value to whole wheat flour diet. Ind. J. Nutr. Dietet. 7: 145.

Kies, C. 1972. Evaluation of the protein value of cereal/plant/oilseed products by human biological assay techniques. In "Symposium: Seed Proteins," Ch. 18, Ed. Inglett, G.E. Avi Publishing Co. Inc., Westport, CT.

Kies, C. and Fox, H.M. 1971. Comparison of the protein nutritional value of TVP, methionine-enriched TVP, and beef at two levels of intake for human adults. J. Food Sci. 36: 841.

Kies, C. and Fox, H.M. 1972. Effect of amino acid supplementation of dehydrated potato flakes on protein nutritive values for human adults. J. Food Sci. 37: 378.

Kies, C., Fox H.M. and Aprahamian, S. 1975. Comparative value of L-, DL-, and D-methionine supplementation of an oat-based diet for humans. J. Nutr. 105: 809.

McCarthy, T.E. and Sullivan, M.X. 1941. A new and highly specific colorimetric test for methionine. J. Biol. Chem. 141: 871.

Miller, E.L. Carpenter, K.J. and Milner, C.K. 1965. Availability of sulfur amino acids in protein foods. 3. Chemical and nutritional changes in heated cod muscle. Br. J. Nutr. 19: 547.

Miller, D.S. and Samuel, P.D. 1970. Effects of the addition of sulfur compounds to the diet on utilization of protein in young rats. J. Sci. Food Agr. 21: 616.

Njaa, L.R. 1962. Utilization of methionine sulfoxide and methionine sulfone by the young rats. J. Nutr. 16: 571.

Onayemi, O. and Potter, N.N. 1976. Cowpea powders dried with methionine: preparation, storage stability, organoleptic properties, nutritional quality. J. Food Sci. 41: 48.

Sauberlich, H.E. 1961. Studies on the toxicity and antagonism of amino acids for weanling rats. J. Nutr. 75: 61.

Shemer, M., Wei, L.S. and Perkins, E.G. 1973. Nutritional and chemical studies on three processed soybean foods. J. Food Sci. 38: 1?2.

Tannenbaum, S.R., Barth, H. and LeRoux, J.P. 1969. Loss of methionine in casein during storage with autoxidizing methyl linoleate. J. Agr. Food Chem. 17: 1353.

Tannenbaum, S.R., Thilly, W.G. and Issenberg P. 1968. Problems in gas chromatographic determination of methionine, methionine sulfoxide, and methionine sulfone. Anal. Chem. 40: 1723.

Ms received 9/1/77; revised 11/10/77; accepted 11/17/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA June 5-8, 1977.

Scientific Journal Series Paper No. 10018, Minnesota Agricultural Experiment Station.

This research was supported in part by the Cooperative State Research Service, USDA under the PL 89-106 grant program.

INNOVATIONS IN SAMPLING AND CULTURING METHODS FOR BACTERIOLOGICAL EXAMINATION OF BROILER CARCASSES

J. E. THOMSON and J. S. BAILEY

ABSTRACT

Broiler carcasses were sampled by excision of the neck skin with the aid of a special clamp, and by swabbing of the breast skin. Samples from both methods were compared for bacteriological evaluation of carcasses by standard plate counts and by two commercial miniature systems (Millipore and Easicult-TTC). In experiment 1, the excised neck skin method showed higher counts than the breast swab method on carcasses stored at 1.5°C for 7–18 days, but not on day of processing; counts did not differ significantly as a function of culturing and counting system (only standard plating and Millipore were compared) on carcasses stored 7–18 days, but were higher by standard plating than by Millipore on day of processing. In experiment 2, counts did not differ significantly as a function of the two sampling methods or three culturing methods, or between counts on 0 and 7 days of storage of carcasses at 1.5°C. The neck skin excise method combined with a miniaturized culturing system was the fastest means to measure the general bacteriological condition of fresh or stored broiler carcasses.

INTRODUCTION

MANY SAMPLING METHODS for determination of bacteriological condition of poultry carcasses have been proposed, devised and evaluated. Avens and Miller (1970a, b) devised a skin tissue blending method for quantifying poultry carcass skin bacteria counts. Barnes (1975) concluded that any of the methods which are reproducible can be used for plant control, but for research purposes, the most accurate answer possible is required. Clark (1965) developed a "spray gun" method, wherein a specific skin area was sprayed with sampling fluid, then the bacteria in a measured volume of this fluid were counted. Cox et al. (1976) compared swabbing and excising for sampling broiler skin for total and *enterobacteriaceae* counts. Kinsley and Mountney (1966) found no significant effect of size of area swabbed, culture medium used, or incubation temperatures used on counts from swab samples. Fromm (1959) preferred swab sampling, while Mallmann et al. (1958) preferred rinse sampling. Mercuri and Kotula (1964) showed that the count of the drip fluid in poultry correlated well with the breast swab count. Notermans et al. (1976) found that cotton and alginate swabs were not significantly different in obtaining carcass skin counts. Patterson (1971) described a sampling method whereby a section of breast skin was excised then shaken in peptone with sand as an abrasive. Patterson (1972) found that neck skin was generally the most heavily contaminated area on the carcass, Simonsen (1971) successfully utilized the neck skin for bacteriological sampling, and Notermans et al. (1975) and Barnes (1975) recommended excised neck skin as a sample source. Thomson et al. (1976) discussed a template-swab sampling method, and Williams (1967) and Ziegler et al. (1954) described methods of sampling by skin scraping. One gram of skin was found to correspond to an area of about 10 cm^2 (Barnes and Shrimpton, 1958; Simonsen, 1971).

Our objectives were (1) to evaluate a device for rapidly excising a sample of broiler carcass neck skin of specific area, in comparison to a standard breast skin swab sampling procedure, and (2) to evaluate two commercial miniaturized methods for culturing and counting bacteria in comparison with the standard plating method for determining total counts of aerobic bacteria on broiler carcasses.

Authors Thomson and Bailey are with the USDA, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30604.

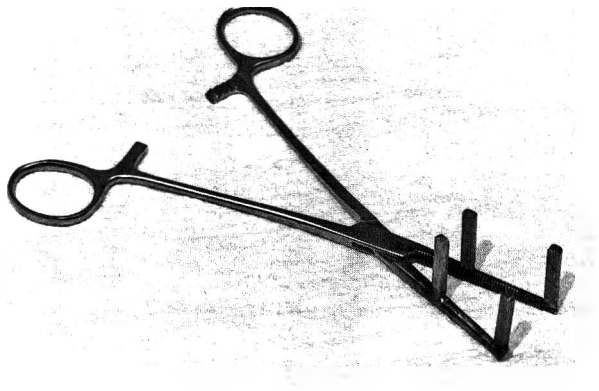


Fig. 1—Modified hemostat designed to aid in obtaining broiler carcass neck skin sample of 5 cm^2 area for bacteriological examination.

MATERIALS & METHODS

Experiment 1

Thirty-two freshly processed broiler carcasses were obtained from a commercial plant. On the day of processing, eight carcasses were sampled by placing a sterile template, with inside area of 12.3 cm^2, on the center of one side of the breast of each carcass, then the area was swabbed for 30 sec with a calcium alginate swab moistened with 1% sodium citrate. The swab was broken into a 20 ml 1% sodium citrate dilution blank, then shaken until dissolved (30–45 sec). Then from the neck skin on each of the same eight carcasses, a 5 cm^2 piece of skin was excised with the aid of the device ("clamper-sampler") shown in Figure 1. This device was constructed by welding four 22 cm long serrated stainless steel prongs 25 cm apart to the jaws of a standard surgical hemostat. Use of the device is shown in Figure 2. The device was dipped in alcohol and flamed to sterilize before each use. It was easiest and fastest to first cut off a piece of skin somewhat larger than 5 cm^2 by clipping with dissecting scissors around the outside of the prongs, then preparing the final 5 cm^2 sample by cutting along the inside periphery of the prongs. With this procedure, the loss of edible product was minimal. Less than 1g of skin was removed from an area not critical to carcass appearance; thus the procedure was considered non-destructive. Total time to obtain each excised sample was about 45 sec, compared to 60 sec for swab sample. The 5 cm^2 skin sample was placed with sterile forceps in a 20 ml 1% sodium citrate dilution blank with 5g of sterile gravel, then shaken well for 30 sec. Calculations of bacteria per cm^2 were based on 10 cm^2 of skin sample, since both sides of the excised sample had been exposed to contamination.

For enumeration of bacteria, both the swab and excised samples were cultured in two ways. One aliquot of each sample (in 20 ml 1% sodium citrate) was serially diluted, then plated with Standard Methods Agar (BBL) and incubated at 20°C for 72 hr. The second aliquot was cultured with the Millipore Total Count Sampler device (left foreground of Fig. 2), manufactured by the Millipore Corporation, Bedford, MA. This is a pre-sterilized disposable device consisting of a membrane filter backed with an absorbent blotter containing dehydrated total count culture medium. The sampler, when dipped for 30 sec in the diluent, absorbs 1 ml through the filter. The remaining diluent was

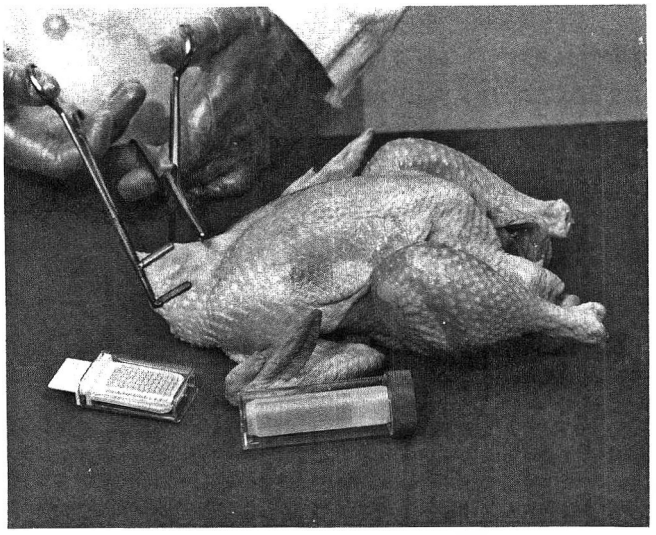


Fig. 2—Neck skin sampler in use. Miniaturized culturing devices in foreground (left: Millipore; center: Easicult-TTC).

discarded, then the sampler was incubated in the case at 20°C for 72 hr. The colonies that developed on the gridded filter of the sampler were counted after incubation.

The above procedures were repeated on eight additional carcasses after each storage time at 1.5°C (7, 15 and 18 days). For replication, the entire experiment was then repeated. Analysis of variance was applied to the data.

Experiment 2

By standard plate count and Millipore sampler, total counts on swab and excised samples were performed as in experiment 1, on eight carcasses on day of processing, and on eight more after 7 days at 1.5°C. In addition, these samples were tested with the Easicult-TTC device (center foreground of Fig. 2) manufactured by Orion Diagnostica, Helsinki, Finland. This is a pre-sterilized disposable device consisting of an agar pad on each side of a plastic slide. The slide is dipped into the diluent to be tested, then the device is incubated in the tube. Bacteria that develop on the agar surface give rise to red colonies that are counted or estimated after incubation. Mulder et al. (1976) used a similar system (Urotube-Roche) to investigate microbiological quality of broiler carcasses as related to continuous immersion chilling.

Analysis of variance was applied to the data.

RESULTS & DISCUSSION

Experiment 1

Counts were significantly higher ($p = 0.01$) on samples from excised neck skin than from breast swab samples on 7, 15 and 18 days of storage, but not on day of processing (Fig. 3). Counts were significantly higher ($p = 0.01$) from the standard plating method than from the Millipore sampler on day of processing only. We, therefore, attributed most of the observed differences in counts to the methods used to sample the carcasses rather than to procedures for bacteriological culturing. Shaking excised neck skin samples in diluent, with gravel as an abrasive to loosen the bacteria, usually gave higher counts per cm^2 than swabbing an intact area on the breast skin. All of the sampling and culturing methods studied, however, if used with care and consistency, would provide useful information on populations of total aerobic bacteria on poultry carcasses. In experiment 1, rapidity of sampling and culturing were greatest with the neck skin excise method and Millipore sampler.

Experiment 2

Mean bacterial counts did not differ significantly as a function of methods of sampling, culturing, and counting, or of days of storage up to 7 days at 1.5°C (Table 1). Sampling the carcass with the aid of the neck skin clamping device, and

Table 1—Bacterial counts per cm^2 of broiler carcass skin as related to skin sampling method, bacteriological culturing and counting method, and days of storage at 1.5°C (Experiment 2)

Sampling method	Culture method	Days of storage 0	7	Mean
Breast swab (12.3 cm^2 delineated by fiberboard template)	Standard plating	3.5[a]	3.2	3.4a[b]
	Millipore	2.8	3.2	3.0a
	Easicult-TTC	2.8	3.1	3.0a
	Mean	3.1a[b]	3.2a	3.1a
Neck skin excise (5 cm^2 delineated by clamping device)	Standard plating	3.3	3.2	3.3a
	Millipore	2.7	3.3	3.0a
	Easicult-TTC	3.1	3.1	3.1a
	Mean	3.1a	3.2a	3.1a
	Overall Mean	3.1a	3.2a	3.1

[a] Mean of eight samples; logarithm$_{10}$ of actual numbers

[b] Means within a column or row followed by the same letter were not significantly different ($P = 0.05$).

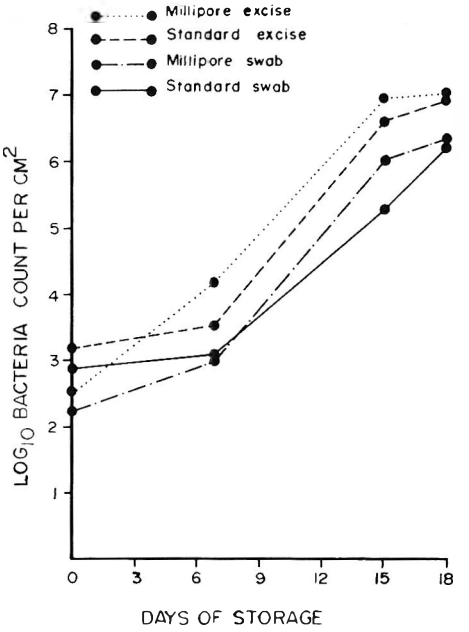


Fig. 3—Total bacteria counts of broiler carcasses during storage at 1.5°C. Carcasses were sampled by breast swab or neck skin excising method, and culturing and counting were by standard plating or miniaturized Millipore system (Experiment 1).

culturing and counting with the Easicult-TTC system when used under our conditions, was a rapid, simple and accurate method to measure the general bacteriological condition of broiler carcasses. However, the procedures have certain limitations. For examination for specific microorganisms of public health significance, such as *Salmonella*, the entire carcass should be sampled, rather than only the neck skin or breast area (Cox et al., 1978). The procedures for sampling and culturing described herein are adequate to detect unsanitary processing practices which are reflected in bacterial counts on the fresh carcass, and development of spoilage bacteria on stored broiler carcasses.

REFERENCES

Avens, J.S. and Miller, B.F. 1970a. Optimum skin blending method for quantifying poultry carcass bacteria. Appl. Micro. 20: 129.

—Continued on page 1306

FATE OF AFLATOXIN B_2 IN A TWO-STEP MICROBIAL RECOVERY SYSTEM FOR FOOD INDUSTRY WASTES

KATHERINE M. JOHNSON and F. F. BUSTA

ABSTRACT

The fate of aflatoxin B_2 in a two-step microbial process for the recovery of single cell protein (SCP) was determined. *Bacillus subtilis* A was used to hydrolyze a protein-starch medium for subsequent growth of *Lactobacilus acidophilus* Farr. Growth of *L. acidophilus* in the modified medium was 100-fold greater than in the initial medium. The medium was spiked with 800 ppb aflatoxin, and high pressure liquid chromatography was employed for quantification of residual aflatoxin. Extraction efficiency of the aflatoxin was 72.4%. No differences were noted in the amounts of aflatoxin present after any of the treatments.

INTRODUCTION

THE DISPOSAL of waste materials generated by the food industry is a problem of economic and environmental concern. There has been increasing interest in the use of these materials to produce utilizable and marketable commodities such as foods or feeds (Litchfield, 1977). Industrial waste materials have been utilized as substrates for the production of single cell protein (SCP)—microbial biomass of bacteria, algae, fungi or yeast. In most systems only one substrate, such as potato wash water (Anon., 1977) or collagenous by-products (Bough et al., 1972), has been utilized for the growth of a single organism. Schmidt et al. (1977) described a system for the production of SCP from a model protein and carbohydrate waste medium utilizing initial growth of *Bacillus subtilis* to provide the amino acids and sugars required for subsequent *Lactobacillus acidophilus* growth.

The normal handling of a waste material does not demand toxicological monitoring, and therefore toxic substances that may be present would remain undetected. If these toxins are degraded or separate from the end product, there is little cause for concern. However, toxins may also become associated with the product and this is unacceptable.

A potential contaminant in some food industry wastes is the mold metabolite, aflatoxin. This substance has been isolated from corn, peanuts, cottonseed, and other products (Stoloff, 1976) and has been demonstrated to be a potent hepatocarcinogen in certain animals. Epidemiological evidence reviewed by Shank (1976) suggests human susceptibility. Studies have indicated that growth of *Lactobacillus delbrueckii* (Maing et al., 1973), *Pseudomonas* species, and *Flavobacterium aurantiacum* (Ciegler et al., 1966) can degrade aflatoxin B_1 in various media.

Microbial recovery of waste materials, such as the production of SCP, may therefore reduce the potential hazard of aflatoxin contamination. The purpose of this work was to note the effect of *B. subtilis* and *L. acidophilus* on aflatoxin in a system for the production of SCP from a protein and starch model waste medium. Aflatoxin B_2 was chosen as a model toxin for this study.

Author Busta is with the Dept. of Food Science & Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108. Author Johnson, Formerly with the Univ. of Minnesota, is now with Capsule Testing Laboratories, Economics Laboratory, Inc., Osborn Building, St. Paul, MN 55102.

0022-1147/78/0004-1303$02.25/0
© 1978 Institute of Food Technologists

MATERIALS & METHODS

Inoculum preparation

A spore suspension of *B. subtilis* strain A was obtained from the culture collection in this department. Spores were maintained in sterile tap water at 4°C. Prior to use, spores were heat shocked at 90°C for 60 min in 0.1% peptone. A 1% inoculum of the shocked suspension was introduced into lactic broth (LB), and growth proceeded for 12–16 hr at 37°C on a rotary shaker at 200 rpm. For inoculation purposes, the culture was diluted using additional LB to obtain an optical density of 0.1 at 650 nm. Lactic broth contained tryptone, 20g; yeast extract, 5g; gelatin, 2.5g; NaCl, 4.0g; sodium acetate, 1.5g; lactose, 10g; and distilled water, 1000 ml.

L. acidophilus variety Farr was obtained from the culture collection in this department. It has been suggested that this strain might better be classified as *L. lactis* (Miller et al., 1970); however, in this paper the organism will continue to be identified as *L. acidophilus*. Frozen stock cultures were maintained in 11% reconstituted nonfat dried milk. Prior to use, a culture was thawed, and 0.1 ml was transferred to LB. After 10–12 hr of growth at 37°C, the culture was centrifuged for 10 min at 23,100 × G and was washed with 0.005M potassium acid phosphate buffer, pH 7.2. For inoculation purposes, the cells were resuspended and diluted in phosphate buffer to an optical density of 0.1 at 650 nm. Phosphate buffer was utilized as a diluent to avoid the addition of nutrients to the medium.

Model waste medium

A model waste medium consisting of 40g Super Dried Meat Solubles (Geo. A. Hormel & Co., Austin, MN) and 5g soluble starch (Mallinckrodt Chemical Works, St. Louis, MO) in 1L of distilled water was prepared, and pH was adjusted to 7.0 ± 0.1 with 1N NaOH. Four hundred ml of this medium were sterilized at 121°C for 15 min in a 1L Erlenmeyer flask, and tempered to 25°C. Aflatoxin B_2 (Sigma Chemical Co., St. Louis, MO) was dissolved in a specific high pressure liquid chromatography (HPLC) elution solvent described later, and was added to obtain a level of 800 ppb. The flask was shaken at 200 rpm for 12–16 hr at 37°C to allow for the evaporation of the solvent. Twenty-five ml portions of the medium were aseptically transferred to sterile 125 ml screw-capped Erlenmeyer flasks immediately prior to inoculation with *B. subtilis* A. For studies involving growth of *L. acidophilus* Farr without prior *B. subtilis* A growth, the medium was first centrifuged at 12,100 × G for 10 min, and 20 ml of the supernatant were aseptically transferred to 18 × 150 mm screw-capped test tubes.

Waste recovery system

The process used was based on a two-step microbial growth system patented by Busta et al. (1977). A 4% inoculum of *B. subtilis* A was grown in the described model waste medium for 4 hr at 37°C and 200 rpm. After centrifugation at 12,100 × G, 20 ml of the supernatant were aseptically transferred to sterile 18 × 150 mm screw-capped test tubes. This modified medium was treated with 0.1 units of penicillin G/ml to inactivate any remaining bacteria. Tubes were incubated at 37°C for 3–4 hr prior to the addition of 50 units of penicillinase/ml. After an additional incubation at 37°C for 3–4 hr, a 4% inoculum of *L. acidophilus* Farr was introduced into the modified medium. Growth proceeded for 12 hr and the cells, or single cell protein, were harvested by centrifugation at 12,100 × G.

Enumeration procedures

In preliminary investigations, no difference was noted between the growth of *L. acidophilus* Farr on tomato juice agar (Difco) and plate count agar (PCA, Difco). Therefore, PCA was employed for the enumeration of both organisms. Procedures for aerobic plate count as described by Speck (1976) were used. Plates were incubated at 37°C for 24 hr and 48 hr for *B. subtilis* A and *L. acidophilus* Farr, respec-

tively. No difference was noted between the growth of either organism in media with or without added aflatoxin.

Extraction of aflatoxin

A modification of the method of Pons (1975) was used for extraction of aflatoxin B_2 from the total, supernatant, and pellet fractions of the medium. Treatments of no growth (control), growth of *B. subtilis* A, growth of *L. acidophilus* Farr, and sequential growth of both organisms were analyzed. From 3 to 13 samples were analyzed for each treatment with a mode of 9.

For the total and supernatant fractions, a 10-ml sample was delivered to a 125 ml Erlenmeyer flask containing 30 ml of acetone and ca. 3 mm diam glass beads. *B. subtilis* A pellets were resuspended in 10 ml of distilled water. *L. acidophilus* Farr pellets were analyzed in one of two ways: (1) the pellet from one culture was resuspended in 5 ml of distilled water; or (2) 5 cultures were combined and the resulting pellet was resuspended in 5 ml of distilled water. The cell suspensions were transferred to acetone as in the total and supernatant procedures, and the resulting suspensions were vigorously shaken on a mechanical shaker for 30 min. The acetone/H_2O extract was filtered through 2 layers of Whatman #1 filter paper, and 30 ml of the filtrate were added to 20 ml of a 2% lead acetate solution. The final solution was stirred and, after 5 min of precipitation, was filtered through 2 layers of Whatman #1 filter paper. Thirty-five ml of this filtrate were added to 10 ml of dichloromethane in a 60 ml separatory funnel. The funnel was gently rolled to avoid the formation of an emulsion; and after separation of the two layers, the lower dichloromethane fraction was drained into a 50 ml beaker. Three more extractions followed, each utilizing 5 ml of dichloromethane, and the combined extracts were dried under a fume hood.

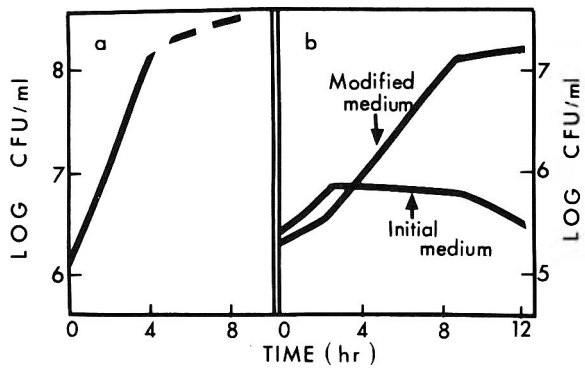


Fig. 1—(a) Growth of B. subtilis A prior to inactivation (solid line), with no inactivation (dashed line); (b) L. acidophilus Farr at 37° C in model waste medium composed of 40 g/L super dried meat solubles and 5 g/L soluble starch. L. acidophilus Farr growth in modified (4 hr) and initial medium.

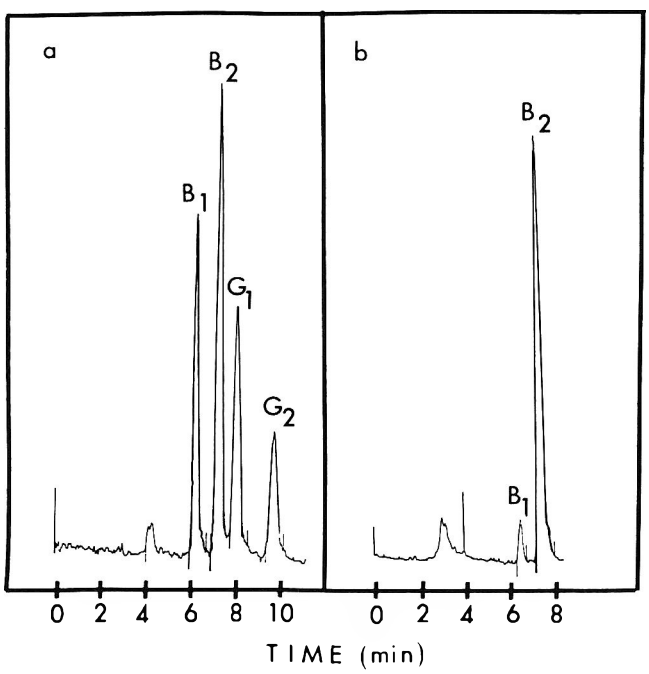


Fig. 2(a)—High pressure liquid chromatogram of aflatoxin standards B_1, B_2, G_1 and G_2; (b) model waste medium extract.

Aflatoxin quantification

The HPLC method of Pons (1976) was used to quantitatively analyze the aflatoxin present. A Waters HPLC (Waters Associates, Inc., Milford, MA), equipped with a model M-6000A pump, a U6K injector and a model 440 absorbance detector at 365 nm was employed. The elution solvent used consisted of water-saturated chloroform, cyclohexane, and acetonitrile in a ratio of 25:7.5:1 with 1% ethanol added to adjust retention time. A Waters Associate μPorasil column (10μm particle size) and a flow rate of 1 ml/min were used to achieve separation of aflatoxin standards. Dried sample extracts were dissolved in 1 ml of elution solvent prior to injection of 10 μl onto the HPLC. The same aflatoxin B_2 solution that was used to spike the medium was also injected separately onto the HPLC, and peaks generated were used to determine the percentage of recovery from the treated samples. Aflatoxin B_1, B_2, G_1, and G_2 standards were obtained from A.E. Pohland, at the Food & Drug Adminstration, Washington, D.C.

Statistical analysis

For statistical analysis of results, 95% confidence intervals were determined using the methods described by Remington and Schork (1970). When the intervals overlap, there is no statistical difference between the treatments at $p = 0.05$.

RESULTS & DISCUSSION

EVALUATION of the influence of individual bacterial strains in a sequential treatment requires the elimination of the first strain when investigating the effects of the second in the sequence. A special approach had to be used in this evaluation of the fate of aflatoxin. While the use of penicillin in a system for the production of SCP would not be acceptable, this method of inactivation of *B. subtilis* A was found to be practical for experimental purposes. Heat, in the presence of air, oxygen, and ozone, has been demonstrated by Dwarakanath et al. (1968) to decrease aflatoxin concentrations. Thus, sterilization at 121°C to inactivate *B. subtilis* A could not be used. Penicillin was therefore used to inactivate *B. subtilis* A, and because penicillin was in turn destroyed by penicillinase, *L. acidophilus* Farr growth and aflatoxin recovery were not affected.

In 4 hr, *B. subtilis* A increased 100-fold with a generation time of 36 min (Fig. 1a). This organism produces proteases and amylases which are capable of degrading this and other media with a wide variety of concentrations of protein and starch (Schmidt et al., 1977). The amino acids, peptides, and sugars produced facilitate the growth of more fastidious organisms.

The growth responses of *L. acidophilus* Farr in the initial and modified media (Fig. 1b) demonstrated the benefits of *B. subtilis* A media modification. Growth of *L. acidophilus* Farr was markedly more rapid in the medium modified by *B. subtilis* A than in the initial medium. In 12 hr of growth, a 100-fold increase was noted in the modified medium, as opposed to a 3-fold increase in the intial medium. No pH change was noted for *L. acidophilus* Farr grown in the initial medium. When grown in the *B. subtilis* A modified medium, pH dropped from 7.0 to 6.5 ± 0.1 in 12 hr. Growth of *L. acidophilus* Farr was not at an optimum in either the modified medium or the initial medium. In preliminary work with the organism in LB, a 1000-fold increase was noted in 12 hr. Furthermore, reduction of the pH to 5.0 was noted in the LB in 12 hr. Work is currently underway in this laboratory to further optimize the waste system to produce greater yields and to produce a biomass on a continuous basis.

Baseline separation of standard solutions of aflatoxins B_1, B_2, G_1, and G_2, was achieved when a composite of all 4 of the compounds was injected on the HPLC (Fig. 2a). Elution time

was approximately 6, 7, 8, and 10 min for aflatoxins B_1, B_2, G_1 and G_2, respectively. When extracted from the experimental medium (Fig. 2b), B_2 was again eluted at about 7 min. The peak at 6 min represents residual B_1 which was present in the commercially prepared toxin used to spike the medium. The peak at 3 min was due to solvent residuals from the extraction procedures, and this peak was considered to be inconsequential in aflatoxin quantification. Standards were injected for each series of samples analyzed to verify the identity of the peaks. No peaks eluted after aflatoxin B_2, thus indicating that no detectable microbiologically or chemically altered form of the aflatoxin B_2 was present.

The mean efficiency of aflatoxin B_2 recovery from uninoculated media was 72.4% from all fractions analyzed (Fig. 3). Ninety-five percent confidence intervals are indicated by vertical bars on this and the following graphs. Recovery refers to the area of the peak produced by the sample on the HPLC, divided by the expected area as calculated. While chloroform is generally used as the final solvent for aflatoxin extraction, Takahashi (1974) reported that dichloromethane was as efficient as chloroform for this purpose. Furthermore, the lower boiling point of dichloromethane as opposed to that of chloroform aided in rapid evaporation. There was no significant difference in extraction efficiency in any of the three portions—total medium, supernatant, or pellet.

Recoveries of aflatoxin B_2 from the total medium for all treatments [uninoculated (control), inoculated with *B. subtilis* A, inoculated with *L. acidophilus* Farr alone, and inoculated with *L. acidophilus* Farr following *B. subtilis* A growth], are shown in Figure 4a. A mean recovery of 55.9% of the expected amount of aflatoxin B_2 was established, and no significant difference was demonstrated between any of the treatments.

A similar trend was noted in the recoveries from the supernatant (Fig. 4b), with a mean recovery of 62.0%. There appears to be a slight difference between the recovery of aflatoxin B_2 from the initial medium inoculated with *L. acidophilus* Farr and from the control. Data presented are an averaged compilation of all analyses run; thus the control extraction includes the controls used in all three treatments. When the recovery of aflatoxin B_2 from the initial medium treated with *L. acidophilus* Farr was compared to controls analyzed at the same time, no difference was indicated.

Recoveries of aflatoxin B_2 from *B. subtilis* A and control pellets are shown in Figure 4c. Only estimates of the amount of aflatoxin B_2 present in the *L. acidophilus* Farr pellets was made. Using the methods described previously for aflatoxin analysis, B_2 was undetectable in an individual *L. acidophilus* Farr pellet due to the small amount of material present. An estimate of the level of B_2 present was made by combining 5 pellets for extraction procedures, and injecting twice the amount of sample (i.e. 20 μl) onto the HPLC. These estimates are indicated on the graph by open bars at the estimated recovery levels. Again, there does not appear to be any difference between the treatments. This is reinforced by the fact that neither concentration nor dilution of aflatoxin was observed in the recovery from the supernatant. Had either been the case, a corresponding opposite change would have been expected in the pellet fraction.

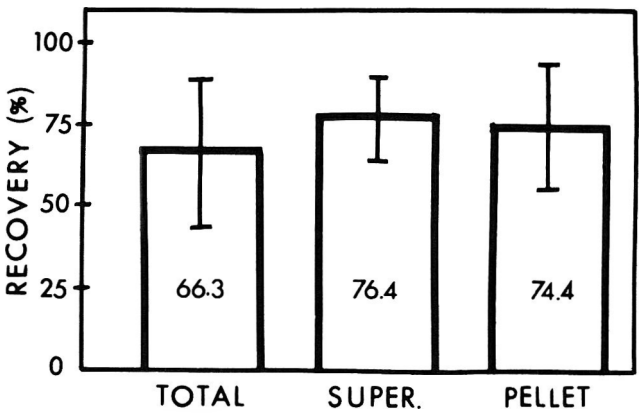


Fig. 3—Efficiency of extraction of aflatoxin B_2 from total, supernatant (Super), and pellet fractions of model waste medium using acetone, PbAc and CH_2Cl_2.

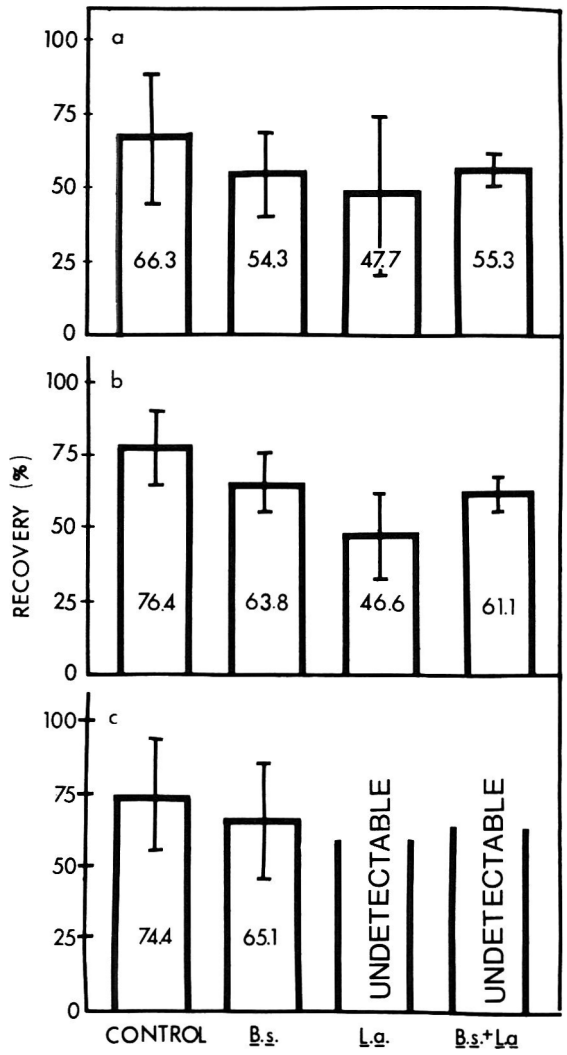


Fig. 4—Effect of growth of B. subtilis A (B.s.), L. acidophilus Farr (L.a.), and L. acidophilus Farr following B. subtilis A modification (B.s. +L.a.) on the recovery of aflatoxin B_2 in model waste medium from the total medium (a), supernatant (b) and pellet (c) fractions.

CONCLUSIONS

WHILE NO SIGNIFICANT reduction in the level of aflatoxin B_2 was demonstrated in this investigation, it is important to note that there was also no concentration of the substance by the cells. It is this concentration that is of most concern in the production of single cell protein from wastes. If toxic substances are present in the waste materials used as substrates, even if the concentrations are undetectable in the initial medium, the ability of the cell to concentrate the toxins could present toxicological hazards in the final product. Since the organisms used in this study did not concentrate the toxin, this hazard is avoided for aflatoxin B_2. With monitoring of the waste materials for aflatoxin prior to inoculation, one can be reasonably assured that aflatoxin B_2 will not be present in the

final product. However, other aflatoxins, notably B_1, may behave differently in this system. Therefore no generalized statement on the fate of all aflatoxins can be made.

The use of waste materials from the food industry remains a feasible method for generating new protein sources and new revenues, while reducing economic and ecologic costs of disposal. The two-step process has been demonstrated to be functional in providing a medium which will support the growth of a fastidious organism suitable for SCP production without the addition of costly growth factors, and does not suffer from the potential for concentration of aflatoxin B_2. In an area with a wide variety of agricultural commodities and processing facilities, an SCP production system of this type has great potential. Investigations concerning the expansion and optimization of this method to a larger scale is the next step in the development of an industrially feasible process.

REFERENCES

Anonymous. 1977. System treats waste water, grows SCP. Canner Packer 146(2): 82.

Bough, W.A., Brown, W.L., Porsche, J.D. and Doty, D.M. 1972. Utilization of collagenous by-products from the meat packing industry: production of single-cell protein by the continuous cultivation of Bacillus megaterium. Appl. Microbiol. 24: 226.

Busta, F.F., Schmidt, B.E. and McKay, L.L. 1977. Method of production and recovery and of protein from food wastes. U.S. Patent 4,018,650.

Ciegler, A., Lillehoj, E.B., Peterson, R.E. and Hall, H.H. 1966. Microbial detoxification of aflatoxin. Appl. Microbiol. 14: 934.

Dwarakanath, C.T., Rayner, E.T., Mann, G.E. and Dollear, F.G. 1968. Reduction of aflatoxin levels in cottonseed and peanut meals by ozonization. J. Amer. Oil Chem. Soc. 45: 93.

Litchfield, J.H. 1977. Single-cell proteins. Food Technol. 31(5): 175. 175.

Maing, I.-Y., Ayres, J.C. and Koehler, P.E. 1973. Persistence of aflatoxin during the fermentation of soy sauce. Appl. Microbiol. 25: 1015.

Miller, A. III, Sandine, W.E. and Elliker, P.R. 1970. Deoxyribonucleic acid base composition of lactobacilli determined by thermal denaturation. J. Bacteriol. 102: 278.

Pons, W.A. Jr. 1975. Collaborative study of a rapid method for determining aflatoxin in cottonseed products. J. Assoc. Off. Anal. Chem. 58: 746.

Pons, W.A. Jr. 1976. Resolution of aflatoxins B_1, B_2, G_1, and G_2 by high-pressure liquid chromatography. J. Assoc. Off. Anal. Chem. 59: 101.

Remington, R.D. and Schork, M.A. 1970. "Statistics with Applications to the Biological and Health Sciences," 1st ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Schmidt, B.E., Busta, F.F. and McKay, L.L. 1977. Protein production by successive growth of Bacillus subtilis and Lactobacillus acidophilus combined food wastes. J. Food Proc. & Preserv. 1: 13.

Shank, R.C. 1976. The role of aflatoxin in human disease. In "Mycotoxins and Other Fungal Related Food Problems," p. 51. Ed. Rodricks, J.V. American Chemical Society, Washington, D.C.

Speck, M.L. ed. 1976. "Compendium of Methods for the Microbiological Examination of Foods" 1st ed. American Public Health Association, Inc., Washington, DC.

Stoloff, L. 1976. Occurrence of mycotoxins in foods and feeds. In "Mycotoxins and Other Fungal Related Food Problems," p. 23. Ed. Rodricks, J.V. American Chemical Society, Washington, DC.

Takahashi, D.M. 1974. Thin layer chromatographic determination of aflatoxins in wine. J. Assoc. Off. Anal. Chem. 57: 875.

Ms received 10/7/77; revised 1/14/78; accepted 1/18/78.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1978.

Scientific Journal Series Paper No. 9965, Minnesota Agricultural Experiment Station. St. Paul, MN 55108.

The authors thank A.E. Pohland and S. Nesheim for aflatoxin standards, Geo. A. Hormel Col. for the dried meat solubles, and J.J. Warthesen and L.S. O'Keefe for their assistance with HPLC methodology.

Avens, J.S. and Miller, B.F. 1970b. Quantifying bacteria on poultry carcass skin. Poultry Sci. 49: 1309.

Barnes, E.M. 1975. The microbiological problems of sampling a poultry carcass. Proc. 2nd European Sym. on Poultry Meat 23: 1.

Barnes, E.M. and Shrimpton, D.H. 1958. The effect of the tetra-cycline compounds on the storage life and microbiology of chilled eviscerated poultry. J. Appl. Bact. 21: 313.

Clark, D.S. 1965. Improvement of spray gun method of estimating bacterial populations on surfaces. Can. J. Microbiol. 11: 1021.

Cox, N.A., Mercuri, A.J., Tanner, D.A., Carson, M.O., Thomson, J.E. and Bailey, J.S. 1978. Effectiveness of sampling methods for Salmonella detection on processed broilers. J. Food Protection. In press.

Cox, N.A., Mercuri, A.J., Thomson, J.E. and Chew, V. 1976. Swab and excised tissue sampling for total and enterobacteriaceae counts of fresh and surface-frozen broiler skin. Poultry Sci. 55: 2405.

Fromm, D. 1959. An evaluation of techniques commonly used to quantitatively determine the bacterial population of chicken carcasses. Poultry Sci. 38: 887.

Kinsley, R.N. and Mountney, G.J. 1966. A comparison of methods used for the microbiological examination of poultry carcasses. Poultry Sci. 45: 1211.

Mallmann, W.L., Dawson, L.E., Sultzer, B.M. and Wright, H.S. 1958. Studies on microbiological methods for predicting shelf life of processed poultry. Food Technol. 12: 122.

Mercuri, A.J. and Kotula, A.W. 1964. Relation of "breast swab" to "drip" bacterial counts in tray-packed chicken fryers. J. Food Sci. 29: 854.

Mulder, R.W.A.W., Dorresteijn, L.W.J., Hofmans, G.J.P. and Veerkamp, C.H. 1976. Experiments with continuous immersion chilling of broiler carcasses according to the code of practice. J. Food Sci. 41: 438.

Notermans, S., Hindle, V. and Kampelmacher, E.H. 1976. Comparison of cotton swab versus alginate swab sampling method in the bacteriological examination of broiler chickens. J. Hyg. Camb. 77: 205.

Notermans, S., Kampelmacher, E.H. and Van Schothorst, M. 1975. Studies on sampling methods used in the control of hygiene in poultry processing. J. Appl. Bact. 39: 55.

Patterson, J.T. 1971. Microbiological assessment of surfaces. J. Food Technol. 6: 63.

Patterson, J.T. 1972. Microbiological sampling of poultry carcasses. J. Appl. Bact. 35: 569.

Simonsen, B. 1971. Methods for determining the microbial counts of ready-to-cook poultry. World's Poultry Sci. J. 27: 368.

Thomson, J.E., Cox, N.A., Bailey, J.S., Holladay, J.H. and Richardson, R.L. 1976. Bacteriological sampling of poultry carcasses by a template-swab method. Poultry Sci. 54: 459.

Williams, M.L.B. 1967. A new method for evaluating surface contamination of raw meat. J. Appl. Bact. 30: 498.

Ziegler, F., Spencer, J.V. and Stadelman, W.J. 1954. A rapid method for determining spoilage in fresh poultry meat. Poultry Sci. 33: 1253.

Ms received 9/19/77; revised 11/21/77; accepted 12/1/77.

Mention of specific brand names does not imply endorsement by the authors or institutions at which they are employed to the exclusion of others not mentioned.

DETERMINING KINETIC PARAMETERS FOR THERMAL INACTIVATION OF HEAT-RESISTANT AND HEAT-LABILE ISOZYMES FROM THERMAL DESTRUCTION CURVES

A. C. LING and D. B. LUND

ABSTRACT

A simple method is proposed to analyze the kinetics of thermal inactivation of enzyme systems which consist of two groups differing in their thermal stability. Using this approach kinetic parameters for thermal destruction for each fraction (the heat labile and heat resistant fraction) can be estimated directly from thermal treatment data. However, to determine the relative concentration of the fractions within the enzyme system, additional information concerning the enzyme assay used to determine enzyme activity is required. Commercially available horseradish peroxidase was used to test the applicability of the method. Analysis of data generated from thermal treatments of the peroxidase using the proposed method were found to be satisfactory. E_a values of 21 kcal/mole and 34 kcal/mole were obtained for the heat resistant and heat labile horseradish peroxidase isozymes, respectively. D_{82C} values were 42 min and 1.2 min, respectively.

INTRODUCTION

HIGH TEMPERATURE–SHORT TIME (HTST) processes in thermal processing of food have long been recognized to result in greater retention of quality factors such as flavor, color, texture and nutrients. However it has also been recognized that foods processed by high temperature-short time treatments frequently undergo a rapid deterioration during storage. This rapid deterioration, evidenced through the development of off odor and taste, has been attributed to enzyme-catalyzed reactions (Joslyn and Cruess, 1929). Peroxidase, an iron-porphyrin organic catalyst which occurs naturally in nearly all plants and animals, is considered to be responsible for off-flavors and off-colors in raw and unblanched vegetables (Joslyn and Bedford, 1940; Joslyn, 1949, 1966; Bedford and Joslyn, 1939; Weaver and Hautala, 1970).

The inadequacy of enzyme inactivation during high temperature short time processes has been explained by suggesting the presence of two peroxidase isozymes with different heat resistances (Yamamoto et al., 1962). This suggestion is supported by the successful separation of peroxidase isozymes. Shannon et al. (1966) isolated seven peroxidase isozymes from horseradish roots and reported that these isozymes might be segregated into two groups on the basis of their chromatographic behavior, electrophoretic migration, spectrophotometric properties, and amino acid and carbohydrate composition. Wang and DiMarco (1972) separated six peroxidase isozymes from horseradish and applying thermal treatments showed that two of these isozymes, A-1 and C have respective Z-values of 23°C (42°F) and 37°C (66°F). Chenchin and Yamamoto (1973) isolated a total of eight peroxidase isozymes from sweet corn and they found that the isozymes of corn consisted of heat labile and heat resistant groups along with some of intermediate heat resistance.

Authors Ling and Lund are with the Dept. of Food Science, 1605 Linden Drive, University of Wisconsin-Madison, Madison, WI 53706.

0022-1147/78/0004-1307$02.25/0
© 1978 Institute of Food Technologists

Due to the presence of peroxidase isozymes of different stabilities, the rate of thermal inactivation of a composite peroxidase will not exhibit first order reaction kinetics. Using high temperatures for inactivation studies allows estimation of the Z-value for the heat resistant fraction but a substantial error may result because of the very short total heating and cooling times (Resende et al., 1969). Isolating the isozymes for inactivation studies would provide an accurate determination of Z-values (activation energies) for each fraction but experimental procedures would demand considerable time and expertise.

By assuming a first-order kinetic model for enzyme destruction and acknowledging the difference in respective inactivation rates, it is possible to analyze conventional thermal destruction data and estimate the thermal inactivation parameters for the heat labile and heat resistant isozymes. This paper describes the method which can be used for estimating kinetic parameters for thermal inactivation of enzyme systems containing isozymes of two different thermal stabilities.

Theory

Three mathematical models describing the possible kinetics of peroxidase isozymes were suggested (Clochard and Guern, 1973). Thermal inactivation studies of peroxidase from vegetable tissues repeatedly resulted in activation curves of unique feature. When the inactivation curves were constructed by plotting logarithm (base 10) of percent of original enzyme activity against heating time, nonlinear curves were obtained. The curve consisted of an initial steep straight line, an intermediate curved portion and a final straight line with a shallow slope (Yamamoto et al., 1962; Resende et al., 1969). The nature of these inactivation curves can be explained by assuming a model consisting of two groups of isozymes with different thermal resistance (E_L and E_R). A simple mathematical scheme describing the inactivation kinetics of the isozymes can be derived when the following assumptions for the model are adopted: (1) there are only two types of isozymes, one characterized by a small thermal inactivation rate (heat resistant, E_R) and one by a large inactivation rate (heat labile, E_L); (2) thermal destruction of the isozymes obeys pseudo first order kinetics; and (3) the dependence of the destruction rate on temperature obeys the Arrhenius equation.

Let E_L represent the concentration of heat labile isozyme and E_R the concentration of heat resistant isozyme at time t of the thermal treatment. At zero heating time, their concentrations are represented by E_{LO} and E_{RO}, respectively. The rate of inactivation of each isozyme at temperature T may be expressed as:

$$\frac{dE_L}{dt} = -k_L E_L \text{ or } E_L = E_{LO} e^{-k_L t} \quad (1)$$

$$\frac{dE_R}{dt} = -k_R E_R \text{ or } E_R = E_{RO} e^{-k_R t} \quad (2)$$

where k_L and k_R are the rate constants for thermal inactivation of heat labile and heat resistant isozymes, respectively.

—Text continued on page 1308

After thermal treatment, residual enzyme activities are determined by an enzyme assay. The kinetics of the enzyme-substrate reaction in the peroxidase assay are:

$$\text{when heating time } t = 0,\ \left[\frac{dS}{d\zeta}\right]_{t=o} = -K_L E_{LO} S - K_R E_{RO} S \quad (3)$$

$$\text{when heating time } t = t,\ \left[\frac{dS}{d\zeta}\right]_{t=t} = -K_L E_L S - K_R E_R S \quad (4)$$

where S = substrate concentration; K_L and K_R = reaction rate constant for the respective isozymes with substrate; and ζ = reaction time in the analysis.

Rate of color development during enzyme assay is a direct measure of the rate of disappearance of the substrate (or the rate of appearance of the colored product) and therefore the percent original activity of enzyme after thermal treatment is actually the measure of the ratio $[dS/d\zeta]_{t=t}/[dS/d\zeta]_{t=o}$. If % original activity = $[dS/d\zeta]_{t=t}/[dS/d\zeta]_{t=o} \times 100$, and combining with Eq (1), (2), (3) and (4),

$$\% \text{ original activity} = \frac{K_L E_{LO} e^{-k_L t} + K_R E_{RO} e^{-k_R t}}{K_L E_{LO} + K_R E_{RO}} \quad (5)$$

Thermal inactivation curves from enzyme systems consisting of heat labile and heat resistant fractions should behave according to Eq (5). To simplify interpretation of thermal inactivation data, two limiting conditions can be applied to Eq (5). When heating time t is long, $e^{-k_L t}$ will be close to zero and Eq (5) becomes:

$$\% \text{ original activity} = \frac{K_R E_{RO}}{K_L E_{LO} + K_R E_{RO} e^{-k_R t}} \quad (6)$$

When the thermal treatment is short, Eq (5) can be simplified and approximated as:

$$\% \text{ original activity} = \frac{K_L E_{LO}}{K_L E_{LO} + K_R E_{RO} e^{k_L t}} + \frac{K_R E_{RO}}{K_L E_{LO} + K_R E_{RO}}$$

or

$$\% \text{ original activity} - \frac{K_R E_{RO}}{K_L E_{LO} + K_R E_{RO}} = \frac{K_L E_{LO}}{K_L E_{LO} + K_R E_{RO} e^{-k_L t}} \quad (7)$$

Applying the limiting conditions at long and short thermal processing time, the thermodynamic parameters of the heat labile and heat resistant isozymes can be determined from the thermal destruction curve. When the thermal destruction curve is similar to that described by Yamamoto et al. (1962), the shallow slope straight line portion of the curve which occurs after a finite heating time is governed by Eq (6). This straight line portion has a slope equal to $-k_R/2.303$ and extrapolating the line to time zero, the intercept will be equal to $K_R E_{RO}/(K_L E_{LO} + K_R E_{RO})$. If the enzyme assays are all performed at the same temperature, the straight line portion of each inactivation curve performed at different treatment temperatures would always intersect at the same point at time zero. This is because K_R and K_L are functions of enzyme assay temperature only and the proportion of heat labile and heat resistant isozymes is constant within the same enzyme system.

Knowing $K_R E_{RO}/(K_L E_{LO} + K_R E_{RO})$, the initial part of the thermal inactivation curve can be replotted according to Eq (7). When the measured percent original activity value is corrected by subtracting the amount $K_R E_{RO}/(K_L E_{LO} + K_R E_{RO})$ and these data are replotted on a logarithmic scale (base 10) against heating time, the slope of the resultant straight line will be $-k_L/2.303$. The thermal inactivation rate of the heat labile isozyme (k_L) and the heat resistant isozyme (k_R) at thermal treatment temperature T can thus be estimated.

To test for the applicability of this mathematical model, a commercially available horseradish peroxidase was used for thermal inactivation studies.

EXPERIMENTAL

HORSERADISH PEROXIDASE was purchased from ICN Nutritional Biochemicals Corporation (Cleveland, OH). The enzyme had been electrophoretically purified (RZ value is 3.0) and lyophilized.

The rate of decomposition of hydrogen peroxide by peroxidase with guaiacol as hydrogen donor was determined by measuring the rate of color development at 430 nm. Five ml of the heat treated enzyme solution were mixed in a cuvette with 0.5 ml of 1% hydrogen peroxide and 0.5 ml of 1% guaiacol in a 50:50 water-ethanol solution. Color development was measured immediately after mixing using a Beckman ACTA III spectrophotometer.

Thermal treatments were performed in a glass container shown in Figure 1. The container is a jacketed cylindrical reaction flask fabricated with inlet and outlet connections for hot water or coolant circulation (Norman Erway Glass Blowing Laboratory, Oregon, WI). Heating and cooling of the container was accomplished by circulating hot or cold water. A block diagram of the circulation system is shown in Figure 2. Two electrical solenoid valves (#VSJ 2470 SR, Skinner Electric Valve Div., New Britain, CT) were used in both the heating and cooling system to direct flow of the heat exchange media. Hot water of desirable temperature was pumped from a constant temperature water bath (No. 6250, Eberbach Corp., Ann Arbor, MI) using an immersible pump (Model 2E-38N, Little Giant Pump Co., Oklahoma City, OK). During the heating period, the solenoid valves in the cooling circuit were closed and hot water flowed only in the heating circuit. When cooling was desired, valves in the cooling circuit were activated and simultaneously the valves in the heating circuit were closed. Cold water was circulated from an ice water bath using an immersible pump. A

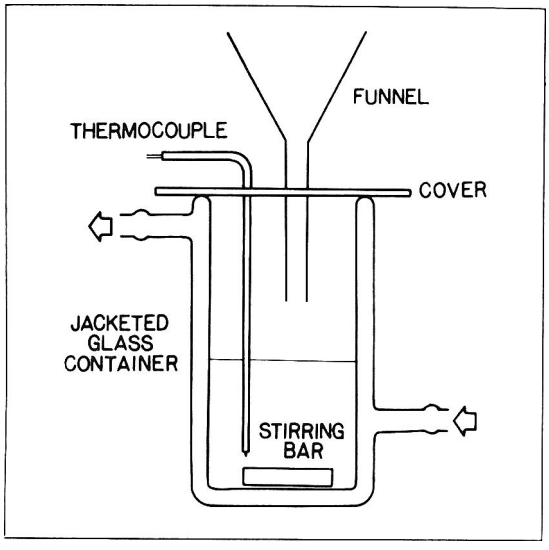


Fig. 1—Schematic diagram of vessel used for thermal inactivation.

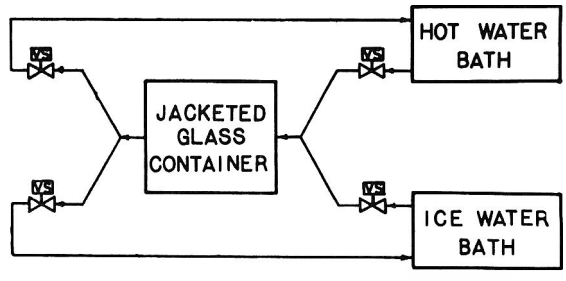


Fig. 2—Block diagram for the heating and cooling system used during thermal inactivation.

Table 1—Thermal inactivation of horseradish peroxidase[a]

Isozyme fraction	Inactivation temperature (°C)	$\frac{K_R E_{RO}}{K_L E_{LO} + K_R E_{RO}}$	k X 10^4 (sec^{-1})	E_a (kcal/mole)	D (sec)	Z (°C)
Heat resistant	76.7 ± 0.5	67.4 ± 10.9%	5.75 ± 1.6		4000 ±1000	
	82.2 ± 0.5	68.4 ± 5.6%	9.10 ± 1.1		2530 ± 300	
	87.2 ± 0.5	66.8 ± 12.5%	14.0 ± 3.2		1640 ± 380	
				21.1 ± 2		27.3 ± 3
Heat labile	76.7 ± 0.5		183.0 ± 30		126 ± 20	
	82.2 ± 0.5		331.0 ± 70		70 ± 15	
	87.2 ± 0.5		768.0 ± 230		30 ± 9	
				33.8 ± 11		17.0 ± 5

[a] Mean and 95% confidence interval are reported.

two-way switch was incorporated in the wiring of electrical equipment so that turning off the heating fluid would automatically activate the cooling fluid circulation. Due to the finite capacity of the flow system, 5–10 sec were required to replace the fluid in the jacketed space.

Twenty ml of distilled water were placed in the glass container and hot water was allowed to circulate. The final temperature of the distilled water was controlled by adjusting the water bath temperature and the flow rate of the hot water. This distilled water was stirred continuously using a magnetic stirrer (Corning PC-351, Hot Plate Stirrer, Scientific Products, Chicago, IL) to assure a uniform bulk temperature. When the temperature of the distilled water in the container stabilized, 0.5 ml of enzyme solution was injected into the container through a 2 cc syringe. The enzyme solution was prepared by dissolving 0.5 mg of enzyme in 10 ml of distilled water and was kept at 0°C prior to use. Introduction of the enzyme solution would lower the temperature of the distilled water by 1–2°C. The mixed solution in the reaction vessel was maintained at this final temperature for a predetermined period of time. The solution could be maintained at this elevated temperature within ± 0.5°C by compensating the heat lost to surrounding environment with manually controlled hot water circulation. To terminate the thermal treatment, the cooling circuit was activated and 20 ml of distilled water at 0°C was added to the container. Cooling the enzyme solution to 40°C was nearly instantaneous and further cooling to 5°C could be accomplished within a few minutes. Corrections for cooling lags were therefore neglected due to the rapid cooling of the enzyme solution to 40°C. Temperature of the enzyme solution was monitored at all times using a 24 gauge copper-constantan thermocouple and a Speedomax XL Recorder (Model 624, Leeds and Northrup Co., North Wales, PA). Triplicate inactivation experiments were performed at 76.7°C (170°F), 82.2°C (180°F) and 87.2°C (189°F).

RESULTS & DISCUSSION

FIGURE 3 shows the residual percent activity of the enzyme system as a function of heating time at three different inactivation temperatures. To analyze for the heat resistant isozymes, the straight line portion of each heating curve at long heating time was analyzed by linear regression. A semilogarithmic plot using Eq (7) was made to analyze for the heat labile fraction (Fig. 4). Information obtained by treatment of experimental data is presented in Table 1. Activation energies for thermal destruction of the heat labile and heat resistant isozymes were determined using the Arrhenius equation and were found to be 33.8 kcal/mole and 21.1 kcal/mole, respectively. Since the range of inactivation temperatures used was narrow (about 10°C), the more commonly used D and Z-values for the enzyme system were also estimated. The Z-value for the heat resistant isozymes was determined to be 27.3°C (49.1°F) and the Z-value for the heat labile isozymes was found to be 17.0°C (30.6°F).

Experimental results indicate that the proposed method provides an adequate description for the horseradish peroxidase system. Limiting conditions employed to simplify data analysis were justified by the vastly different k values between the heat labile and heat resistant isozymes. At long heating time (e.g. 3,000 sec at 77°C) only 0.4% of the heat labile isozyme fraction was still active and the assumption that all heat labile isozyme was inactivated was acceptable.

A slightly larger error occurs in the analysis of the heat labile fraction. During the short heating period (less than 30 sec) used to estimate k_L values for the heat labile fraction, the heat resistant fraction did undergo some thermal inactivation. Based on the experimental k_R values and assuming an equal portion of heat labile and heat resistant isozymes, the

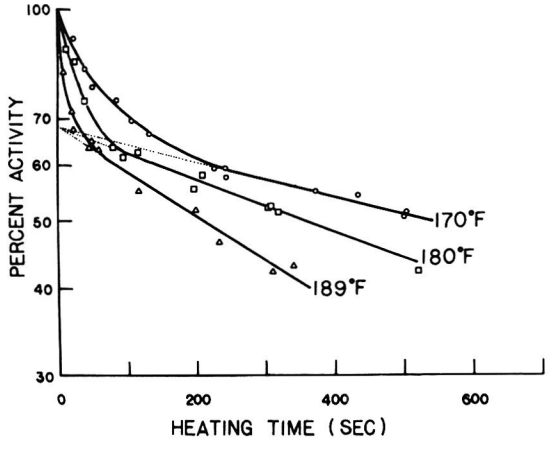


Fig. 3—Thermal inactivation curves for horseradish peroxidase.

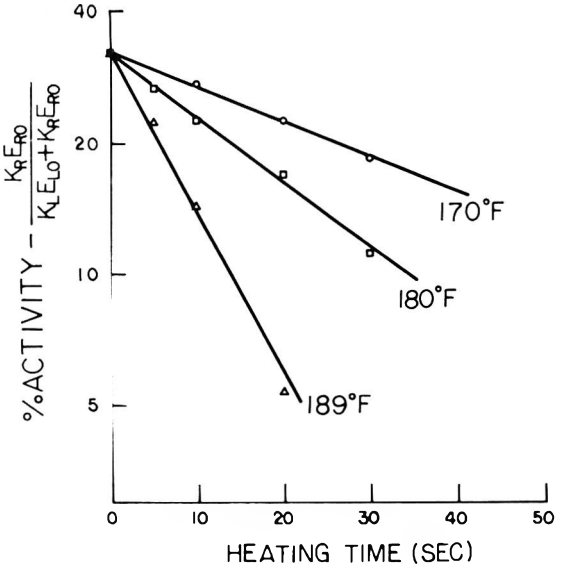


Fig. 4—Modified thermal inactivation curves for determining kinetic parameters for the heat labile isozymes.

theoretical percent activity (assuming no inactivation of the heat resistant fraction) would be 1% greater than the measured activity at a heating temperature of 76.7 and 3.6% greater at 87.2°C. This overestimation, however, was small compared to experimental uncertainties.

Z-values reported for horseradish peroxidase vary widely. Joffe et al. (1962) reported that Z-values for horseradish peroxidase ranged from 27.7°–35.7°C. Since very high temperatures were used for thermal inactivation in their study (up to 150°C), the Z-values reported should be for the heat resistant isozymes. These values compare favorably with the 27.3°C (49.1°F) found in this study. When experimental results were compared with values reported by Wang and DiMarco (1972), Z-values in this study were lower [27.3°C vs 38°C (49°F vs 66°F) for the heat resistant isozymes and 17.2°C vs 23.3°C (31°F vs 42°F) for the heat labile isozymes]. However, Wang and DiMarco used two isolated isozymes in their study and their heat treatment procedure is not detailed.

While the thermodynamic parameters of the isozymes can be determined using the described method, the fraction of the peroxidase system which is heat resistant (or heat labile) can be identified only if values for K_R and K_L are known. If K_L and K_R are identical, the Y-intercept from the straight line portion of the thermal inactivation curves at long heating time will indicate the relative concentration of the fraction. This technique had been used by Yamamoto et al. (1962). However, this Y-intercept should not automatically be considered as an indication of the heat resistant isozyme fraction until K_L and K_R are determined. As indicated by Wang and DiMarco (1972), the specific activities of the isozymes in enzyme assays were different. When the rate of decomposition of hydrogen peroxide by peroxidase with O-dianisidine as hydrogen donor was determined, the specific activity of isozyme fraction A-1 (heat labile isozyme) was found to be six times greater than that of isozyme fraction C (heat resistant isozyme). If K_L is assumed to be six times larger than K_R in this study, the relative concentration of the enzyme system can be estimated to contain 7.6% heat labile isozymes and 92.4% heat resistant isozymes. This estimation differs significantly from the conclusion that 67% of the isozymes are heat resistant when K_L and K_R are assumed to be identical.

CONCLUSION

THE THERMAL INACTIVATION data generated from commercially obtained horseradish peroxidase were analyzed by assuming that the enzyme system under investigation can be separated into two fractions, the heat resistant and the heat labile isozymes. Z-values determined for the heat labile and heat resistant horseradish peroxidase isozymes are 27.3°C (49.1°F) ± 3°C and 17.0°C (30.6°F) ± 5°C, respectively. While the kinetic parameters for thermal destruction of the isozymes can be determined, estimation of the relative concentration of the fractions is not readily possible. The distribution of the two isozyme fractions within the enzyme system can be estimated only if the reaction rate constants (K_L and K_R) between the isozymes and the substrate used in enzyme assay are known. It must be noted that the method proposed does not apply to systems containing more than two fractions of isozymes. For more complicated systems, a different means of analysis is required.

REFERENCES

Bedford, C.L. and Joslyn, M.A. 1939. Enzyme activity in frozen vegetable string beans. Ind. Eng. Chem. 31(1): 751.

Chenchin, E.E. and Yamamoto, H.Y. 1973. Distribution and heat inactivation of peroxidase isoenzymes in sweet corn. J. Food Sci. 38: 40.

Clochard, A. and Guern, J. 1973. Destruction Thermique De L'activite peroxydasique interpretation Des courbes experimentales. Revue Generale Du Froid. No. 8, Sept. 1973.

Joffe, F.M. and Ball, C.O. 1962. Kinetics and energetics of thermal inactivation and the regeneration rates of a peroxidase system. J. Food Sci. 27(6): 587.

Joslyn, M.A. 1949. Enzyme activity on frozen vegetable tissue. Adv. Enzymol. 9: 613.

Joslyn, M.A. 1966. Cryobiology, In "Freezing of Fruits and Vegetables," p. 583. Academic Press, New York.

Joslyn, M.A. and Bedford, C.L. 1940. Enzyme activity in frozen vegetables, asparagus. Ind. Eng. Chem. 32(1): 702.

Joslyn, M.A. and Cruess, W.V. 1929. Freezing storage of fruit and vegetable for retail distribution in paraffined paper containers. Fruit Prods. J. 8(7): 9; 8(8): 9.

Resende, R., Francis, F.J. and Stumbo, C.R. 1969. Thermal destruction and regeneration of enzymes in green bean and spinach puree. Food Technol. 23: 63.

Shannon, L.M., Kay, E. and Lew, J.Y. 1966. Peroxidase isozymes from horseradish roots. J. Biol. Chem. 241: 2166.

Wang, S.S. and DiMarco, G.R. 1972. Isolation and characterization of the native, thermally inactivated and regenerated horseradish peroxidase isozymes. J. Food Sci. 37: 574.

Weaver, M.L. and Hautala, E. 1970. Study of hydrogen peroxide, potato enzymes and blackspot. Am. Potato J. 47: 457.

Yamamoto, H.Y., Steinberg, M.P. and Nelson, A.I. 1962. Kinetic studies on the heat inactivation of peroxidase in sweet corn. J. Food Sci. 27: 113.

Ms received 7/30/77; revised 12/9/77; accepted 12/14/77.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by USDA, Hatch Act Research Grant.

ULTRASONIC PEPTIZATION OF SOYBEAN PROTEINS FROM AUTOCLAVED FLAKES, ALCOHOL-WASHED FLAKES AND COMMERCIAL SAMPLES

L. C. WANG

ABSTRACT

An ultrasonic method to peptize soybean proteins from defatted, autoclaved and alcohol-washed flakes and commercial concentrates and isolates was studied. Extraction conditions were optimized by examining factors including sonic power, meal-to-water ratio, salt concentration, pH and sonic time. The present study reaffirmed the fact that sonication can disperse 80% of total proteins from autocalved flakes with one extraction. Also, this method solublilized over 90% of the proteins from alcohol-washed flakes. Results from commercial concentrates and isolates varied. The low solubility of autoclaved commercial concentrates is likely due to high acidity rather than the inability to disperse proteins in water by ultrasound.

INTRODUCTION

SOYBEANS PROVIDE inexpensive proteins for food. To extract proteins from soybeans, the whole beans are usually cracked, dehulled, flaked, defatted and then dispersed in water. The method of dispersing often includes mechanical stirring (Circle et al., 1959) which extracts proteins effectively from unheated raw soybean flakes, but poorly from heated ones. Heat denatures proteins, lowers their solubility and alters their functionality, but heat-denatured proteins usually have improved flavor, increased nutritive values, less unfavorable biological factors and better acceptability for food uses.

To retain desirable functional properties such as solubility in soybean proteins the amount of heat treatment is usually carefully controlled. However, a recent finding (Wang, 1975) shows that applying ultrasonic energy during extraction improves water solubility of heat-denatured soybean proteins. Since then, studies have been continued to compare ultrasonic extraction with stirring extraction in order to explore the possible advantages and efficiency of the sonication method.

Ultrasonic treatment gives a new approach to improve soybean protein extraction. Previous results indicated that this technique extracted 80% of the protein from autocalved soybean flakes, while the stirring method yielded about 20% (Wang, 1975). Childs and Forte (1976), using combined ultrasonic and enzymatic techniques, effectively extracted proteins from cottonseed meal with low expenditure of time, enzyme and energy. Thus, the ultrasonic method can be applied to other meals than soybeans.

Ultrasounds induce cavitation (El'Piner, 1964) which can be affected by factors such as sonic power, sonic time, solvent, pH and salt concentration. These factors and their effects on cavitation have been studied in systems other than soybean protein extraction. Studies reported here include testing combinations of factors to establish an optimum extracting condition whereby the maximum amount of soybean proteins can be solubilized in water. The optimized extracting condition was then applied to soybean flakes of four different varieties, alcohol-washed flakes and commercially prepared samples. This paper presents results of these studies.

MATERIALS & METHODS

Materials

Soybeans of Harosoy, Amsoy, Hawkeye and Kanrich varieties were purchased from local suppliers. The whole soybeans were cracked, dehulled, flaked and defatted in the laboratory. Moisture contents of these defatted flakes ranged from 8–12% and their respective total protein contents were 48.1%, 50.2%, 50.1% and 46.4% (as-is basis). Other samples including one each of commercial defatted flakes and flour, two soybean isolates and three soybean concentrates were obtained from different commercial sources. Their protein contents were 52.7% and 54.9%, respectively, for flakes and flour, a range of 90.9–91.1% for isolates and 63.8–67.7% for concentrates (all as-is basis).

Autoclaving was carried out at 120°C for 20 min on defatted flakes, and the heated samples were stored at room temperature for further use.

Defatted Kanrich soybean flakes were also washed at room temperature with varied concentrations of alcohol (Smith et al., 1951). The flakes were air dried and ground to a powder before protein extraction by sonication or stirring.

In one case, 2N HCl and 2N NaOH were used to adjust the pH of extracting solution. Unadjusted water extracts had a pH of 6.8. Analytical grade of NaCl was used to adjust salt concentration when desired.

Methods

Sonication. Conditions were similar to previous experiments (Wang, 1975) except that a sonifier model J32A (Heat Systems-Ultrasonics, Inc., Plainview, NY) was used. The sonifier delivers, at a maximum input, 480 watts power at frequency of 20 KHz. Other sonifiers which delivered 125, 110 and 60 watts were used in one experiment to study the effect of varying sonic power. Sonication was carried out for 8 min except in one case where sonic time was varied from 2–10 min. After sonication, the mixture was centrifuged at 10,000 × G for 15 min to clarify the extract. Protein concentration in the supernatant was measured by the biuret method (Layne, 1957). The amount of soluble protein in the total amount of water used was calculated, and results reported here are means of triplicate analyses.

Stirring extraction. A mechanical stirrer with three ½-in. blades was used. Usually in 50-ml water, a 5-g sample was stirred for 1 hr at 1200 rpm at room temperature. The samples were handled the same way as sonicated samples in centrifugation, collecting supernatant and determining the protein content in the supernatant.

RESULTS & DISCUSSION

Dispersion of denatured proteins

The following two experiments further substantiate the ability of sonication to disperse denatured soybean proteins in water. The sonifier used had an output power of 480 watts which is higher than the one used before (Wang, 1975). Extracting conditions for each experiment are given with corresponding figures.

Alcohol-denatured proteins. With or without heat, maximum denaturation of soybean proteins occurs with 40–60% alcohol (Smith et al., 1951; Mann and Briggs, 1950; Roberts and Briggs, 1963). Sonication, however, peptized over 90% of the proteins in all flakes treated with alcohol concentrations from 20–80% (Fig. 1).

Heat-denatured proteins. Sonication can solubilize all proteins from autoclaved soybean flakes in water with two or three consecutive extractions (Fig. 2). With a sonifier of 480 watt power and meal-to-water ratio of 1:40, two extractions reached 100% (curve A), while that with 1:10 ratio required three extractions (curve B). Mechanical stirring failed to come close to these levels (curve C). Results in Figure 2 suggest that sonic power and meal-to-water ratio clearly affected the quantity of protein peptized, because previous results with a low power sonifier yielded less protein (Wang, 1975). To ensure maximum level of proteins dispersed in water, factors such as

Author Wang is with the USDA Science & Education Adm., Federal Research, North Central Region, Northern Regional Research Center, 1815 N. University St., Peoria IL 61604.

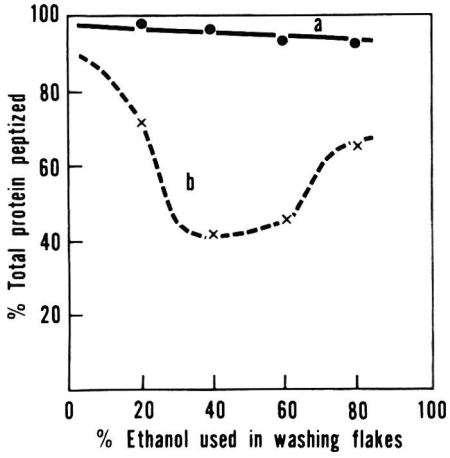


Fig. 1—Amount of protein peptized in water from defatted Kanrich soybean flakes washed with different concentrations of ethanol: (a) Sonication (Sonifier 480 watts for 8 min, meal-to-water ratio 1:40); (b) Stirring for 1 hr at room temperature, meal-to-water ratio at 1:10.

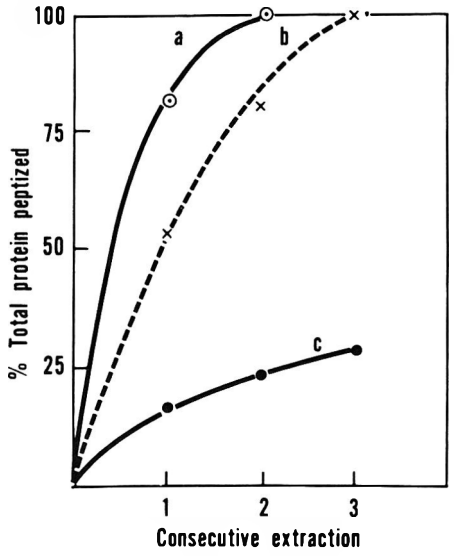


Fig. 2—Percent of protein solubilized from autocalved, defatted Kanrich soybean flakes with consecutive extractions. Extracting conditions were the same as Fig. 1: (a) Sonication; (b) Sonication but with 1:10 meal-to-water ratio; and (c) Stirring with 1:10 meal-to-water ratio.

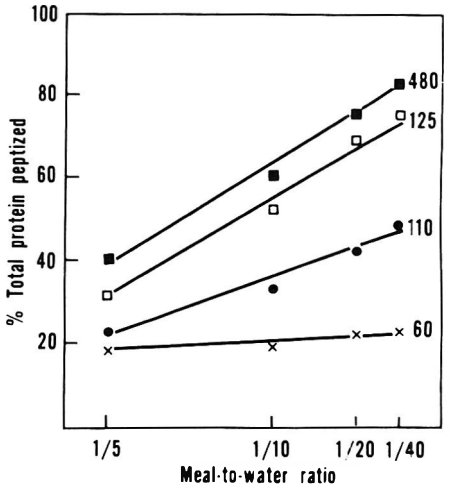


Fig. 3—Effect of sonic power (in watts, shown on curves) and meal-to-water ratio on protein peptization from autoclaved, defatted Kanrich soybean flakes.

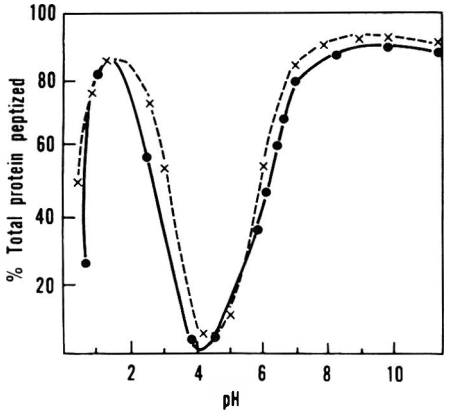


Fig. 4—Effect of pH on protein extraction by sonication (ratio, 1:40; time, 8 min) from autoclaved, defatted Kanrich soybean flakes (•——•) and by stirring (ratio, 1:10; time, 1 hr) from unautoclaved, defatted Kanrich soybean flakes (×——×).

Table 1—Effect of NaCl concentration of soy protein peptization by ultrasonic treatment from defatted, autoclaved flakes (meal-to-water ratio 1:40, sonicated for 8 min)

Ionic strength, μ (calculated)	Total protein extracted, %	
	Unautoclaved	Autoclaved
0.000	90.0	80.2
0.025	84.4	75.7
0.100	80.0	60.9
0.200	88.7	70.9
0.500	89.2	75.9
1.000	89.4	74.5

sonic time, pH and salt concentration were examined as a function of sonic power and meal-to-water ratio.

Factors affecting protein peptization

Sonic power and meal-to-water ratio. Figure 3 presents the relationship of sonic power and meal-to-water ratio to the amount of protein peptized. Within the ranges of sonic power and meal-to-water ratio tested, the amount of protein found in water increased with increasing power and decreasing ratio. At each power level, the protein solubility increased linearly with decreased ratios. The solubility rose as the power changed from 60 watts to 125 watts, and then not as much from 125 watts to 480 watts. Solubility of protein in water approached a maximum of 82% at the best combination of sonic power of 480 watts and meal-to-water ratio at 1:40. These two factors affected the quantity of proteins peptized in water more than all other factors tested.

Salt concentration and pH of solvent. Soybean proteins are more soluble in water than in neutral salt solution. The protein dispersion curve in salt solution shows a minimum at about 0.1N NaCl and at 0.02N $CaCl_2$ solution (Smith et al., 1938). The minimum occurs in solution with ionic strength at 0.1μ. The low yield at the minimum is due to the loss of 11S and 15S proteins as shown by ultracentrifugal analysis (Wolf and Briggs, 1956). Previous investigators used mechanical stirring extraction. When sonication (480 watts for 8 min, meal-to-water ratio at 1:40) was used, the same minimum was found at 0.1μ ionic strength level on both autoclaved or unautoclaved, defatted flakes (Table 1). Sonication enabled an extension of the study to heat-denatured proteins.

Stirring-extracted proteins from unautoclaved soybean flakes have a minimum solubility at pH 4 to 5 (Smith et al., 1938). Sonicated soybean proteins from autoclaved flakes also have the same low solubility in this pH range. Figure 4 gives comparative results for samples of two differently treated flakes with two different extracting methods.

Results presented in Table 1 and Figure 4 agree qualitatively with previous findings (Smith et al., 1938), and suggest that soybean proteins from autoclaved flakes retain their low

solubility in salt solution at 0.1μ ionic strength and in water at pH 4–5 after sonication.

Sonication time. Length of time to sonicate soybean flakes is interrelated to meal-to-water ratio in affecting the amount of protein peptized in water. For example, with a sonic power of 480 watts and a ratio of 1:20 the amount of protein dispersed in water rose rapidly as time increased from 2 to 6 min, and then reached a plateau. When the meal-to-water ratio is 1:40, over 80% of the total protein was extracted within 8 min. Further increase in length of time did not change the figure. but at 1:60, the amount of protein peptized decreased. Clearly an optimum is reached with a sonifier of 480 watts at meal-to-water ratio of 1:40 and time at 8 min.

Other factors investigated include particle size, temperature of extraction, container size and shape, and type of solvent. None of these factors gave a significant increase in protein solubility.

Application to commercial soybean materials

Sonication efficiency was tested with a number of samples that included soybean flakes of four varieties prepared in the laboratory, one each of commercial flakes and flour, two soybean isolates and three concentrates. Sonicating conditions were kept at a meal-to-water ratio of 1:40, and sonication time at 8 min with a 480-watt sonifier. Since stirring-extraction results were consistently lower than those by sonicated extraction, only results from sonication of unautoclaved and autocalved samples are presented in Table 2. It is not surprising to note that one sonication dispersed 83.4–97.7% of the total proteins from unautoclaved flakes and flour. The fact that sonication dispersed 70.1–80.7% of total proteins from autoclaved flakes and flour supports the finding that sonication improves water dispersibility of denatured soybean proteins.

Results with commercial isolates and concentrates are included in Table 2. Results (expressed as means of triplicate determinations) show greater variations with concentrates than isolates. Commercial isolates have high solubility (96.6% and 101.1%). Their respective extract pH's were 6.7 and 6.9. Commercial concentrates yielded 65.3%, 8.7% and 2.0% of total protein. Respective pH's of their extracts were 6.7, 5.8 and 4.3. When the pH of the extracting solution was adjusted to 7.0, they all yielded over 70% extractable protein. When these isolates and concentrates were autoclaved and sonicated, respective results of protein peptized were lower than those from unautocalved samples.

Results reported in this paper substantiate the effectiveness of sonication for dispersing soybean proteins in water and extend the effect to alcohol-denatured soybean proteins. Sonication has its greatest effect in improving dispersiblility of proteins from autocalved soybean flakes. This fact, so far, has not been reported by other investigators.

Sonication may break down protein molecules and alter their functional and physical properties. Proteins are changed in their size and charge distribution under enormous sonic energy inputs. Increases in solubility of the denatured proteins with increased energy input (Fig. 1 and 2) suggest that possibility. Although ultracentrifugal studies showed no apparent sedimentation constant difference in sonicated proteins (Wang, 1975), gel filtration and disc electrophoresis may help to reveal pertinent answers. A later paper will report studies on size and charge distribution of sonicated proteins.

Table 2–Ultrasonic peptization of soybean proteins from autoclaved, defatted flakes, flour, concentrates and isolates. (Sonic power 480 watts, sonic time 8 min and meal-to-water 1:40)

Sample	Ultrasonic peptized soybean protein,%	
	Unautoclaved	Autoclaved
Harosoy (flakes)	83.4	80.7
Kanrich (flakes)	90.0	80.2
Amsoy (flakes)	93.6	70.1
Hawkeye (flakes)	93.9	76.3
Commercial A (flakes)	95.8	77.4
Commercial B (flour)	97.7	71.4
Concentrate A[a]	65.3	34.6
Concentrate B	74.5	–
Concentrate C	76.5	–
Isolate A[b]	101.1	70.0
Isolate B	96.6	68.6

[a] Concentrate A, B and C were prepared by alcohol-leached (A) and dilute acid leached (B and C), respectively. Sample A was not readily wetted with water; B and C were pretreated with heat and determined in water with pH adjusted to 6.9.

[b] Protein isolate A and B are in the form of sodium proteinate.

REFERENCES

Childs, E.A. and Forte, J.F. 1976. Enzymatic and ultrasonic techniques for solubilization of protein from heat-treated cottonseed products. J. Food Sci. 41: 652.

Circle, S.J., Julian, P.L. and Whitney, R.W. 1959. Process for isolating soya protein. U.S. Patent 2,881,159.

El'Piner, I.E. 1964. Action of ultrasonic waves on biomacromolecules. In "Ultrasound: Physical, Chemical and Biological Effects," Ch. 7. Consultants Bureau, New York.

Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. 3. Biuret method. Methods Enzymol. 3: 450.

Mann, R.I. and Briggs, D.R. 1950. Effects of solvent and heat treatment on soybean proteins as evidenced by electrophoretic analysis. Cereal Chem. 27: 258.

Roberts, R.C. and Briggs, D.R. 1963. Characteristics of the various soybean globulin components with respect to denaturation by ethanol. Cereal Chem. 40: 450.

Smith, A.K., Circle, S.J. and Brother, G.H. 1938. Peptization of soybean proteins. The effect of neutral salts on the quantity of nitrogenous constitutents extracted from oil-free meal. J. Am. Chem. Soc. 60: 1316.

Smith, A.K., Johnsen, V.L. and Derges, R.E. 1951. Denaturation of soybean protein with alcohols and with acetone. Cereal Chem. 28(4): 325.

Wang, L.C. 1975. Ultrasonic extraction of proteins from autoclaved soybean flakes. J. Food Sci. 40: 549.

Wolf, W.J. and Briggs, D.R. 1956. Ultracentrifugal investigation of the effect of neutral salts on the extraction of soybean proteins. Arch. Biochem. Biophys. 63(1): 40.

Ms received 9/22/77; revised 12/31/77; accepted 1/4/78.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

PROXIMATE ANALYSIS, FATTY ACID AND AMINO ACID COMPOSITION OF NIGELLA SATIVA L. SEEDS

V. K. BABAYAN, D. KOOTTUNGAL and G. A. HALABY

ABSTRACT

Proximate analysis of *Nigella sativa* L. seeds showed a composition of 21% protein, 35.5% fat, 5.5% moisture, 3.7% ash and the rest being total carbohydrate. Fatty acid analysis of the extracted oil was determined using gas-liquid chromatography; it showed 56% linoleic acid, 24.6% oleic acid, 12% palmitic acid, 3% stearic acid, 2.5% eicosadienoic acid, 0.7% linolenic acid and 0.16% myristic acid. Traces of few unidentified fatty acids were also found. Amino acid analysis of the seed protein hydrolysate by gas chromatography of the n-propyl, N-acetyl derivatives showed the presence of 15 amino acids including 9 essential amino acids. Given the significant content of fat, protein and minerals in the seeds, it should be investigated as a source of these nutrients and its potential applications in flavoring various types of foods.

INTRODUCTION

Nigella sativa L. seeds, also known as black cumin or black caraway, is a member of the Ranunculaceae family and native to some parts of the Mediterranean region. These seeds are not related to cumin or caraway, both of which belong to the Umbelleferous family. The plant is also grown in India and the seed is used for sprinkling on bread, flavoring vinegar and as a carminative and stomachic in native medicine (Redgrove, 1933). The seeds are used by the Egyptians as a diuretic, carminative and flavoring agent, by the Syrians for cheese flavoring and by the Armenians for flavoring cheese and bakery products. The expressed oil has been used for treatment of asthma (Muschler, 1912).

A proximate analysis of the Russian *Nigella sativa* L. seeds was made by Greenish (1880) and found that they contain 4.35% nitrogen, 37% fixed oil, 7.43% moisture and 4.14% ash. Qualitative analysis of the ash showed that it consisted mainly of calcium salts. Crossley and Le Sueur (1898) were the first to prepare the oil from the Indian seeds by expression and to study its physical and chemical properties. Later, Bures and Mladkova (1928, 1930 quoted by Gad et al., 1963) extracted the oil with cold and boiling ether in 30% and 33.4% yield respectively and reported the physico-chemical characteristics and fatty acid composition of the oil. According to their study the fatty acids consisted mainly of telfairic and myristic acids. Tiwari and Singh (1942) reported that the saturated fatty acids of the extracted oil were composed of myristic, palmitic, stearic and arachidic acids and the unsaturated acids determined by the elaidin test, oxidation with potassium permanganate and quantitative bromination, were oleic 55.26% and linoleic 44.74%.

Gad et al. (1963) studied the physical and chemical characteristics and fatty acid composition of the oil extracted from the Egyptian *Nigella sativa* L. seeds. According to their study, the saturated fatty acids constituted 11.8% of the total fatty acids and consisted of myristic, palmitic and stearic acids, while the spectrophotometric determination of the unsaturated acids showed the presence of oleic 48.76%, linoleic 37.56% and linolenic 1.88%. Some studies on the chemical constitution of the volatile oil of *Nigella sativa* L. were conducted by the Egyptian workers and they reported the isolation and identification of nigellone (Mahfouz and El-Dakhakhny, 1960), thymoquinone (El-Dakhakhny, 1963) and thymohydroquinone (El-Alfy et al., 1975) from the volatile oil. These compounds were shown to possess antimicrobial and pharmacological activities.

The purpose of the present investigation is to study the proximate and mineral composition of the *Nigella sativa* L. seeds, the fatty acid composition of the fixed oil fraction and the amino acid composition of the seed protein.

EXPERIMENTAL

THE *Nigella sativa* L. SEEDS were obtained from a local ethnic food store, reportedly imported from the Middle East, which is typical of the commercially available product. Total nitrogen content was determined by the standard Kjeldahl method and total fat by soxhlet extraction of the ground seeds for 24 hr using petroleum ether (35–60°C). Moisture, ash and fiber were determined by standard AOAC procedures. Mineral analysis was done on the hydrochloric acid solution of the ash; calcium and iron were determined using a Perkin Elmer 303 atomic absorption spectrophotometer, and sodium and potassium on an Instrumentation Laboratory model 143 flame photometer.

The dark yellow colored oil obtained by soxhlet extraction which contained 3.76% free fatty acid as oleic, was refined by treating a 50% solution of the oil in hexane with 14% caustic soda. The physical and chemical properties of the refined oil such as refractive index, specific gravity, saponification number, iodine value and free fatty acid were determined using standard AOAC methods. Fatty acid composition of the oil was determined by gas liquid chromatography of their methyl esters. The oil was hydrolyzed with methanolic sodium hydroxide on a steam bath, and the methyl esters were prepared by the boron trifluoride-methanol procedure of Metcalf et al. (1966). Gas chromatographic analysis of the methyl esters was conducted on a Hewlett Packard 5830A reporting gas chromatograph equipped with a flame ionization detector. A 6-ft × 1/8-in. stainless steel column, packed with 10% diethylene glycol succinate on 80–100 mesh Chromosorb W.H.P., was used for fatty acid analysis. The run was temperature programmed from 80–200°C at a rate of 5° per min, with a carrier gas nitrogen flow rate of 30 ml per min. Integration of the peak areas was done by the microprocessor built into the GLC unit. Identification and percent composition of the fatty acids were determined by reference to a standard of known composition.

The acid hydrolysis of the seed protein was carried out in a mini-reaction vial using 0.2g of the oil free meal and 3 ml of 6N hydrochloric acid at 110°C for 16 hr under nitrogen atmosphere. After the hydrolysis was complete, the contents of the vial was filtered, and the filtrate made up to 10 ml. One hundred microliters of this hydrolysate, along with 10 µl of the internal standard alpha amino adipic acid, was passed through a micro column of Dowex 50-X8, 100–120 mesh cation-exchange resin. The amino acids and the internal standard were eluted with 2 ml of 2N ammonium hydroxide. The dry eluate was derivatized with propanolic HCl and then with acylation reagent, and the n-propyl, N-acetyl esters obtained were determined by GLC according to the method described by Adams (1974). The 2-ft × 1/8-in. stainless steel column was packed with 0.31% Carbowax 20M, 0.28% Silar 5CP and 0.06% Lexan on 120–140 mesh Chromosorb WAW. The analysis was temperature programmed from 120–250°C at a rate of 8° per min with a nitrogen flow rate of 20 ml per min. The amino acids were identified by comparing their retention times with those of the standards analyzed under identical conditions. Quantitation was done automatically by the microprocessor using the internal standard method.

All authors are with Stokely-Van Camp, Inc., Central Laboratories, Indianapolis, IN 46226.

0022-1147/78/0004-1314$02.25/0
© 1978 Institute of Food Technologists

RESULTS & DISCUSSION

PROXIMATE ANALYSIS and mineral composition of the *Nigella sativa* L. seeds is presented in Table 1 along with the values obtained by Greenish (1880). The results indicate a percentage composition of crude protein 21.26 (33% on oil free basis), fat 35.49, moisture 5.52, ash 3.77, total carbohydrate 33.96, fiber 5.5, calcium 1.06, iron 0.014, sodium 0.098 and potassium 0.582 respectively. A comparison of these results with the literature values indicate that the major difference is in the amount of protein. This may be due to the geographic and climatic differences of places where the seeds are grown or due to the differences in the analytical techniques used. Quantitative determination of crude fiber, calcium, iron, sodium and potassium is not reported in the literature. The proximate and mineral composition shows that the seeds have high protein, fat and calcium content.

The general physical and chemical characteristics of the refined oil extracted from the seeds are summarized in Table 2 along with the literature values. The table shows 0.55% free fatty acids calculated as oleic, a saponification value of 189.4, an iodine value of 124.63, a specific gravity of 0.9201 and a refractive index of 1.4728 at 25°C, which shows a general agreement with the reported values. The fatty acid composition of the *Nigella sativa* L. seed oil is presented in Table 3. The fatty acids consist of 56.12% linoleic, 24.64% oleic, 12.08% palmitic, 3.11% stearic, 2.53% eicosadienoic, 0.7% linolenic and 0.16% myristic acids. Traces of few unidentified fatty acids were also found which amounted to less than 0.7% of the total fatty acids. For comparison of these results with those obtained by Tiwari and Singh (1942) and Gad et al. (1963) their values are included in Table 3. The fatty acid composition determined by the present investigation is entirely different from the literature values which differ themselves considerably. For example, according to the present study linoleic acid is the major fatty acid while the literature values indicate oleic acid as the major one. The oleic acid content is almost half the reported value (24.64% vs 48.76%). The linolenic acid is also less (0.7% vs 1.88%). The presence of eicosadienoic acid is not reported in the literature. The total unsaturated fatty acids are less (84% vs 88.2%) and saturated fatty acids more (15.35% vs 11.8%) than the literature values. No arachidic acid was found in the present investigation as reported by some of the previous workers.

The amino acid composition of the seed protein, determined by gas liquid chromatography, is summarized in Table 4. The protein consists of 15 amino acids namely, alanine, valine, glycine, isoleucine, leucine, proline, threonine, serine, aspartic acid, methoionine, phenylalanine, glutamic acid, tyrosine, lysine and arginine. It is interesting to note that among the 15 amino acids found, 9 are essential amino acids. The seed protein is rich in arginine, glutamic acid, leucine, phenylalanine and lysine, arginine being the major amino acid. The amino acid composition has not been reported elsewhere.

The use of *Nigella sativa* L. seeds as a carminative and flavoring agent should be cultivated in the Western cuisine. Some possible applications for the seeds have been attempted in making bakery-type products. Breads were made in both loaf and bun forms on which the seeds have been sprinkled. A loaf of bread was also made with approximately 3% of the seed meal. Some pizza, a favorite American dish, was made with the seeds sprinkled on top with the other pizza flavors. In all instances, organoleptic evaluation indicated that the seeds contribute a very unique flavor to the products, but very little aroma, which may be due to the low flavor volatiles levels and the fact that whatever is left is lost during the baking process. Sensory evaluation of the above products indicated a good potential for the use of these seeds in many bakery and dairy flavoring meat products such as sausage and luncheon meats. products. The further potential use of the seeds could be for

Table 1—Proximate analysis and mineral composition of Nigella sativa L. seeds

Analysis	Percent composition	
	Found	Literature values[a]
Moisture	5.52	7.43
Crude protein (N X 6.25)	21.26	27.19
Fat	35.49	37.00
Ash	3.77	4.14
Carbohydrate	33.96	–
Crude fiber	5.50	–
Calcium	1.06	–
Iron	0.014	–
Sodium	0.098	–
Potassium	0.582	–

[a] Greenish (1880)

Table 2—Physical and chemical properties of Nigella sativa L. oil

Properties	Found	Literature[a]
Free fatty acids (% as oleic)	0.55	–
Specific gravity (25°C)	0.9201	0.9207
Refractive index (25°C)	1.4728	1.4718
Saponification number	189.40	196.30
Iodine value (Wij's 60 min)	124.63	114.50

[a] Gad et al. (1963)

Table 3—Fatty acid composition of Nigella sativa L. seed oil

Fatty acids	Percent composition		
	Found	Literature values[a]	
Myristic	0.16	–	–
Palmitic	12.08	–	–
Stearic	3.11	–	–
Oleic	24.64	55.26	48.76
Linoleic	56.12	44.74	37.56
Linolenic	0.70	–	1.88
Eicosadienoic	2.53	–	–
Total saturated fatty acids	15.35	–	11.80

[a] Tiwari and Singh (1942); Gad et al. (1963)

Table 4—Amino acid composition of Nigella sativa L. seed protein

Amino acids	Percent composition
Alanine	3.77
Valine	3.06
Glycine	4.17
Isoleucine	4.03
Leucine	10.88
Proline	5.34
Threonine	1.23
Serine	1.98
Aspartic acid	5.02
Methionine	6.16
Phenylalanine	7.93
Glutamic acid	13.21
Tyrosine	6.08
Lysine	7.62
Arginine	19.52

REFERENCES

Adams, R.F. 1974. Determination of amino acid profiles in biological samples by gas chromatography. J. Chromatog. 95: 189.

Crossley, A.W. and Le Sueur, H.R. 1898. Chemical and physical characters of some Indian edible oils. J. Soc. Chem. Industr. 17: 989.

—Continued on page 1319

CHANGES IN KUNITZ TRYPSIN INHIBITOR DURING GERMINATION OF SOYBEANS: AN IMMUNOELECTROPHORESIS ASSAY SYSTEM

ROBERT C. FREED and DALE S. RYAN

ABSTRACT

A rocket immunoelectrophoresis system was developed for assay of the major trypsin inhibitor of soybean seeds, the Kunitz soybean trypsin inhibitor (STI). Antibodies specific for purified Kunitz inhibitor were produced by subcutaneous injection of the purified inhibitor in rabbits. Electrophoresis of samples containing STI into agarose gels containing antisera to STI resulted in the formation of precipitin bands (rockets) whose height was linearly related to the amount of STI in the sample in the range of 35–175 nanograms. The smallest amount of STI which can be detected is approximately 5–10 nanograms. For many purposes, this assay system represents a substantial improvement over enzyme assays because only a single protein is recognized rather than all proteins with trypsin inhibitory activity. Trypsin-STI complexes are also recognized in the immunoelectrophoresis assay, suggesting that at least two of the antigenic determinants of STI are accessible to antibodies when the inhibitor is complexed with enzyme. The Kunitz trypsin inhibitor concentration of soybeans (*Glycine max* L. var. Steele) decreased by 13% on a dry weight basis during the first 9 days of germination. Enzymatic assay of total trypsin inhibitor activity showed similar decreases during germination. Since rapid decreases in Kunitz inhibitor content do not take place during germination it seems improbable that the Kunitz inhibitor functions as a storage protein in the seed.

INTRODUCTION

LEGUME SEEDS are known to contain a number of constituents which may have deleterious effects on their nutritional value including proteinase inhibitors, phytohemagglutinins, tannins, phytic acid and others (Rackis, 1974). Of these constituents, proteinase inhibitors have been the most thoroughly studied. Both the mechanism of action on a molecular level (Sweet et al., 1974; Means et al., 1974) and the nutritional significance (Liener, 1976; Lyman et al., 1974) of these molecules have been thoroughly examined.

The use of germinated or green-mature soybeans in the United States is insignificant compared to the usage of dry-mature beans. Interest in the use of germinated soybeans in foods has increased considerably, however, because of improved vitamin content (Fordham et al., 1975) and because of improved functional properties of the soybean protein after germination (Pomeranz et al., 1977). Proteinase inhibitors are not likely to be a major determinant of nutritional quality in properly heat treated seeds but they may be more important in sprouts which frequently receive only mild heat treatments before consumption.

The physiological function of proteinase inhibitors in legume seeds is poorly understood (Ryan, 1973; Richardson, 1977). Several possible functions have been discussed and include roles as storage proteins, as regulators of endogenous proteinases, or as defense mechanisms directed against insect proteinases, microbial proteinases or delocalized endogenous proteinases. The presence of very large quantities of proteinase inhibitors in seeds and in tubers suggests a possible storage function for these molecules. Proteinase inhibitors make up roughly 6% of the protein in soybeans and about 10% of the soluble proteins of barley grains or potato tubers (Ryan, 1973). It has been suggested that a distinct advantage of proteinase inhibitors as storage proteins may lie in their high content of sulfur-containing amino acids (Pusztai, 1972). Studies of changes in the level of Chymotrypsin Inhibitor I during the life cycle of the potato plant are consistent with the idea that the molecule acts as a storage protein (Ryan, 1973). Inhibitor I present in vegetative tissues decreases rapidly during tuber development while concentrations in the tubers increase. The high concentration of the inhibitor in tubers decreases rapidly in turn when germination begins (Ryan et al., 1968). Analogous changes have been observed with inhibitor levels in tobacco callus tissue cultures. For example, inhibitor which had accumulated in aging callus cultures decreased rapidly when tissue was transferred to a new medium, suggesting degradation of the inhibitor to support new growth (Wong et al., 1975). Decreases in proteinase inhibitor content during seed germination or increases during seed maturation have also been observed with barley (Kirsi and Mikola, 1971), lettuce (Shain and Mayer, 1968) maize (Melville and Scandalios, 1972), and pea (Hobday et al., 1973). The trypsin inhibitors of kidney bean appear to change independently of each other, some increasing and some decreasing during germination (Pusztai, 1972).

The total proteinase inhibitor content appears to decrease during the germination of soybeans (Collins and Sanders, 1976; Bates et al., 1977). As with most other studies, however, studies with soybeans have all used nonspecific enzymatic assays and it is possible that certain of the inhibitors undergo changes different from the changes in total proteinase inhibitor content. Any differences of this kind could influence considerably conclusions as to the physiological function of these molecules in the seed. Conclusions as to the nutritional significance of proteinase inhibitors could also be influenced by the use of nonspecific enzyme assays. Total trypsin inhibitor activity of different soybean varieties does not correlate at all with the PER of unheated seeds. Since pancreatic hypertrophy does correlate well with PER for these varieties, it is possible that only certain inhibitors are responsible for pancreatic enlargement and therefore that total trypsin inhibitor activity may be misleading (Kakade et al., 1972). The present report demonstrates using a rocket immunoelectrophoresis assay that only minor changes in Kunitz soybean trypsin inhibitor content take place during germination.

MATERIALS & METHODS

CELLEX-D anion exchange cellulose was obtained from Bio-Rad Laboratories (catalogue No. 748-0530). Crude Soybean Trypsin Inhibitor type IIS, Bovine Trypsin type III, low electro-endo-osmotic Agarose and α-n-Benzoyl-DL-arginine-p-nitroanilide HCl (BAPNA) were purchased from Sigma.

Soybeans (var. Steele, lot LA 1–4) were obtained from Olds Seed Co., Madison, WI. Varieties Harosoy, T-245, PI 196.172, and PI 246.367 were obtained from Dr. Richard L. Bernard, U.S. Regional Soybean Laboratory, Urbana, IL. Korean soybean varieties *Kum du* and *Baik tae* were obtained from Dr. K. Kitamura, Tohoku University, Japan.

Authors Freed and Ryan are with the Dept. of Food Science, 123A Babcock Hall, University of Wisconsin-Madison, Madison, WI 53706.

0022-1147/78/0004-1316$02.25/0
© 1978 Institute of Food Technologists

Kunitz soybean trypsin inhibitor isolation

A 22 × 1.5 cm column of Cellex-D (Bio-Rad DEAE-Cellulose) was equilibrated with 0.2M ammonium acetate (NH_4OAc) at pH 6.5. A sample of 400–500 mg of crude Soybean Trypsin Inhibitor (Sigma), dialyzed overnight against the initial buffer (0.2M NH_4OAc pH 6.5), was applied to the column and eluted at room temperature with a linear gradient of 0.2M NH_4OAc (pH 6.5) to 0.5M NH_4OAc (pH 6.5) in a total volume of 600 ml. Fractions of approximately 5 ml were collected on a Gilson FC-80H Micro Fractionator. Optical density readings were taken on a Beckman 25 Spectrophotometer at 280 nm. Fractions corresponding to Kunitz Soybean trypsin inhibitor (STI) were collected, dialyzed and lyophilized. Purified Kunitz inhibitor was stored at room temperature until used. Discontinuous polyacrylamide gel electrophoresis of the inhibitor showed a single band. Antibodies produced against purified Kunitz STI showed only a single precipitin band on double diffusion against crude extracts of a number of varieties of soybean seeds, demonstrating the immunochemical homogeneity of the preparation used for production of antibodies. Crossed immunoelectrophoresis of crude extracts of ungerminated soybean seeds against antisera to STI also showed a single peak which confirms the homogeneity of the antiserum.

Antibody production

Purified Kunitz soybean trypsin inhibitor (2 ml of 4.5 mg/ml in 0.9% NaCl) was sterilized by passage through a Millipore filter (Metrical GA-8). Female white rabbits received a total of 1.5 to 2.0 mg Kunitz STI in an emulsion with Freund's complete adjuvant. Rabbits were reinjected twice monthly and were bled every two weeks from the peripheral blood vessel of the ear. The antiserum appeared homogeneous by Ouchterlony double diffusion.

Rocket immunoelectrophoresis

One percent agarose gels containing 2% (v/v) antiserum and Barbital-Glycine-Tris buffer (0.037M Barbital, 0.37M Glycine, 0.19M Tris, pH 8.8) were prepared with dimensions of 180 × 80 × 1.5 mm on agarose-coated glass plates. Sample wells (2½ mm diam) were spaced about 3 mm apart. The gel plates were cooled to 4°C on a cooling plate, through which cold water was circulated from a Haake FK 2 refrigerated water bath. Ten volts per cm gel width was supplied to each gel. Three millimeter thick agarose gels with Barbital-Glycine-Tris buffer were used for wicks to connect the gel with the buffer reservoirs of a Gelman flat bed electrophoresis chamber. Sample wells were filled with 7 µl samples. After 6 hr of electrophoresis, the gel was removed and six layers of moistened Whatman No. 2 filter paper were pressed against the gel with a 10-lb weight overnight. The gel was then dried under an infrared lamp, stained with Coomassie Blue (5% Coomassie Brilliant Blue R-250, 10% glacial acetic acid, 45% ethanol), and destained by diffusion against a 10% glacial acetic acid, 45% ethanol solution. Peak heights on the dried and stained gel were measured from the top of the sample wells with calipers.

Seed germination

Soybeans were washed with water, rinsed with 10% chlorox, and soaked for 5 hr in deionized H_2O at 30°C. Seeds were spaced evenly on trays covered with successive layers of aluminum foil, filter paper, and cheese cloth. Seeds were sprayed with deionized H_2O every 12 hr. The tray was covered with moistened cheese cloth and incubated at 25°C in the dark.

Seed extraction and dry weights

Approximately 15g of seed were crushed with a mortar and pestle and divided into two samples, for extraction, and for dry weight measurements. Dry weights were determined after drying at 100°C in a Thelco vacuum chamber. Dry weights were taken until they stabilized.

The soybeans for extraction were weighed and homogenized for 1 min in 20 ml 0.01N NaOH with a Polytron PT 10 at a setting 4. The homogenate was diluted with another 20 ml 0.01N NaOH and centrifuged at 9°C and 15,000 rpm for 20 min in a SS-34 rotor (27,138 × G) using a Sorvall RC 2 B centrifuge. The supernatant was collected. The pellets were homogenized with another 20 ml of 0.01N NaOH for 30 sec at a setting 4. This homogenate was centrifuged as before. The supernatant was collected and the pellet was extracted a third time with another 20 ml 0.01N NaOH. The supernatant from all three centrifugations was combined and the total volume measured in a graduated cylinder. Additional extractions contained only traces of the Kunitz inhibitor. The combined extracts were frozen at −20°C until used.

Enzyme assay of trypsin inhibitor activity

Soybeans were extracted in 0.01N NaOH as described above and assayed for trypsin inhibitor activity using the method of Kakade et al. (1974). Optical density at 410 nm was determined with a Beckman 25 spectrophotometer and compared to a reaction mixture without inhibitor. Trypsin inhibitor activity was determined from dilutions which gave between 40 and 60% trypsin inhibition. One Trypsin Unit (T.U.) is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of reaction mixture. Trypsin inhibitor activity is expressed in terms of Trypsin Units Inhibited (T.U.I.).

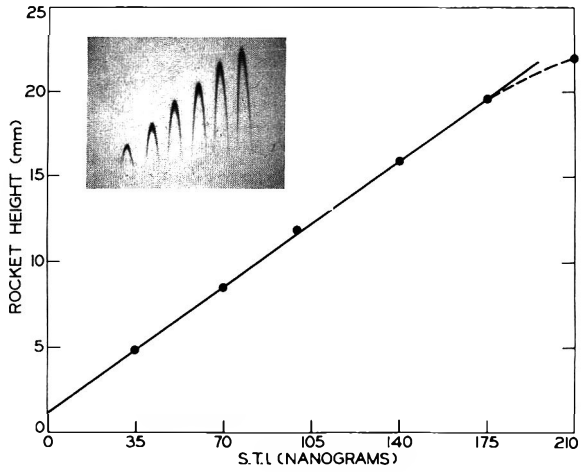


Fig. 1—Standard curve for rocket immunoelectrophoresis. Seven microliter samples of 5–30 µg STI per ml electrophoresed into a 1.5 mm thick 1% agarose gel in Barbital-Glycine-Tris buffer containing approximately 2% rabbit antisera. Insert shows typical result from standard solutions.

Table 1—Recovery of purified STI added to a 1:30 dilution of crude soybean extract

Amount added (µg STI)	Amount expected (µg/ml × 10^2)	Amount observed (µg/ml × 10^2)
0	–	2.07
46.6	2.54	2.39
93.2	3.00	2.95
139.8	3.47	3.42
186.4	3.93	3.90

RESULTS & DISCUSSION

ROCKET IMMUNOELECTROPHORESIS of purified STI showed a linear relationship between rocket height and amount of STI between 35 and 175 nanograms of STI (Fig. 1). Rocket heights are inversely proportional to the concentration of antibody in the gel, so relatively small amounts of serum were necessary for quantitation of STI levels. Approximately 0.5 ml serum was sufficient for a 180 × 80 × 1.5 mm plate which can accommodate 32–80 samples. Reproducibility of rocket height within a gel was quite good (± 2%). Reproducibility of rocket height from one gel to another, however, was not nearly as good, so standards of 5–25 µg STI per ml were run on each plate. Recovery of STI added to diluted crude extract as measured by the rocket immunoelectrophoresis showed excellent recoveries (Table 1).

Incubation of trypsin with soybean trypsin inhibitor (Fig. 2) reduces the apparent content of STI. Concentrations of both enzyme and inhibitor were in the range of 1×10^{-5} M. At these concentrations, complex formation between enzyme and inhibitor should be essentially stoichiometric even at an enzyme-inhibitor ratio of one. The reason that rocket height does not reach a minimum at an enzyme-inhibitor ratio of one is not clear. It is most likely, however, that dissociation of

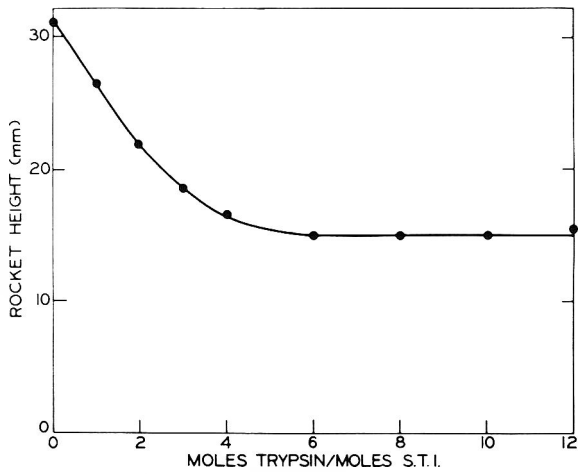


Fig. 2—Rocket immunoelectrophoresis of solutions containing increasing concentrations of bovine trypsin in the presence of a constant concentration of Kunitz soybean trypsin inhibitor.

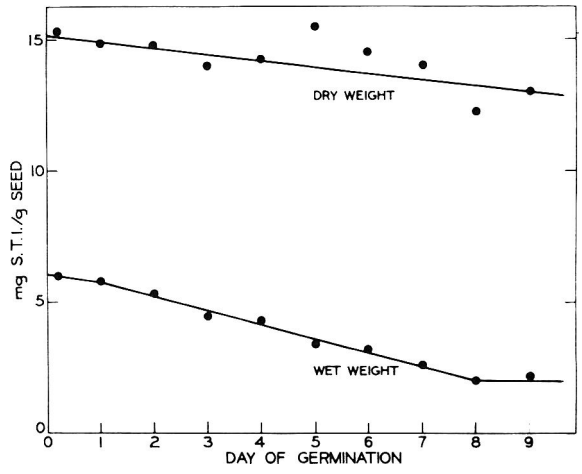


Fig. 3—Kunitz soybean trypsin inhibitor content of Steele variety soybeans as a function of germination time measured by rocket immunoelectrophoresis.

enzyme-inhibitor complexes take place during immunoelectrophoresis and diffusion of trypsin outside the area of antigen excess releases free inhibitor. The fact that an excess of trypsin does not reduce apparent STI levels below about 50% of maximum suggests that antibodies to STI can recognize STI in a complex with trypsin. This must mean that at least two of the antigenic determinants of STI are exposed after complex formation. This result also suggests that endogenous seed proteinases could interfere to some extent with determinations of STI. This problem is also encountered with classical enzymatic assays for proteinase inhibitors in seeds. The fact that immunochemical determination of STI content can be reduced by only 50% in the presence of a very large excess of enzyme is a substantial improvement over enzymatic assays in which even a slight excess of endogenous enzyme could reduce apparent STI levels to zero.

Rocket immunoelectrophoresis provides a rapid quantitative assay for Kunitz soybean trypsin inhibitor in the concentration range of 5–25 μg/ml. The smallest amount which can be detected is approximately 5–10 nanograms. This sensitivity exceeds considerably the sensitivity necessary for assaying STI content of seeds, and has the advantage of observing a single molecular species rather than observing a mixture of proteins all of which have inhibitory activity against a particular enzyme. Enzyme assays are indirect and for that reason cannot detect the presence of inactive inhibitors such as inhibitors complexed with endogenous enzymes.

The content of Kunitz soybean trypsin inhibitor varies considerably in different varieties of soybean (Table 2). Steele and Harosoy soybeans contain about 12–13 mg Kunitz inhibitor per gram of ungerminated seed. Two Korean varieties, *Kum du* and *Baik tae*, which had been reported (K. Kitamura, personal communication) not to contain trypsin inhibitors, were confirmed to be lacking in the Kunitz inhibitor. Varieties known to have genetic variants of the Kunitz inhibitor with altered electrophoretic mobility (T-245, PI 196.172, and PI 246.367) contained between 9.5 and 13.5 mg Kunitz inhibitor per gram seed (Hymowitz, 1973). The behavior of Kunitz inhibitor from T-245 seeds on rocket immunoelectrophoresis varied slightly from Kunitz inhibitor from other varieties in that stained rockets were considerably fainter. The reason for this difference is not known but is presumably related to genetic changes in one or more of the antigenic determinants of the molecule.

Germination of Steele soybeans showed a gradual decrease in Kunitz soybean trypsin inhibitor content on a fresh weight basis as a function of germination time (Fig. 3). After 9 days of germination, STI content was 50% of the original. On a dry weight basis, however, the decrease was considerably smaller, only a 13% decrease after 9 days. Very similar changes were observed in the total trypsin inhibitor content of the seed as measured with an enzymatic assay (Fig. 4). The fact that both assays yield similar results suggests that the Kunitz inhibitor is responsible for most of the trypsin inhibitor activity of the seed.

The magnitude of the changes which take place in the Kunitz inhibitor are considerably less than those which might be expected for a storage protein. The total protein content of

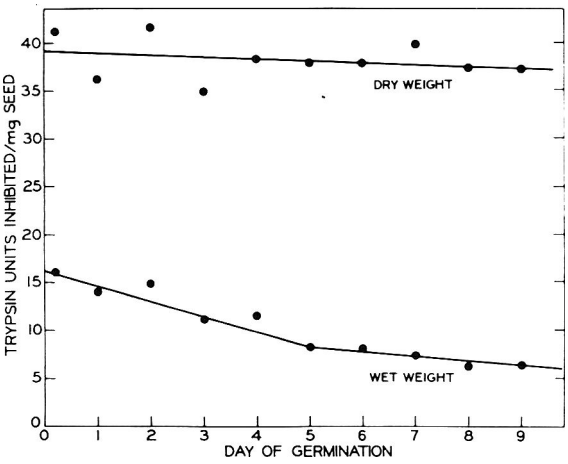


Fig. 4—Total trypsin inhibitor content of Steele variety soybeans as a function of germination time as measured by enzyme assay.

Table 2—Kunitz soybean trypsin inhibitor content of soybean varieties

Seed variety	STI content (mg STI/g fresh seed)
Steele	12.9
Harosoy	12.5
T-245	13.5
PI 196.172	9.5
PI 246.367	10.2
Baik tae	no STI detected
Kum du	no STI detected

germinating soybean cotyledons, for example, has been reported to decrease by 60% (mg protein per cotyledon) during nine days of germination (Treffry et al., 1967). The 7S component of soybean seeds is no longer detectable by immunodiffusion after 9 days of germination, and the 11S component although it is still detectable after 16 days of germination is present in considerably reduced amounts after 9 days (Catsimpoolas et al., 1968a, b). It seems unlikely therefore that the Kunitz soybean trypsin inhibitor functions as a storage protein in the seed.

REFERENCES

Bates, R.P., Knapp, F.W. and Araujo, P.E. 1977. Protein quality of green-mature, dry mature and sprouted soybeans. J. Food Sci. 42: 271.

Catsimpoolas, N., Campbell, T.G. and Meyer, E.W. 1968a. Immunochemical study of changes in reserve proteins of germinating soybean seeds. Plant Physiol. 43: 799.

Catsimpoolas, N., Ekenstam, C., Rogers, D.A. and Meyer, E.W. 1968b. Protein subunits in dormant and germinating soybean seeds. Biochim. Biophys. Acta 168: 122.

Collins, J.L. and Sanders, G.G. 1976. Changes in trypsin inhibitory activity in some soybean varieties during maturation and germination. J. Food Sci. 41: 168.

Fordham, J.R., Wells, C.E. and Chen, L.H. 1975. Sprouting of seeds and nutrient composition of seeds and sprouts. J. Food Sci. 40: 552.

Hobday, S.M., Thurman, D.A. and Barber, D.J. 1973. Proteolytic and trypsin inhibitory activities in extracts of germinating Pisum sativum seeds. Phytochem. 12: 1041.

Hymowitz, T. 1973. Electrophoretic analysis of SBTI-A_2 in the USDA soybean germplasm collection. Crop. Sci. 13: 420.

Kakade, M.L., Simons, N.R., Liener, I.E. and Lambert, J.W. 1972. Biochemical and nutritional assessment of different varieties of soybeans. J. Agr. Food Chem. 20: 87.

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.

Kirsi, M. and Mikola, J. 1971. Occurrence of proteolytic inhibitors in various tissues of barley. Planta 96: 281.

Liener, I.E. 1976. Legume toxins in relation to protein digestibility. J. Food Sci. 41: 1076.

Lyman, R.L., Olds, B.A. and Green, G.M. 1974. Chymotrypsinogen in the intestine of rats fed soybean trypsin inhibitor and its inability to suppress pancreatic enzyme secretions. J. Nutr. 104: 105.

Means, G.E., Ryan, D.S. and Feeney, R.F. 1974. Protein inhibitors of proteolytic enzymes. Accounts of Chem. Res. 7: 315.

Melville, J.C. and Scandalios, J.G. 1972. Maize endopeptidase: genetic control, chemical characterization, and relationship to an endogenous trypsin inhibitor. Biochem. Genet. 7: 15.

Pomeranz, Y., Shogren, M.D. and Finney, K.F. 1977. Flour from germinated soybeans in high-protein bread. J. Food Sci. 42: 824.

Pusztai, A. 1972. Metabolism of trypsin-inhibitory proteins in the germinating seeds of kidney bean. Planta 107: 121.

Rackis, J.J. 1974. Biological and physiological factors in soybeans. J. Amer. Oil Chem. Soc. 51: 161A.

Richardson, M. 1977. The proteinase inhibitors of plants and microorganisms. Phytochemistry 16: 159.

Ryan, C.A. 1973. Proteolytic enzymes and their inhibitors in plants. Ann. Rev. Plant Physiol. 24: 1073.

Ryan, C.A., Huisman, O.C. and Van Denburgh, R.W. 1968. Transitory aspects of a single protein in tissues of Solanum tuberosum and its coincidence with the establishment of new growth. Plant Physiol. 43: 589.

Shain, Y. and Mayer, A.M. 1968. Activation of enzymes during germination, trypsin-like enzyme in lettuce. Phytochemistry 7: 1491.

Sweet, R.M., Wright, H.T., Janin, J., Chothia, C.H. and Blow, D.M. 1974. Crystal structure of the complex of porcine trypsin with soybean trypsin inhibitor (Kunitz) at 2.6A resolution. Biochemistry 13: 4212.

Treffry, T., Klein, S. and Abrahamsen, M. 1967. Studies of fine structural and biochemical changes in cotyledons of germinating soybeans. Aust. J. Bio. Sci. 20: 859.

Wong, P.P., Kuo, T. and Ryan, C.A. 1975. Growth-dependent accumulation and utilization of proteinase inhibitor I in tobacco callus tissues. Biochem. Biophys. Res. Commun. 63: 121.

Ms received 9/23/77; revised 12/9/77; accepted 12/14/77.

Support from the University of Wisconsin Graduate School Research Committee (Project Number 180116) and from the College of Agricultural and Life Sciences, UW Madison, is gratefully acknowledged. This material was submitted by R. Freed to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the M.S. degree in Food Chemistry.

COMPOSITION OF Nigella sativa L. SEEDS . . . From page 1315

El-Alfy, T., El-Fatatry, H. and Toama, M. 1975. Isolation and structure assignment of an antimicrobial principle from the volatile oil of Nigella sativa L. seeds. Pharmazie 30: 109.

El-Dakhakhny, M. 1963. Studies on the chemical composition of Egyptian Nigella sativa L. seeds. 2. The essential oil. Planta Medica 11: 465.

Gad, A., El-Dakhakhny, M. and Hassan, M. 1963. Studies on the chemical constitution of Egyptain Nigella sativa L. oil. Planta Medica 11: 134.

Greenish, H.G. 1880. Contribution to the chemistry of Nigella sativa. Pharm. J. & Trans. 10: 909.

Mahfouz, M. and El-Dakhakhny, M. 1960. Some chemical and pharmacological properties of the new anti-asthmatic drug "Nigellone." Alexandria Med. J. 6: 357.

Metcalfe, L., Schmitz, A. and Pelka, R. 1966. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem. 38: 514.

Muschler, R. 1912. "A Manual Flora of Egypt." R. Friedlander Un Sohn, Berlin.

Redgrove, S. 1933. "Spices and Condiments." Isaac Pitman Co.

Tiwari, R.D. and Singh, B.K. 1942. Chemical examination of the seeds of Nigella sativa, Linn (Margel) Part 1. Fatty oil. Proc. Acad. Sci. India 12: 141.

Ms received 9/16/77; revised 12/14/77; accepted 12/20/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

CLASSIFICATION OF WINES BY APPLYING PATTERN RECOGNITION TO CHEMICAL COMPOSITION DATA

W. O. KWAN and B. R. KOWALSKI

ABSTRACT

Pattern recognition techniques are applied to the classification of wines by their chemical compositions. A study has been completed on 49 samples of 1970 and 1971 vintage Rhine and Moselle German white wines. The previously published data include atomic absorption analysis for elemental compositions, gas chromatographic analysis for alcohols, and the determination of total acids, solids and ash. Visual examination of the data showed no apparent separation. Several classification procedures were used and Least Squares Multilinear Classifier (LEAST) proved to be the best method for this study. Separations by vintage years and wine regions are made possible by these methods. The best chemical features used for each separation are different, which reflects the wide variations between regions and between years within the same region. Ratios of chemical features are found to be more useful for classification than values of individual measurements.

INTRODUCTION

GERMANY exports more white wines to the United States than most of the major wine producing countries. Many experts agree that the finest German wines are of excellent quality, and even the less expensive ones have their special character and charm. Valleys along the Rhine and its most important tributary, the Moselle, constitute the major wine regions in Germany. Although only a few varieties of grape can be successfully cultivated, almost all of these—the Riesling, the Muller-Thurgau, the Gewürztraminer, the Sylvaner, plus a few others—yield superior white wines. German wines owe their reputation to a unique interplay of climate and soil. The temperate climate allows the grapes a far longer growing and ripening period to be nourished with the minerals of the soil and to develop taste and flavor substances more intensely. According to Amerine and Joslyn (1970), the Rhine wines are slightly higher in alcohol and less tart than the wines of Moselle. They know of no critical experiments, but their impression is that the Rhine wines may have a little more color. But differences in processing, particularly in the amount of sulfur dioxide used, may and frequently do make it difficult to generalize.

A study was carried out by Dyer and Ansher (1977) of the Bureau of Alcohol, Tobacco, and Firearms, Department of the Treasury to distinguish Rhine and Moselle wines by their chemical compositions. Forty-nine samples of 1970 and 1971 vintage Rhine and Moselle German white wines were studied and a comparison of the data for the 1970 and 1971 vintage years and the Rhine and Moselle wine regions was presented. It was concluded that the data were insufficient to determine distinguishing and identifying characteristics of either vintage year or wine region.

In this study, techniques of pattern recognition are used to analyze this data set to classify these wine samples according to vintage year and wine region. Pattern recognition techniques have been applied to and proven successful in a wide variety of chemical problems. These include analysis of mass (Jurs et al., 1969; Isenhour et al., 1974), infrared (Kowalski et al., 1969; Liddell and Jurs, 1974; Preuss and Jurs, 1974), and nuclear magnetic resonance spectra (Kowalski and Reilly, 1971; Kowalski and Bender, 1972a); material quality control (Kowalski, 1974) and materials source identification (Duewer and Kowalski, 1975; Duewer et al., 1975; Kowalski et al., 1972). General reviews of the theory and application of pattern recognition in chemistry have been published by Kowalski (1975) and Jurs and Isenhour (1975). In general, pattern recognition can be applied to problems where there is a collection of objects and a list of measurements made on each object. The question to be answered will then be whether it is possible to find and/or predict a property of the objects that is not directly measurable but is known to be related to the measurements via some unknown relationship.

Pattern recognition can also be described as a collection of techniques that analyze n-dimensional plots. There are four types of operations in pattern recognition performed on the n-dimensional data structure. The first process, feature reduction, generates features by forming linear or nonlinear combinations of measurements. This preprocessing step is often a necessary one. The generated features are more amenable to data analysis by other pattern recognition methods than the original measurements. In the second process, the data in n-space are mapped to two- or three-dimensional space by display methods that seek to minimize information loss. These mappings make it possible for scientists to examine and recognize patterns in the two- or three-dimensional space. The last two processes are called supervised learning and unsupervised learning. In supervised learning, the sought-for property of some of the objects is known. These objects, collectively known as the training set, are used to develop classification rules that can be used to predict the property of the rest of the objects. If there is no training set, unsupervised learning methods are then used to discover a useful property of the objects.

Of particular interest to this study is the ability of pattern recognition to test whether or not this set of measurements contains the information necessary to determine distinguishing and identifying characteristics of the wine samples by vintage year or wine region and, if it does, what minimum subset of the measurements is sufficient. The techniques involved are largely nonparametric, making no or minimal assumptions about the underlying statistics of the data, and are quite insensitive to the nature of the data as long as it is numerical.

Terminology

In the terminology of pattern recognition, each individual wine sample analyzed is an "object" and each of the 16 chemical compositions is a "measurement." The measurements are used to generate "features" which may be the simple measurements, some combinations of measurements, some mathematical transformations of the measurements and/or combinations of the above. The feature values define a "data vector" for each of the objects. When data vectors belonging to defined groups, such as wine region and vintage year, are analyzed, the groups are called "categories."

Data

There are 49 samples of 1970 and 1971 vintage Rhine and Moselle German white wines. These samples are classified into four categories, namely Moselle vintage 1970 (M70), Moselle vintage 1971 (M71), Rhine vintage 1970 (R70), and Rhine vintage 1971 (R71) having 10, 16, 14 and 9 samples respectively. For each category, the set of average values of each measurement is calculated and added to its category as another sample. The 49 samples and 4 sets of average values from each category combine to give a total of 53 data vectors. Nearly all data vectors of the data set are complete, with less than 1%

Authors Kwan and Kowalski are with the Laboratory for Chemometrics, Dept. of Chemistry, University of Washington, Seattle, WA 98195.

0022-1147/78/0004-1320$02.25/0
© 1978 Institute of Food Technologists

missing values. The missing values are substituted by average values of that particular measurement from that category.

The data include atomic absorption analysis for the elements K, Na, Mg, Ca, Fe, Cu, and Zn; gas-liquid chromatographic analysis for methanol, ethyl acetate, n-propyl alcohol, iso-butyl alcohol, and iso-amyl alcohol; the traditional specific gravity determination of ethanol; and the determination for total acids, solids, and ash.

System

The data were analyzed using our batch-process version of ARTHUR (Harper et al., 1977) which contains more than 30 statistical and pattern recognition methods written in FORTRAN. An earlier version of this system is available upon request (Duewer et al., 1976). All processing was done on the University of Washington Academic Computer Center's CDC 6400.

EXPERIMENTAL

AUTOSCALE

The effect of the differing measurement magnitudes and units was compensated by normalizing the measurements. Sixteen features with uniform means of zero and variances of unity are formed by first subtracting the measurement mean from the individual values, then dividing by the total measurement variance (Kowalski and Bender, 1972b).

CORRELATION MATRIX

The interdependence of the 16 chemical measurements was investigated using the standard statistical intermeasurement correlation matrix.

WEIGHT

Weight is a preprocessing method that weighs each feature on the basis of its individual importance to the solution of a pattern recognition problem. Once the various measurements have been autoscaled to allow comparisons, weighing methods determine which of these new features are useful for separaton of the categories and should be enhanced or weighed, and which new features are not useful and can be deemphasized, or, even eliminated. The utility of each feature in separating the various wine samples was assessed by calculating for each feature the multicategory variance and Fisher weights which are the geometric and arithmetic averages of the inter-intra category weights, respectively (Kowalski and Bender, 1972b).

SELECT

This method is an iterative feature selection procedure that generates orthogonal features based on their importance to classification (Kowalski and Bender, 1976). The criterion for importance for categorized data is the variance weight. The feature with the highest weight is selected as the first feature. The remaining features are then decorrelated from it. The decorrelated features are reweighed and the feature now with the highest weight becomes the second selected feature. The process continues until either a specified number of features is chosen or the weight of the last selected feature falls below a preset limit.

KARLOV (Karhunen-Loeve Transform)

The K-L transform consists of a combination of subroutines. It is often used as a method of reducing the dimensionality of a data set (Kowalski and Bender, 1972b). It is used in this study to project data onto two dimensions for display.

VARVAR – Feature by feature line printer plots are produced from a data matrix. Two options are available. Either two features may be plotted against one another or one feature may be plotted against other properties of the data vectors.

TUNE

This method generates a new data file with features formed from the n(n – 1) ratios of the original measurements. This simple transform not only offers more features to the various selection algorithms, but also, in many cases, lends stability to the features.

K-NEAREST NEIGHBOR ANALYSIS (KNN)

KNN classifies unknown data vectors using their interpattern distances to data vectors in the training set as the decision criterion (Cover and Hart, 1967). Euclidean n-space distance is used in this study. A data vector is classified into the category which contributes the greatest number of the k nearest data vectors in the training set. For this study, the 1-, 3-, 5-, 7-, and 9-Nearest Neighbor classifications were considered. In case of a tie, the data vector is assigned to the category whose sum distance over k neighbors is the smallest.

STATISTICAL ISOLINEAR MULTIPLE COMPONENT ANALYSIS (SIMCA)

SIMCA is a classification method made up of five independent routines to facilitate disjoint principal component analysis of categorized data (Wold, 1976). It extracts a specified number of principal components from each category in turn, then classifies on the basis of euclidean distance from each data vector to the function defined by the components of each category.

LEAST

LEAST utilizes the least-squares multi-linear regression method to calculate weight vectors which are used for property prediction or category classification (Harper et al., 1977).

RESULTS & DISCUSSION

NINE SEPARATIONS were performed on the 4 categories of wine samples. First, classification was attempted for all 4 categories simultaneously. Then, classification by wine region was carried out by combining samples from different vintage years of the same region into one category. Similarly, samples from different regions but of the same vintage year were combined into one category to allow classification by vintage year. The rest are pair-wise separations between two categories by wine region and/or vintage year.

The correlation matrix (Table 1) shows that positive correlations over the entire data above 0.5 were observed for the following measurement combinations (total acids, methanol), (ash, K), (ethyl acetate, i-butyl alcohol, i-amyl alcohol). Correlation matrices on data subsets for the pairwise separations also show the same results as well as correlations for the elemental measurements (Na, Cu), and (Ca, Zn). The magnitude of these correlations is not so high that one measurement can be used in place of another, nor can they be considered as independent.

The variance weights of the 16 features for the nine separations are shown in Table 2. i-Butyl alcohol, ethanol, ethyl acetate, and Na possess high variance weights in most of the separations. Low variance weight features include ash, and all elemental measurements except Na and K. Three features are selected from the original 16 by SELECT. Features having high variance weights in Table 2 may not be the features selected. In separation 2, for example, i-butyl alcohol and ethanol rank first and second in the order of decreasing magnitude in variance weight while n-propyl alcohol ranks tenth. These three features are chosen by SELECT to be the three features containing the most information for classification by wine region. The reason that n-propyl alcohol is selected over other features with weight ranking between third and ninth is that these features are correlated to i-butyl alcohol and ethanol. When they are decorrelated after the first two features have been selected, their weights become lower than that of n-propyl alcohol which is then selected as the third feature. It should also be pointed out that even though most of the elemental compositions possess relatively low variance weights, as many of these features are selected for the nine separations as features of alcoholic compositions which have high variance weights. These two types of features obviously contain discriminatory information. Thus, classification can best be achieved by choosing a combination of features from these two types.

Three classification methods, KNN, SIMCA, and LEAST are applied to the data set utilizing three feature subsets, namely (a) 16 original features; (b) 3 selected features from (a); and (c) 3 selected features from 256 features which consist of the original features and all possible ratios of the features created in TUNE. Complete separation of 4 categories simultaneously is not achieved by any of the three classification methods. This result is not surprising as the chemical composition of wine depends on many factors. In addition to the

Table 1—Correlation matrix of the entire data set

	Total acids	Solids	Ash	Ethanol	Methanol	Ethyl acetate	n-Propyl alcohol	i-Butyl alcohol	i-Amyl alcohol	Na	K	Mg	Ca	Fe	Cu	Zn
Total acids	1.00															
Solids	0.115	1.00														
Ash	0.329	0.215	1.00													
Ethanol	0.315	−0.442	0.120	1.00												
Methanol	0.540	0.257	0.131	0.054	1.00											
Ethyl acetate	−0.072	−0.296	−0.099	0.388	−0.017	1.00										
n-Propyl alcohol	0.180	−0.301	0.124	0.029	0.033	0.277	1.00									
i-Butyl alcohol	−0.051	−0.051	−0.011	0.214	0.234	0.647	0.300	1.00								
i-Amyl alcohol	−0.106	−0.238	0.019	0.263	−0.029	0.771	0.412	0.737	1.00							
Na	−0.399	−0.148	0.076	−0.169	−0.549	−0.022	0.054	−0.316	−0.051	1.00						
K	0.313	0.037	0.742	0.284	0.092	0.074	0.065	−0.028	0.049	0.072	1.00					
Mg	0.141	−0.178	0.351	0.296	0.066	0.094	0.234	0.074	0.088	0.195	0.260	1.00				
Ca	−0.126	0.131	0.125	−0.291	−0.035	−0.293	−0.258	−0.210	−0.319	0.202	0.048	−0.035	1.00			
Fe	−0.263	−0.188	−0.046	−0.043	−0.325	0.125	0.084	0.094	0.158	0.309	−0.008	−0.015	−0.010	1.00		
Cu	0.134	−0.264	0.022	0.282	0.247	0.058	−0.079	0.212	0.075	−0.345	0.110	0.122	0.135	−0.120	1.00	
Zn	−0.188	−0.140	0.046	−0.036	−0.050	0.083	0.142	0.203	0.050	0.066	−0.028	0.247	0.125	0.160	0.147	1.00

diversities due to varieties of vines and different soils in specific regions and subregions, there is the diversity of climate over successive years. Other factors such as the method of wine making and a host of other conditions which may be unknown contribute significantly to the chemical composition diversities in wine. There may be a vast difference in most of these factors between Moselle and Rhine, but other factors which vary yearly in both regions may offset these differences thus making it difficult to differentiate all 4 categories from each other simultaneously based on chemical compositions. Better results are obtained for separation by region than by vintage year suggesting that differences due to geographic location dominate yearly variation within the same region. Pairwise classifications are considerably better and in a few cases, exceed 90% from more than one method.

Figures 1 and 2 allow a visual inspection of the data by 2-dimensional projections. The degree of clustering of data vectors in these plots reflects to some extent the percentage of correct classification by the three classification methods. No apparent pattern can be observed in Figure 1 which shows the distribution of the whole data set. This agrees with the classification results which are only about 50% utilizing the three features subsets. Figure 2 displays a distinctive separation of two categories which can be separated on this 2-dimensional plot with only one misclassification.

A comparison of the overall average classification results using the three classification methods and three feature subsets is given in Table 3. LEAST is the best method for this study. Results from SIMCA and KNN are not as good as those of LEAST, although they are no more than 10 percentage points lower. Results of the three classification methods using feature subset (a) are better than (b) and (c). But the low data vector/feature ratio makes these results questionable. The results from using subsets (b) and (c) are more acceptable since the ratio is raised to 7 or higher when only 3 selected features are used in both subsets. Results from subset (c) are consistently better than those from (b). This is not surprising as the variance weights of the features from (c) are slightly higher than

Table 2—Variance weights of 16 features in 9 separations (1.000 = no discriminatory information)

	(1) M70/M71/R70/R71	(2) (M70+M71)/(R70+R71)	(3) (M70+R70)/(M71+R71)	(4) M70/R70	(5) M71/R71	(6) M70/M71	(7) R70/R71	(8) M70/R71	(9) M71/R70
Total acids	1.134[a] (9)[b]	1.051 (8)	1.170 (3)	1.000 (16)	1.151 (7)	1.243 (6)	1.052 (8)	1.037 ($^{15}_{16}$)	1.359 (2)
Solids	1.173 (8)	1.007 (14)	1.270 (1)	1.006 (13)	1.003 (15)	1.261 (5)	1.268 (1)	1.231 (6)	1.309 (3)
Ash	1.069 ($^{14}_{15}$)	1.000 (16)	1.055 (8)	1.085 (10)	1.004 ($^{13}_{14}$)	1.150 ($^{8}_{9}$)	1.011 ($^{11}_{12}$)	1.148 ($^{8}_{9}$)	1.024 (12)
Ethanol	1.302 (2)	1.355 (2)	1.020 (11)	1.356 (4)	1.530 (1)	1.077 (11)	1.087 (7)	1.707 (2)	1.176 (5)
Methanol	1.204 (5)	1.059 (7)	1.143 (4)	1.193 (6)	1.086 (9)	1.359 (2)	1.141 (4)	1.496 (4)	1.015 (15)
Ethyl acetate	1.210 (4)	1.318 (3)	1.032 (9)	1.425 (3)	1.192 (3)	1.004 (16)	1.007 (14)	1.148 ($^{8}_{9}$)	1.594 (1)
n-Propyl alcohol	1.077 (13)	1.033 (10)	1.031 (10)	1.025 (12)	1.172 (4)	1.026 (14)	1.131 (5)	1.121 (12)	1.000 (16)
i-Butyl alcohol	1.342 (1)	1.445 (1)	1.001 ($^{13}_{14}$)	1.607 (2)	1.401 (2)	1.150 ($^{8}_{9}$)	1.010 (13)	1.705 (3)	1.307 (4)
i-Amylalcohol	1.090 (11)	1.122 (5)	1.000 ($^{15}_{16}$)	1.105 (9)	1.153 (6)	1.008 (15)	1.025 (9)	1.138 (10)	1.118 (7)
Na	1.286 (3)	1.034 (9)	1.244 (2)	1.137 (8)	1.071 (11)	1.419 (1)	1.256 (2)	1.974 (1)	1.056 (11)
K	1.203 (6)	1.086 (6)	1.013 (12)	1.922 (1)	1.001 (16)	1.289 (4)	1.023 (10)	1.171 (7)	1.023 (13)
Mg	1.110 (10)	1.010 (13)	1.070 (7)	1.045 (11)	1.150 (8)	1.311 (3)	1.001 (15)	1.120 (13)	1.060 (10)
Ca	1.088 (12)	1.028 (11)	1.001 ($^{13}_{14}$)	1.176 (7)	1.062 (12)	1.134 (10)	1.104 (6)	1.037 ($^{15}_{16}$)	1.022 (14)
Fe	1.069 ($^{14}_{15}$)	1.001 (15)	1.082 (5)	1.001 (15)	1.004 ($^{13}_{14}$)	1.048 (12)	1.180 (3)	1.129 (11)	1.066 (9)
Cu	1.178 (7)	1.217 (4)	1.000 ($^{15}_{16}$)	1.354 (5)	1.164 (5)	1.046 (13)	1.000 (16)	1.428 (5)	1.135 (6)
Zn	1.068 (16)	1.013 (12)	1.075 (6)	1.004 (14)	1.081 (10)	1.187 (7)	1.011 ($^{11}_{12}$)	1.039 (14)	1.096 (8)

[a] variance weight
[b] Order in decreasing variance weight

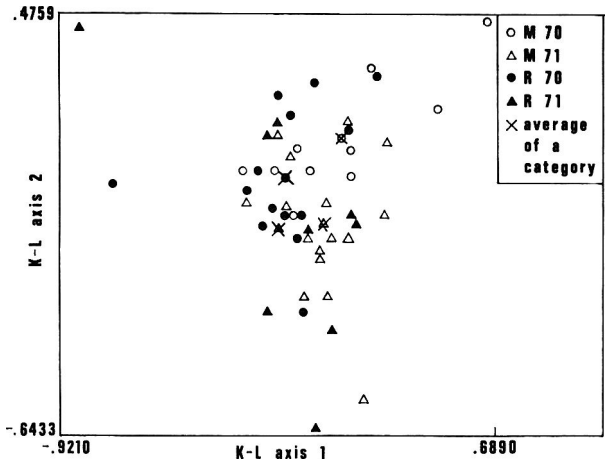


Fig. 1—K-L projection of 16-space onto 2-space for separation 1 (M70/M71/R70/R71) preserving 37.2% of the total variance.

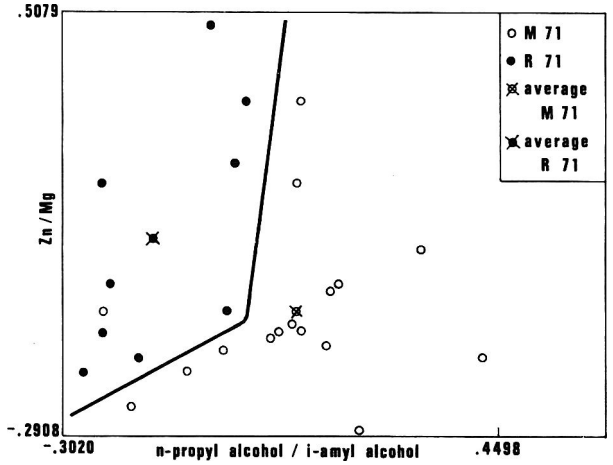


Fig. 2—Plot of 1st selected feature (n-propyl alcohol/i-amyl alcohol) versus 2nd selected feature (Zn/Mg) from feature (Zn/Mg) from feature subset (c) for separation 5 (M71/R71). (All measurements have been autoscaled).

those from (b) thus demonstrating a higher discriminatory power for classification. This is true because the features in (c) are nonlinear combinations of the measurements. Nonlinear features tend to bring more information to bear on a problem but often lead to considerable interpretation difficulties.

CONCLUSION

THE CHARACTER and quality of a wine depend on the kind of vine, the natural setting of the vineyards, and the human effort in the care of the vines in the vineyard and in the production and storage of the wine in the cellar. The natural prerequisites for the vine to grow and thrive are the local climate, the location and topography of the vineyard sites, and the kind of soil. Variations of all these factors contribute to the diversities of wine and are reflected in the differences of their chemical compositions. Sometimes the differences are so subtle that they can not be revealed by visual examination of chemical data. In this study, pattern recognition techniques are shown to be a remedy for this deficiency. Three classification methods. LEAST, SIMCA, and KNN are able to classify wines from Moselle and Rhine of 1970 and 1971 vintage with a respectable accuracy. Features which are ratios between alcohols and elemental compositions are found to be most useful for classification. Different features are selected for separating different pairs of categories reflecting the variations among the four categories. Study of the 2-dimensional plots of data vector distributions reveals some inherent information about the samples.

In order to have a better understanding of the data, more should be known about the history of the samples. In the original study (Dyer and Ansher, 1977), factors which contribute to the variations were not mentioned. These include aspects such as grape variety, soil type, weather during the two vintages, and the origin of the samples. Even though pattern recognition techniques can successfully classify these samples, we feel that such information is important to make the classifications more meaningful. Research is in progress in our laboratory to take further advantage of the multivariate data analysis methods of pattern recognition and much more highly resolved wine composition profiles using modern analytical chemistry methodology.

Table 3—Average classification results

Feature subset	Classification method		
	KNN	SIMCA	LEAST
a	60.3%	79.7%	91.2%
b	68.8%	79.1%	83.4%
c	74.5%	80.3%	84.6%

REFERENCES

Amerine, M.A. and Joslyn, M.A. 1970. "Table Wines — The Technology of Their Production," 2nd ed. U. of California Press, Davis, CA.
Cover, T.M. and Hart, P.E. 1967. The Nearest Neighbor Pattern Classification. IEEE Trans. Inform. Theory. IT-13: 21.
Duewer, D.L. and Kowalski, B.R. 1975. Forensic data analysis by pattern recognition. Categorization of white bond papers by elemental composition. Anal. Chem. 47: 526.
Duewer, D.L., Kowalski, B.R. and Schatzki, T.F. 1975. Source identification of oil spills by pattern recognition analysis of natural elemental composition. Anal. Chem. 47: 1573.
Duewer, D.L., Koskinen, J.R. and Kowalski, B.R. 1976. "ARTHUR" available from B.R. Kowalski, Lab. for Chemometrics, Dept. of Chemistry, Univ. of Washington, Seattle, WA 98195.
Dyer, D.H. and Ansher, A.F. 1977. Analysis of German wines by gas chromatography and atomic absorption. J. Food Sci. 42: 534.
Harper, A.M., Duewer, D.L., Kowalski, B.R. and Fasching, J.L. 1977. ARTHUR and experimental data analysis: The heuristic use of a polyalgorithm." In "ACS Symposium No. 52," American Chemical Society, Washington, DC.
Isenhour, T.L., Kowalski, B.R. and Jurs, P.C. 1974. "Critical Reviews in Analytical Chemistry," Vol. 4, p. 1. CRC Press Inc.
Jurs, P.C., Kowalski, B.R. and Isenhour, T.L. 1969. Computerized learning machines applied to chemical problem—Molecular formula determination from low resolution mass spectrometry. Anal. Chem. 41: 21.
Jurs, P.C. and Isenhour, T.L. 1975. "Chemical Applications of Pattern Recognition," Wiley-Interscience, New York, NY.
Kowalski, B.R. 1974. Pattern recognition techniques for predicting performance. ChemTech. 5: 300.
Kowalski, B.R. 1975. Measurement analysis by pattern recognition. Anal. Chem. 47: 1152A.
Kowalski, B.R., Jurs, P.C., Isenhour, T.L. and Reilly, C.N. 1969. Computerized learning machines applied to chemical problems—Interpretation of infrared spectrometry data. Anal. Chem. 41: 1945.
Kowalski, B.R. and Reilly, C.A. 1971. Nuclear magnetic resonance spectral interpretation by pattern recognition. J. Phy. Chem. 75: 1402.
Kowalski, B.R., Schatzki, T.F. and Stross, F.H. 1972. Classification of archaeological artifacts by applying pattern recognition to trace element data. Anal. Chem. 44: 2176.
Kowalski, B.R. and Bender, C.F. 1972a. The K-nearest neighbor classification rule (pattern recognition) applied to nuclear magnetic resonance spectral interpretation. Anal. Chem. 44: 1405.
Kowalski, B.R. and Bender, C.F. 1972b. Pattern recognition: A powerful approach to interpreting chemical data. J. Am. Chem. Soc. 94: 5632.
Kowalski, B.R. and Bender, C.F. 1976. An orthogonal feature selection method. J. Pattern Recog. 8: 1.
Liddell, R.W. III and Jurs, P.C. 1974. Interpretation of infrared spectra using pattern recognition techniques. Anal. Chem. 46: 2126.
Preuss, D.R. and Jurs, P.C. 1974. Pattern recognition techniques applied to the interpretation of infrared spectra. Anal. Chem. 46: 520.
Wold, S. 1976. Pattern recognition by means of disjoint principal components models. J. Pattern Recog. 8: 127.

Ms received 9/29/77; revised 1/20/78; accepted 1/30/78.

The authors express their gratitude to A. Harper, for her counsel and criticism; J. Ng, for her help in preparing the manuscript; and to Maynarhs daKoven for his valuable observations. This paper was presented at the 1977 Pacific Conference on Chemistry and Spectroscopy at Anaheim, CA, Oct. 12—14, 1977.

CHANGES IN CYTOKININ ACTIVITY IN THE RIPENING TOMATO FRUIT

N. DESAI and G. W. CHISM

ABSTRACT

The endogenous cytokinin activity of tomato fruits (*Lycopersicon esculentum* var. Heinz 1439) as measured by the *Amaranthus* bioassay decreases from 10.35 μg benzylaminopurine equivalents/1000g mature green fruit to 0.15 μg benzylaminopurine equivalents/1000g for the fully ripe fruits. Chromatography of the various tomato fruit extracts indicates changes in cytokinins during the ripening process. Liquid chromatography and gas chromatography of the tomato extracts indicate the presence of zeatin, zeatin riboside and isopentenyl adenine riboside like compounds in the extracts. The presence of zeatin riboside was confirmed by mass spectroscopy.

INTRODUCTION

THE RIPENING OF FRUITS is a process which is of great commercial importance. Plant growth substances are involved in the control of fruit ripening and the process is now considered to be essentially a senescence phenomenon (Frenkel et al., 1968; Hulme, 1972; Pratt, 1975). Since cytokinins are known to be involved in delaying senescence, it is suggested that cytokinins play a role in controlling fruit ripening (Dilley, 1969).

Exogenous application of kinetin to tomato fruits delayed ripening and extended the shelf life of the treated tomato fruits (Abdel-Kader et al., 1966). However, treatment of Rutger's fruit and *rin* mutant tomatoes with 6-benzylaminopurine gave no response (Mizrahi et al., 1975).

The endogenous cytokinin levels in young developing cherry tomato fruits were studied and it was found that the cytokinin levels were much higher in the developing fruits than the more mature fruits (Abdel-Rahman et al., 1975). The cytokinin levels of the cherry fruits declined rapidly after the first two weeks of fruit development. Varga and Bruinsma (1974) concluded in their study that increasing levels of endogenous cytokinins progressively retarded ripening of tomato fruits. Mizrahi et al. (1975) suggested that the rate of ripening of tomato fruits examined in the Varga and Bruinsma study when considered in relation to differences in endogenous cytokinin levels in the tomato fruits is not physiologically significant.

This study was conducted to determine the endogenous cytokinin levels present in tomato fruits at different stages of ripening and to study the nature of the endogenous cytokinins in tomato fruits.

EXPERIMENTAL

Fruit material utilized

Mature green tomato fruits (*Lycopersicon esculentum* var. Heinz 1439) were harvested from The Ohio State University farm and allowed to ripen under ambient conditions. Three batches of fruits were chosen for the extraction of cytokinins: (a) Mature green fruits, (b) Intermediate ripened fruits – "breaker stage," and (c) Fully ripe fruits – "full red stage." Approximately 1 kg of tomatoes were chosen at random from lots of 100 fruits and sliced into liquid nitrogen. Five hundred gram portions of the frozen fruit pieces were then extracted as described below. Analyses were performed in duplicate.

Chemicals

Standard cytokinins, zeatin, zeatin riboside (ZR) isopentenyl adenine (IPA), isopentenyl adenine riboside (IPR) and benzylaminopurine (BAP) were from Sigma Chemicals. Bis-(trimethyl silyl) acetamide (BSA), bis-(trimethyl silyl) trifluoracetamide (BSTFA) were from Pierce Chemical Co. Insoluble polyvinylpolypyrrolidone (PVP) was from Sigma Chemicals and Sephadex LH-20 was purchased from Pharmacia Fine Chemicals. All other chemicals and solvents were of reagent grade.

Cytokinin bioassay

The modified *Amaranthus* betacyanin bioassay (Biddington and Thomas, 1973) was used to measure endogenous cytokinin levels of tomato extracts. The bioassay was modified in our experiments as follows:

1. *Amaranthus caudatus* (Love Lies Bleeding-608D, Stokes Seeds, Inc., Buffalo, NY) were used for the betacyanin bioassay.
2. The explants were incubated in glass petri dish (9 cm i.d.) for 24 hr in dark on a single Whatman No. 1 filter paper with 2 ml phosphate buffer containing 1 mg/ml tyrosine and the test material.
3. Betacyanin pigment was extracted in 2 ml distilled water in a Potter-Elvehjem tissue grinder, centrifuged and the optical density of the supernatant measured at 537 and 620 nm.

The *Amaranthus* bioassay for cytokinins was chosen as a simple and accurate method for assaying the presence of cytokinins in the fractions obtained after liquid chromatography of the tomato extracts. The bioassay is based on the cytokinin-induced formation of betacyanin in *Amaranthus caudatus* explants in the presence of tyrosine in dark. It was found that the response of the explants to cytokinins was BAP > IPA > Zeatin > IPR > ZR.

Extraction procedure

The cytokinins were extracted from tomato fruits according to the procedure outlined in Figure 1.

Separation of cytokinins by liquid chromatography

The three tomato extracts as obtained by the extraction procedure were further purified. Each fraction was treated separately. The extract was evaporated to dryness (in vacuo at 40°C) extracted 3× with 80% methanol, evaporated to dryness and finally reconstituted in 15 ml, 35% ethanol. The partially purified ethanol extracts were separated on a Sephadex LH-20 column (32 cm × 2 cm i.d.) using ethanol at a flow rate of 0.66 ml/min (Armstrong et al., 1969). The eluent was monitored at 254 nm against 35% ethanol blank and 7.5 ml fractions collected. The fractions were evaporated (in vacuo at 45°C), dissolved in 2 ml phosphate buffer containing 1 mg/ml tyrosine and the cytokinin activity was determined using the *Amaranthus* bioassay.

Gas-liquid chromatography

Gas-liquid chromatography of cytokinin standards and "active" fractions was carried out on a Hewlett-Packard 402 gas chromatograph. Standard conditions for cytokinin analysis modified after Most et al. (1968) were: 2% OV-17 (on Chromosorb W-HP mesh 100–120) glass column (1.6 m/4 mm), isothermal at 250°C or temperature programmed 180–250°C at 10°C/min. after a 1 min. delay. Trimethyl silyl (TMS) derivatives were prepared by reacting the dried extract of cytokinin active fractions with BSA (25 μl), BSTFA (25 μl) and dry pyridine (100 μl) at 73°C for 1 hour in a reacti-vial.

Authors Desai and Chism are with the Dept. of Food Science & Nutrition, Ohio Agricultural Research and Development Center and The Ohio State University, Columbus, OH 43210.

0022-1147/78/0004-1324$02.25/0
© 1978 Institute of Food Technologists

RESULTS AND DISCUSSION

BY THE MODIFIED EXTRACTION procedure three fractions of the tomato extract are obtained, a basic n-butanol fraction, acidic n-butanol fraction and an aqueous acid fraction. These fractions will be referred to as basic fraction, acidic fraction and aqueous fraction, respectively. The naturally occurring cytokinins zeatin, ZR, IPA and IPR are extracted into the basic fraction (Carnes et al., 1975). Some cytokinins having glucose in the 7 or 9 positions on the purine ring are also extracted into the basic fraction. The acidic fraction would contain cytokinins having low pK_a values such as N-substituted purines with carboxylated side chain (Letham, 1973; Carnes et al., 1975). The cytokinin ribotides would normally remain in the aqueous fraction. Recovery of added BAP from the basic fraction by this procedure was 25%.

Table 1—Changes in cytokinin activity in basic, acidic and aqueous fractions from tomatoes at different stages of ripening measured by the Amaranthus bioassay correspond to BAP equivalents per 1000g fruit

Tomato ripening stage	Basic n-butanol fraction	Acidic n-butanol fraction	Aqueous fraction	Total cytokinin activity µg/1000g fruit
Mature green	9.1	0.50	0.75	10.35
"Breaker"	0.90	0.41	0.31	1.62
Ripe	0.06	0.05	0.05	0.16

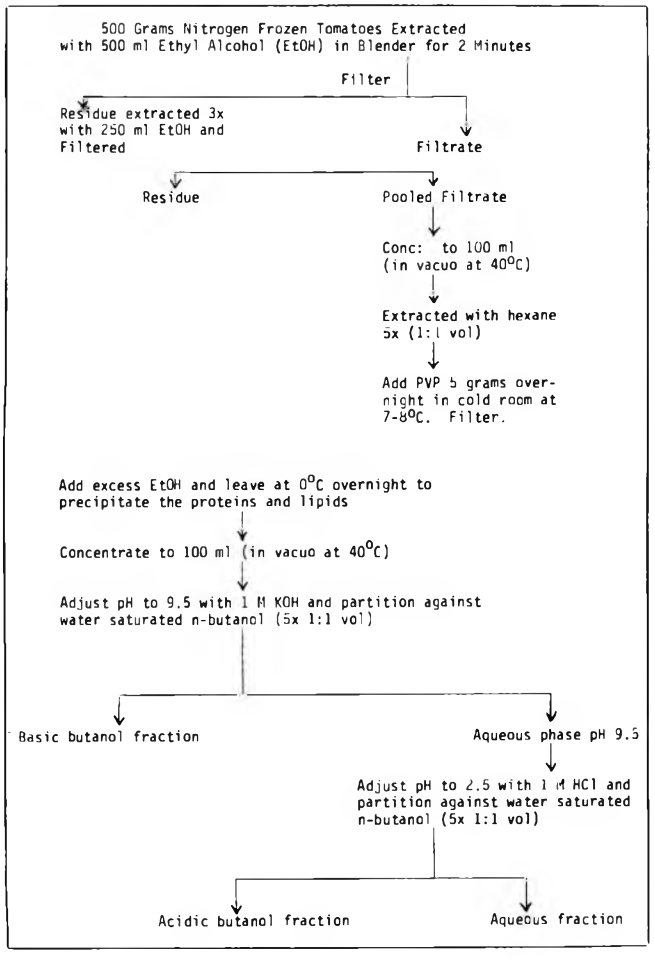


Fig. 1—Partition scheme for extraction of cytokinins of tomato fruit.

The total cytokinin activity declined dramatically during ripening of the fruits (Table 1). All three fractions—basic, acidic and aqueous—showed reduced cytokinin activity in the ripe fruits as compared to the mature green fruits, but the reductions were greatest in the basic fraction which contained about 90% of the cytokinin activity of the mature green fruits.

The chromatographic profiles and cytokinin activity of the basic fraction of the mature green, "breaker" and ripe fruit extracts are shown in Figure 2. As ripening proceeded there was a reduction in all of the zones of cytokinin activity present in the extracts of mature green fruits. A new zone of cytokinin activity appeared in the extracts of "breaker" fruits and was also present in the extracts of ripe fruits. The elution volume of this new zone was the same as that of IPR standards. The majority of the cytokinin activity in the extracts of mature green and "breaker" fruits was found where zeatin and ZR standards eluted.

Further analysis of the basic fraction by GLC and comparison of data with authentic standards indicated the presence of zeatin, ZR, IPA, and IPR-like compounds in the extracts of mature green tomato fruits. The presence of ZR was confirmed by GC-Mass spectroscopy.

Separation of the acidic fraction (Fig. 3) showed two zones

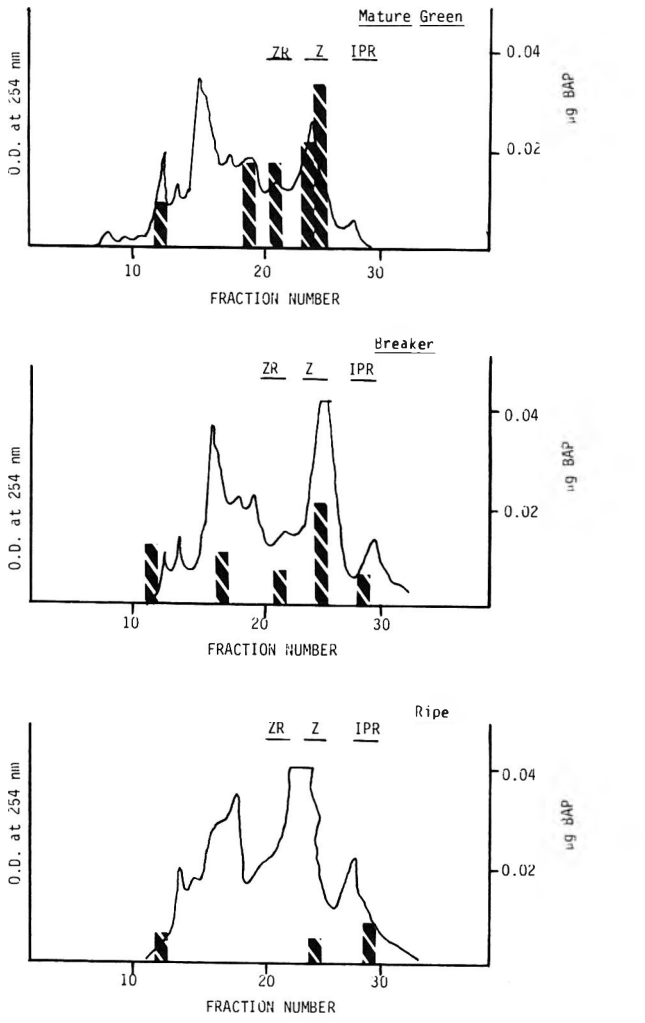


Fig. 2—Chromatograms of the basic butanol fraction of partitioned extracts of mature green, "breaker" and ripe tomato fruits on Sephadex LH-20. Bars indicate cytokinin activity in the Amaranthus bioassay. The elution zones for the following cytokinin standards: Zeatin (Z), zeatin riboside (ZR) and isopentenyl adenine riboside (IPR) are indicated.

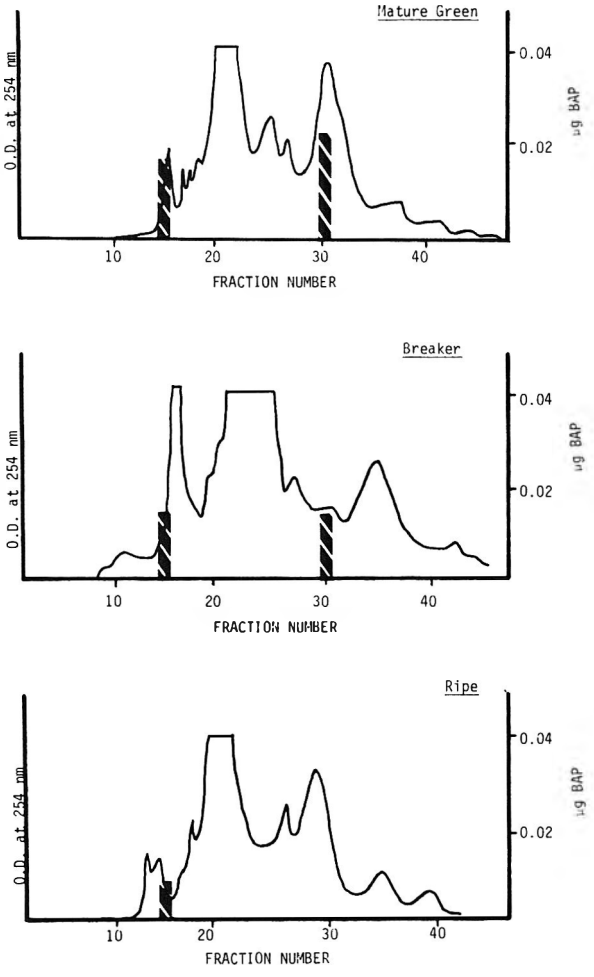


Fig. 3—Chromatograms of the acidic butanol fraction of partitioned extracts of mature green, "breaker" and ripe tomato fruits on Sephadex LH-20. Bars indicate cytokinin activity in the Amaranthus bioassay.

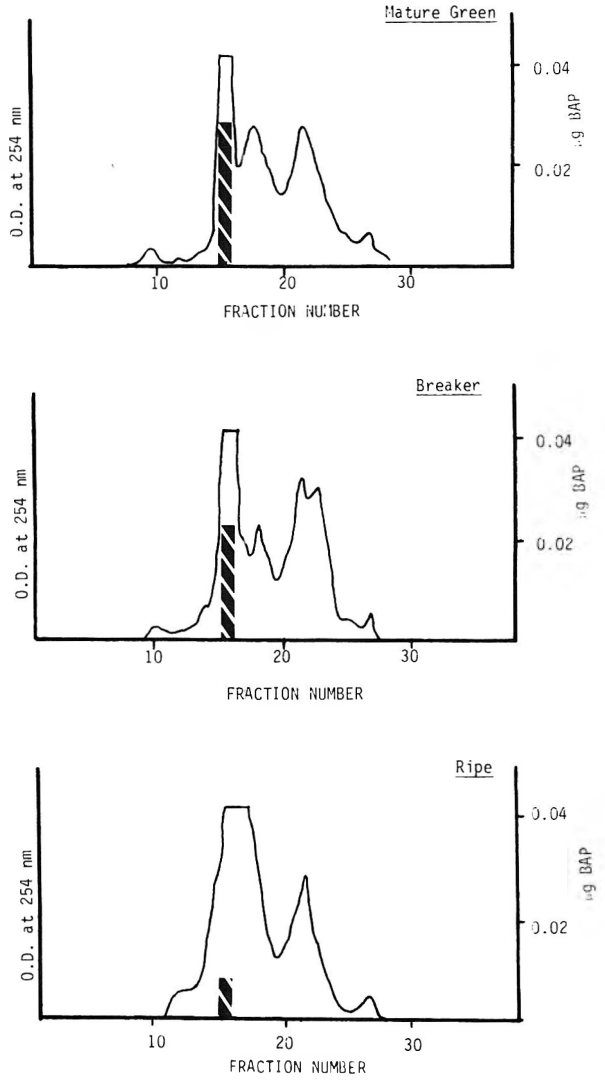


Fig. 4—Chromatograms of the aqueous fraction of partitioned extracts of mature green, "breaker" and ripe tomato fruits on Sephadex LH-20. Bars indicate cytokinin activity in the Amaranthus bioassay.

of cytokinin activity in extracts of mature green and "breaker" fruits but only a single zone in the extract of ripe fruits. Unlike the basic fraction, the most dramatic changes in cytokinin activity occurred after the "breaker" stage.

Separation of the aqueous fraction (Fig. 4) showed only a single zone of cytokinin activity that declined as the fruits ripened. This fraction should contain the ribotides of cytokinins. The cytokinin activity may be under-estimated because the bioassay is less sensitive to ribotides (Abdel-Rahman et al., 1975).

It is concluded from this study that the endogenous cytokinins present in the mature green tomato fruits decrease rapidly as the fruit ripens. Since cytokinins are involved in delaying senescence, these findings are consistent with the thesis that changes in cytokinins are involved in fruit ripening.

REFERENCES

Abdel-Kader, A.S., Leonard, L.M. and Maxie, E.C. 1966. Effect of growth-regulating substances on the ripening and shelf-life of tomatoes. HortScience 1: 90.

Abdel-Rahman, M., Thomas, T.H., Doss, D.J. and Howell, L. 1975. Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiol. Plant. 34: 39.

Armstrong, D.J., Burrows, W.J., Evans, P.K. and Skoog, G.F. 1969. Isolation of cytokinins from t-RNA. Biochem. Biophys. Res. Comm. 37: 451.

Biddington, N.L. and Thomas, T.H. 1973. A modified Amaranthus betacyanin bioassay for the rapid determination of cytokinins in plant extracts. Planta. 111: 183.

Carnes, M.G., Brenner, M.L. and Andersen, C.R. 1975. Comparison of reversed-phase high-pressure liquid chromatography with Sephadex LH-20 for cytokinin analysis of tomato root pressure exudate. J. Chromatography 108: 95.

Dilley, D.R. 1969. Hormonal control of fruit ripening. HortScience 4:11.

Frenkel, C., Klein, I. and Dilley, D.R. 1968. Protein synthesis in relation to ripening of some fruits. Plant Physiol. 43: 1146.

Hulme, A.C. 1972. The proteins of fruits: their involvements as enzymes in ripening. J. Food Technol. 7: 343.

Letham, D.S. 1973. Cytokinin from Zea mays. Phytochemistry. 12: 2445.

Mizrahi, Y., Dostal, H.C., McGlasson, W.B., and Cherry, J.H. 1975. Effects of abscisic acid and benzyladenine on fruits of normal and rin mutant tomatoes. Plant Physiol. 56: 544.

Most, B.H., Williams, J.C., and Parker, K.J. 1968. Gas chromatography of cytokinins. J. Chromatography 38: 136.

Pratt, H.K. 1975. The role of ethylene in fruit ripening. In "Facters et Regulation de la Maturation des Fruits." Centre National De La Recherche Scientifigue, Paris. 153.

Varga, A. and Bruinsma, J. 1974. The growth and ripening of tomato fruits at different levels of endogenous cytokinins. J. Hort. Sci. 49: 135.

Ms received 8/29/77; revised 11/27/77; accepted 12/1/77.

Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5-8, 1977.

Journal Article No. 132-77, Ohio Agricultural R&D Center. Supported in part by The Ohio State University Graduate School.

A Research Note

STORAGE LIFE OF PRE–CUT, REFORMED, VACUUM PACKAGED LAMB LOINS

J. D. TATUM, G. C. SMITH and Z. L. CARPENTER

ABSTRACT

Thirty-two lamb loins were cut into retail chops, the chops were stacked together to reform the original configuration of the primal cut and the pre-cut, reformed loins were vacuum packaged. The 32 pre-cut, reformed, vacuum packaged loins and 32 intact, vacuum packaged loins were transported-stored at 0–2°C for 7, 14, 21 or 28 days. Following storage, one retail chop from each of the 64 loins was displayed under retail conditions for 4 days. Chops from pre-cut, reformed loins stored for 14–28 days sustained greater ($P < 0.05$) surface discoloration and were less desirable ($P < 0.05$) in overall appearance than chops from intact loins. Chops from pre-cut, reformed loins stored for 21 or 28 days had a higher ($P < 0.05$) incidence of off-odor than chops from intact loins. If transport-storage intervals of 14 days or longer are needed to distribute lamb loins, pre-cutting of chops and distribution as reformed, vacuum packaged loins cannot be recommended.

INTRODUCTION

WITH THE IDENTIFICATION of more effective systems for maintaining fresh lamb quality during transport and distribution, more attention is now being focused on the implementation of centralized processing concepts. Presently, almost three-fourths of the lamb produced in the U.S. leaves the packer in whole carcass form, 11% as saddles and the remaining 15% as primals, although a 3-to-5 year projection indicates a trend toward the use of centralized breaking concepts (Dean and Wyckoff, 1975). Advantages frequently cited for centralized breaking include: (1) reduced transportation costs, (2) more efficient use of labor and by-products, (3) greater merchandising flexibility, (4) improved inventory control and (5) simplification of retail operations (Menzie, 1975; Smith, 1975; Volz and Marsden, 1963). A relatively recent concept involves the fabrication and packaging of retail cuts prior to distribution, thereby facilitating additional reductions in labor and equipment costs at the retail level. Correspondingly, the present investigation was conducted to determine the efficacy of pre-cutting, reforming and vacuum packaging lamb loins prior to distribution and subsequent retail display.

EXPERIMENTAL

SIXTY-FOUR WHOLE LAMB LOINS (from U.S. Choice, yield grade 2 or 3, 23–27 kg carcasses, selected 18–24 hr postmortem) were obtained at a commercial packing plant. Thirty-two of the loins (randomly selected) were cut into retail chops (3.2 cm in thickness) and chops (sawdust was not removed from cut surfaces) were stacked together to reform the original configuration of the primal cut prior to vacuum packaging; the remaining 32 loins were vacuum packaged (in Cryovac B-620 bags) in their intact form. The vacuum packaged loins were placed in cardboard containers and transported 1590 km in a refrigerated trailer with a thermostat setting of 0°C. Intransit temperature was monitored by placing continuous temperature recorders in 4 boxes of the loins; temperature in the cardboard containers ranged from −1 to 2°C during shipment. The loins were randomly assigned to one of four transport-storage intervals (7, 14, 21 or 28 days); upon arrival at the meat laboratory, loins were stored in a 0–2°C cooler for the appropriate periods of time. Time in storage after arrival at T.A.E.S. and cut form (pre-cut vs intact loins) were the variables studied; all other conditions of transport-storage (temperature, relative humidity, etc.) were identical for the 64 loins. At the end of each storage period, one chop was obtained from the interior of each loin, placed on a styrofoam backing board, wrapped with polyvinyl chloride film and observed under simulated retail display conditions. Loin chops were displayed for a period of 4 days at 0–2°C under illumination with 883 lux of incandescent light for 12 hr during each day.

Subjective evaluations of the retail chops were made daily by a trained, 3-member panel. Scores assigned to the retail loin chops included surface discoloration (scored by use of a 7-point scale; 7 = no surface discoloration, 1 = complete surface discoloration), odor (scored by use of a 4-point scale; 4 = no detectable off-odor, 1 = extreme off-odor) and overall appearance (scored by use of an 8-point scale; 8 = extremely desirable, 1 = extremely undesirable).

Samples for aerobic plate counts were obtained at 0 (immediately after sawing), 7, 14 and 21 days of storage from the anterior longissimus muscle surfaces of 16 loins (8 intact and 8 pre-cut, reformed loins). Procedures for obtaining microbial counts and for diagnostics were identical to those described by Seideman et al. (1976). Data were analyzed using analysis of variance (Snedecor and Cochran, 1967); mean separation analyses were performed using the Duncan (1955) Multiple Range Test.

Table 1—Comparison of mean values for surface discoloration, off-odor incidence and overall appearance of loin chops from intact vs pre-cut, reformed loins

Trait	Storage interval (days)	Day of retail display	Wholesale lamb loin[d]	
			Intact	Pre-cut, reformed
Surface discoloration[a]	7	4	5.3d	5.3d
Surface discoloration[a]	14	4	5.6d	4.3ef
Surface discoloration[a]	21	4	5.6d	4.8de
Surface discoloration[a]	28	4	4.7e	4.1f
Off-odor incidence[b]	7	4	3.8d	3.8d
Off-odor incidence[b]	14	4	2.9e	3.6d
Off-odor incidence[b]	21	4	4.0d	3.1e
Off-odor incidence[b]	28	4	3.0e	2.4f
Overall appearance[c]	7	4	5.7d	5.5d
Overall appearance[c]	14	4	5.5d	3.9e
Overall appearance[c]	21	4	5.3d	4.0e
Overall appearance[c]	28	4	4.6e	4.2e

[a] Means based on a 7-point scoring system (7 = no surface discoloration, 4 = 50% surface discoloration, 1 = complete surface discoloration).

[b] Means based on a 4-point scoring system (4 = no detectable off-odor, 3 = slight off-odor, 1 = extreme off-odor).

[c] Means based on an 8-point scoring system (8 = extremely desirable, 5 = slightly desirable, 1 = extremely undesirable).

[d] Means in the same column and for the same trait followed by the same letter are not different ($P > 0.05$). Means in the same row underscored by a common line do not differ ($P > 0.05$).

RESULTS

A COMPARISON of ratings for surface discoloration, odor incidence and overall appearance of retail chops from intact loins and pre-cut, reformed loins is presented in Table 1. Among loins stored for periods of 14 days or longer, chops

—Continued on page 1329

Authors Tatum, Smith and Carpenter are with the Meats & Meat Chemistry Section, Dept. of Animal Science, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843.

0022-1147/78/0004-1327$02.25/0
© 1978 Institute of Food Technologists

RESEARCH NOTES

A Research Note

EFFECT OF VARIOUS SELECTIVE AGARS ON THE GROWTH OF RARE AND COMMON SALMONELLAE

DAVID L. KRONINGER and GEORGE J. BANWART

ABSTRACT

The growth of four serotypes of salmonellae was determined on various selective and differential agars. Statistical analysis was used to compare the data. Generally, there was significantly less growth on the selective agars as compared to plate count agar. Two of the four serotypes revealed significantly less growth on Salmonella-Shigella (SS) agar than on any of the other agars tested. On some selective media, one or two of the rare serotypes were inhibited as compared to *S. typhimurium*. However, on three of the selective agars tested, *S. typhimurium* was inhibited to a greater extent than at least one of the rare serotypes. It does not seem that selective agars are involved in the lack of isolation of rare salmonella serotypes.

INTRODUCTION

ACCORDING TO THE Center for Disease Control there are over 1700 known serotypes of *Salmonella* (U.S. Dept. HEW 1976). Of these serotypes, comparatively few are involved in outbreaks of salmonellosis. The reason has not been explained for the overwhelming presence of certain serotypes while other serotypes are isolated rarely after their first detection.

If, in fact, the serotypes are different, perhaps the rarer types do not grow as readily as common serotypes in the selective media used in *Salmonella* methodology. The growth on selective agars is necessary for the detection and isolation of these microorganisms. Therefore, the growth of rare serotypes was compared with a common serotype using selective agar media.

MATERIALS & METHODS

Microorganisms

The three rare serotypes used were *Salmonella coeln* (CDC 751-75), *S. singapore* (CDC 755-75), and *S. goettingen* (CDC 1376-75) obtained from the Center for Disease Control, Atlanta, GA. A culture of the most prevalent serotype, *S. typhimurium*, was obtained from the culture collection of The Ohio State University.

Agars

Plate count (PC) agar (Difco) was used as the nonselective agar. The selective agars tested were brilliant green (BG) agar (Difco), brilliant green sulfa (BGS) agar (BBL), MacConkey (MC) agar (Difco), bismuth sulfite (BS) agar (Difco), Salmonella-Shigella (SS) agar (Difco), desoxycholate citrate (DC) agar (Difco), Hektoen enteric (HE) agar (Pfizer), and selenite brilliant green sulfa (SBGS) broth (BBL) plus 1.5% agar for solidification.

Inoculum and test

The salmonellae were grown in sterile nutrient broth (Difco) at 37°C for 24 hr. Growth in the broth was diluted to 300–3,000 cells/ml with sterile 0.1% peptone water and 0.1 ml was spread plated onto solidified surfaces of the agars with the aid of a glass hockey stick. The inoculated agars were incubated at 37°C for 24 hr and the colonies counted with the aid of a Quebec colony counter. Three separate trials were completed, each with duplicate plates. Hence, the mean colony counts are averages of the six plates involved in each of the tests.

Statistical analysis

An analysis of variance was used to detect the homogeneity of the treatment means. Duncan's multiple range and multiple F test were the specific tests used to determine significant differences among the means (Duncan, 1955).

RESULTS & DISCUSSION

THE MEAN COLONY COUNTS of the growth of the salmonellae on the agars is shown in Table 1. A statistical analysis, relating the variation of the means, was used to detect any significant differences among the organisms on the nine media, and within the media for each individual organism. Table 1 shows the statistical differences which existed among the media, and among the organisms. The horizontal subscripts indicate possible differences between the media while the superscripts in the vertical columns indicate differences within the four salmonellae. The statistical differences were determined at the 5% level of significance.

The effects exerted by the ten media, individually, on the salmonellae varied from medium to medium. On plate count agar and desoxycholate citrate agar, there were no significant differences in growth of the four salmonellae at the 5% level. Only on SS agar did the most common serotype, *S. typhimurium*, produce significantly more colonies than the three other serotypes. With the other six media, all four serotypes, at one time or another were more seriously affected than one or more of the other organisms, but there was no definite pattern showing differences among the serotypes. These observations and the statistical analyses showed only slight differences between the organisms on any of the media. Thus, the ability to grow on selective agars does not seem to be the reason that rare serotypes are not isolated more frequently.

Growth of the four salmonellae individually on the ten media showed varying degrees of the selective effect by the media. Plate count agar had the highest means with all the serotypes. Brilliant green agar and BGS agar were significantly different only for *S. singapore*. For these organisms, BG agar was significantly different from PC agar. Salmonella-Shigella agar was nearly always the most selective medium. The other media varied in their selectivity from organism to organism. Similar results have been recorded in the literature. Banwart and Ayres (1953) found that SS agar was the most selective of the agars used in the isolation of *Salmonella*. Osborne and Stokes (1955), Banwart and Ayres (1953) and Moats and Kinnear (1974) reported that BG agar gave maximum recovery and minimum inhibition of *Salmonella*.

SUMMARY

ONLY SLIGHT GROWTH differences were observed among the four serotypes on the various selective agars used in this study. Except for SS agar, the rare serotypes were not inhib-

Authors Kroninger and Banwart are with the Dept. of Microbiology, The Ohio State University, Columbus, OH 43210.

0022-1147/78/0004-1328$02.25/0
© 1978 Institute of Food Technologists

Table 1—Mean values[1] of four serotypes of Salmonella grown in ten selective agar media

Organism	Agars used								
	PC[2]	BG	BGS	MC	BS	SS	SBGS	HE	DC
S. typhimurium	404_{a}[3]	$^{a}124_{b}$	$^{a}160_{b}$	$^{b}21.5_{d,c}$	$^{b}76.2_{c}$	$^{a}20.8_{d,e}$	$^{b}16.5_{e}$	$^{a}39.0_{c,d}$	$22.3_{d,e}$
S. singapore	211_{a}	$^{b}58.3_{b}$	$^{a}158_{a}$	$^{a}73.8_{b}$	$^{b}56.8_{b}$	$^{b}7.67_{d}$	$^{b}28.0_{c}$	$^{a}42.2_{b,c}$	$40.2_{b,c}$
S. goettingen	382_{a}	$^{a}140_{b,c}$	$^{a}232_{a,b}$	$^{a}81.7_{c,d}$	$^{a}131_{b,c}$	$^{b}7.42_{g}$	$^{a}56.7_{d,e}$	$^{b}14.0_{f}$	35.5_{e}
S. coeln	343_{a}	$^{a}92.8_{b}$	$^{b}62.7_{b,c}$	$^{b}32.7_{c}$	$^{a,b}87.7_{b}$	$^{b}7.81_{f}$	$^{b}16.2_{d,e}$	$^{b}12.1_{e,f}$	$30.3_{c,d}$

[1] Mean values listed are the average of the colony forming units on six plates, using three replications, each with duplicate plates. The numbers are the colonies counted equivalent to the 10^{-7} dilution.

[2] The selective media used are as follows: PC (plate count), BG (brilliant green), BGS (brilliant green sulfa, MC (MacConkey), BS (bismuth sulfite), SS (Salmonella-Shigella), SBGS (selenite brilliant green sulfa), HE (Hektoen enteric), DC (desoxycholate citrate).

[3] Subscripts (lower right of mean value) in horizontal rows indicate significant differences in the media. Superscripts (upper left of mean value) vertical columns indicate significant differences among the organisms (at the 5% level of significance), for a particular medium. Where no superscript is present, no statistical differences were found.

ited to a greater extent than was the common serotype, *S. typhimurium*. Statistical analysis revealed that differences did exist within the media and within the organisms.

REFERENCES

Banwart, G.J. and Ayres, J.C. 1953. Effect of various enrichment broths and selective agars upon the growth of several species of Salmonellae. Appl. Microbiol. 1: 296.

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

Moats, W.A. and Kinner, J.A. 1974. Factors affecting selectivity of brilliant green-phenol red agar for salmonellae. Appl. Microbiol. 27: 118.

Osborne, W.W. and Stokes, J.L. 1955. A modified selenite brilliant green medium for the isolation of Salmonella from egg products. Appl. Microbiol. 3: 295.

U.S. Dept. of Health, Education & Welfare, Public Health Service. 1976. "Salmonella Surveillance Annual Summary, 1975." Center for Disease Control, Atlanta, GA.

Ms received 11/4/77; revised 2/11/78; accepted 2/15/78.

PRE-CUT, REFORMED LAMB LOINS . . . From page 1327

from pre-cut, reformed loins sustained significantly ($P < 0.05$) greater surface discoloration than did chops from intact loins.

Chops from pre-cut, reformed loins stored for 21 or 28 days had a higher ($P < 0.05$) incidence of off-odor than chops from intact loins. Chops from pre-cut, reformed loins were significantly less desirable in overall appearance than were chops from intact loins stored for 14, 21 or 28 days. Chops from pre-cut, reformed loins stored for 14 days or longer had overall appearance ratings indicative of unacceptability to consumers following 4 days of retail display. General increases in off-odor incidence and decreases in overall appearance were observed as storage time increased for both intact and pre-cut loins, but the decline in ratings was greater for pre-cut loins than for intact loins.

Aerobic plate counts (per 6.45 sq cm) were 4×10^2 on day 0, 7×10^2 on day 7, 8×10^3 on day 14 and 3.4×10^4 on day 21; counts did not differ between intact and pre-cut, reformed loins. Initially (day 0), *Microbacterium* and *Corynebacterium* species predominated; after 21 days of storage *Lactobacillus* and *Moraxella-Acinetobacter* species predominated.

DISCUSSION

ACCORDING TO Dean and Wyckoff (1975), distribution cycles for fresh lamb in the U.S. range from 1 to 10 days, with the majority of lamb reaching the consumer 5 to 7 days following slaughter. Results of the present investigation indicate that vacuum packaged, pre-cut, reformed loins can be stored for 7 days without substantial reductions in product quality. Transport-storage of pre-cut, reformed lamb loins for 14 days or longer, however, resulted in loin chops which were unacceptable in appearance and odor following 4 days of retail display. Consequently, if extended transport-storage intervals are anticipated, the use of centralized retail fabrication involving interim storage of pre-cut, reformed lamb loins in vacuum packages cannot be recommended.

REFERENCES

Dean, B. and Wyckoff, G. 1975. A survey of industry opinion on lamb marketing. Proc. Lamb Marketing Seminars, p. 11, American Sheep Producers Council, Inc. Denver, CO.

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

Menzie, E.L. 1975. Changes in meat merchandising. Proc. Meat Ind. Res. Conf., p. 19. American Meat Institute, Washington, DC.

Seideman, S.C., Vanderzant, C., Hanna, M.O. Carpenter, Z.L. and Smith, G.C. 1976. Effects of various types of vacuum packages and length of storage on the microbial flora of wholesale and retail cuts. J. Milk Food Technol. 39: 745.

Smith, G.C. 1975. A comparison of five marketing and distribution systems for lamb-technology, costs and storage life. Proc. Lamb Marketing Seminars, p. 49. American Sheep Producers Council, Inc., Denver, CO.

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

Volz, M.D. and Marsden, J.A. 1963. Centralized processing of fresh meat for retail stores. USDA Marketing Research Report No. 628. Washington, DC.

Ms received 11/4/77; revised 2/4/78; accepted 2/8/78.

Technical Article 13805, Texas Agricultural Experiment Station This study was supported in part by The Natural Fibers & Food Protein Commission of Texas and Monfort of Colorado, Inc.

A Research Note

AFTEREFFECT OF SUBMERSION IN WATER ON GREENING AND GLYCOALKALOID FORMATION OF POTATO TUBERS

M. T. WU and D. K. SALUNKHE

ABSTRACT

Treatment with submersion in water prior to light exposure effectively inhibited the light-induced greening and glycoalkaloid formation of potato tubers of three cultivars tested. The extent of inhibition depended mostly on the duration of submersion treatment up to 20 days and less on the temperature and the cultivar of tubers. Treatment resulted in more inhibition in formation of glycoalkaloid than that of chlorophyll formation. Submersion treatment of potato tubers prior to wounding also inhibited the wound-induced glycoalkaloid formation of the tuber tissue. Incorporation of low concentration of sodium hypochlorite in the soaking water effectively retarded the microbial population and consequently retarded the microbial decay of potato tubers.

INTRODUCTION

GLYCOALKALOIDS which are potent cholinesterase inhibitors occur in potato tubers in various amounts depending upon the variety, stage of development and environmental conditions. Light and mechanical injury are the two major postharvest environmental factors which stimulate glycoalkaloid formation of potato tubers (Gull and Isenberg, 1958, 1960; Forsyth and Eaves, 1968; Wu and Salunkhe, 1972a, b, 1976b; Salunkhe et al., 1972). Recently, we found that temporary anoxia created by submerging potato tubers in water completely inhibited light-induced greening and glycoalkaloid formation of three cultivars of potato tubers (Wu and Salunkhe, 1977). We also observed that treatments had some residual effect on tuber greening even after removal from water. This investigation was conducted to study the extent of after effect of submerging water treatment on photoinduction of greening and glycoalkaloid formation and wound-induced glycoalkaloid formation of three cultivars of potato tubers. Incorporation of sodium hypochlorite in the soaking water for reducing microbial population and consequently tuber deterioration were also reported.

MATERIALS & METHODS

RUSSET BURBANK, Pontiac and White Rose cultivars were used for the study. Potato tubers with uniform size (150g ± 10g) and without any mechanical and pathological injuries were selected. There were 14 tubers for each treatment. Treatments were conducted by submerging potato tubers in water in plastic cups (7.5 cm ht, 10 cm diam) with or without 200 ppm NaOCl at 4° and 13°C for 2–20 days in the absence of light. The surface of water was always maintained about 1 cm above the top of the tubers. A preliminary experiment was conducted to determine the approximate duration at different temperatures under which potato tubers could tolerate submerging treatment. Tubers were removed from water periodically and exposed to white fluorescent light (200 ft-c) at 13°C and 80% R.H. for 8 days. Completely randomized block design was employed to arrange tubers for light exposure. Control tubers were directly exposed to light for the same duration as treated but without prior submerging treatment. After light exposure, tubers were withdrawn and peels of light exposing part were removed for chlorophyll and total glycoalkaloid analysis.

For the study of the aftereffect of submerging treatment on wound-induced glycoalkaloid formation, tubers were incubated in water with 200 ppm NaOCl at 4°C for 10 and 20 days in the absence of light. Tubers were then peeled and cut into cubes (0.5 × 0.5 × 0.5 cm) and incubated at 13°C for 3 days in the dark. After incubation, potato cubes were frozen for total glycoalkaloid determination.

Total chlorophylls were determined by the method of AOAC (1965). The Gull and Isenberg method (1960) was used for glycoalkaloid extraction and determination. An automatic shaking device with constant volume pipettes was used for the color reaction in total glycoalkaloid determination (Wu and Salunkhe, 1976a). Triplicate analyses were conducted. The content of total glycoalkaloids was expressed on a fresh weight basis. Analysis of variance was made and means were compared according to Tukey ω-procedure (Steel and Torrie, 1960).

For determining the effect of incorporation of 200 ppm sodium hypochlorite on the viable microbial counts of the soaking water for potato tubers, both control (without NaOCl) and NaOCl added soaking water were withdrawn periodically for viable microbial counts. This was done by plating 1 ml of the serial dilution of soaking water in Trypticase Soy Agar (Baltimore Biological Laboratories, Cockeysville, MD) and counting the colonies present after aerobic incubation at 37°C for 48 hr (Dotson et al., 1977). Triplicate analyses were performed for each sample.

RESULTS & DISCUSSION

THE EXTENT of aftereffect of submerging water treatment on light-induced greening and glycoalkaloid formation of potato tubers depended mostly upon the duration of treatment and less on the temperature and the cultivar of the tubers (Table 1). Treatments prior to light exposure all significantly reduced light-induced formation of chlorophylls and glycoalkaloid of all three cultivars when compared with the controls. For Russet Burbank cultivar treatment at 4°C for 2 days resulted in 48% inhibition in chlorophyll formation and 65% inhibition in glycoalkaloid formation. For White Rose cultivar, same treatments resulted in 17% and 61% inhibition in chlorophyll and glycoalkaloid formation, respectively. Similar results have also been shown on Pontiac cultivar. Increasing the duration of submerging treatment resulted in more inhibition in both chlorophyll and glycoalkaloid formation. It is interesting to note that in most cases, the same treatment resulted in more inhibition in the formation of glycoalkaloid than that of chlorophyll. For Russet Burbank cultivar, submerging treatment of a minimum of 5 days was necessary for effective control of glycoalkaloid formation, while a minimum of 10 days was needed for both White Rose and Pontiac cultivars. Submerging treatments at elevated temperature (13°C) had less inhibition on both glycoalkaloid and chlorophyll formation when compared with the same treatment at 4°C. The addition of sodium hypochlorite in the soaking water had slight but no consistant effect on chlorophyll and glycoalkaloid formation of tubers.

Submersion water treatments also had extended inhibitory effect on wound-induced glycoalkaloid formation of potato tuber tissue (Table 2). Submersion water treatment of three cultivars of potato tubers for 10 days prior to wounding result-

Authors Wu and Salunkhe are with the Department of Nutrition & Food Science, Utah State University, Logan, UT 84322.

0022-1147/78/0004-1330$02.25/0
© 1978 Institute of Food Technologists

Table 1—Aftereffect of submersion water treatment at various durations on light-induced greening and glycoalkaloid formation on three cultivars of potato tubers. After submersion water treatment, tubers were exposed to 200 ft-c light at 13° C and 80% RH for 8 days

Treatment	Submersion temp (°C)	Russet Burbank		White Rose		Pontiac	
		Chlorophylls mg/100g peels	Total glycoalkaloids mg/100g peels	Chlorophylls mg/100g peels	Total glycoalkaloids mg/100g peels	Chlorophylls mg/100g peels	Total glycoalkaloids mg/100g peels
Zero time	–	0.113**	21.99**	0.180**	19.33**	0.230**	30.69**
Control	–	3.815	40.38	4.554	37.65	3.119	52.28
2 days submersion	4	2.033**	28.45**	3.833**	26.39**	2.442**	36.65**
5 days submersion	4	1.843**	22.84**	3.656**	25.44**	1.249**	36.20**
10 days submersion	4	0.665**	22.09**	0.666**	21.96**	0.336**	37.58**
20 days submersion	4	0.438**	22.36**	0.432**	19.78**	0.304**	32.69**
2 days submersion (200 ppm NaOCl)	4	2.364**	26.31**	3.690**	27.71**	2.533**	36.00**
5 days submersion (200 ppm NaOCl)	4	2.019**	23.09**	3.524**	26.93**	1.306**	36.24**
10 days submersion (200 ppm NaOCl)	4	0.758**	22.68**	0.775**	22.38**	0.278**	33.78**
20 days submersion (200 ppm NaOCl)	4	0.475**	22.50**	0.334**	21.05**	0.234**	31.90**
5 days submersion	13	2.384**	22.45**	3.386**	28.46**	1.336**	34.84**
2 days submersion (200 ppm NaOCl)	13	3.145**	29.33**	3.918**	29.44**	2.738**	35.52**
5 days submersion (200 ppm NaOCl)	13	2.667**	22.79**	3.406**	27.30**	1.544**	34.39**
10 days submersion (200 ppm NaOCl)	13	0.885**	22.74**	0.748**	22.18**	0.258**	33.86**

** Significantly different from control at 1% level

ed in 80–82% inhibition of wound-induced glycoalkaloid formation, while 20 day anoxia treatment resulted in 97–100% inhibition.

Incorporation of 200 ppm sodium hypochlorite in the soaking water effectively retarded the microbial population. Microbes from the surface of potato tubers were the major microbial source in the soaking water. Without sodium hypochlorite, rapid increases in microbial population took place especially at elevated temperature. With 200 ppm sodium hypochlorite in the soaking water, no microbial count could be detected even after 20 days at 4°C. Addition of sodium hypochlorite in the soaking water retarded microbes and consequently retarded the microbial decay of potato tubers.

Submersion water treatment has been previously shown to inhibit sugar accumulation of postharvest potato tubers (Samotus, 1971). Under anaerobic condition, potato tubers has been reported to accumulate lactic acid (Baker and El Saifi, 1953). Submersion water treatment resulted in anaerobic respiration of potato tubers and the accumulated metabolite(s) through anaerobic respiration of tuber tissues as lactic acid may account for the later inhibition in light-induced chlorophyll and glycoalkaloid formation in the potato tubers.

Table 2—Aftereffect of submersion water treatment at 4° C on wound-induced glycoalkaloid formation of potato tuber tissue[a]

Cultivar	Submersion treatment	Total glycoalkaloid mg/100g
Russet Burbank	Zero time	0.36**
	Control	12.61
	10 days submersion	2.58**
	20 days submersion	0.39**
Pontiac	Zero time	0.32**
	Control	15.02
	10 days submersion	3.26**
	20 days submersion	0.81**
White Rose	Zero time	0.44**
	Control	11.18
	10 days submersion	2.30**
	20 days submersion	0.66**

[a] After submersion water treatment, cut cubes were incubated at 13° C, in the absence of light.

** Significantly different from control at 0.01

REFERENCES

AOAC. 1965. "Methods of Analysis," 10th ed. Assoc. Official Agr. Chemists, Washington, D.C.

Barker, J. and El Saifi, A.F. 1953. Studies in the respiratory and carbohydrate metabolism of plant tissue. 3. Experimental studies of the formation of carbon dioxide and of the changes in the lactic acid and other products in potato tubers in air following anaerobic conditions. Proc. Roy. Soc. (London) series 140: 508.

Doston, C.R., Frank, H.A. and Cavaletto, C.G. 1977. Indirect methods as criteria of spoilage in tofu (soybean curd). J. Food Sci. 42: 273.

Forsyth, F.R. and Eaves, C.A. 1968. Greening of potatoes: CA cure. Food Technol. 22: 48.

Gull, D.D. and Isenberg, F.M. 1958. Light burn and off-flavor development in potato tubers exposed to fluorescent light. Proc. Amer. Soc. Hort. Sci. 71: 446.

Gull, D.D. and Isenberg, F.M. 1960. Chlorophyll and solanine content and distribution in four varieties of potato tubers. Proc. Amer. Soc. Hort. Sci. 75: 545.

Salunkhe, D.K., Wu, M.T. and Jadhav, S.J. 1972. Effects of light and temperature on the formation of solanine in potato slices. J. Food Sci. 37: 969.

Samotus, B. 1971. Storage of potato tubers under water. Preliminary investigation. Potato Res. 14: 145.

Steel, R.G.D., and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., New York, NY.

Wu, M.T. and Salunkhe, D.K. 1972a. Control of chlorophyll and solanine synthesis and sprouting of potato tubers by hot paraffin wax. J. Food Sci. 37: 629.

Wu, M.T. and Salunkhe, D.K. 1972b. Inhibition of chlorophyll and solanine formation and sprouting of potato tubers by oil dipping. J. Amer. Soc. Hort. Sci. 97: 614.

Wu, M.T. and Salunkhe, D.K. 1976a. Devices for improving color development in glycoalkaloid determination. J. Food Sci. 41: 220.

Wu, M.T. and Salunkhe, D.K. 1976b. Changes in glycoalkaloid content following mechanical injuries to potato tubers. J. Amer. Soc. Hort. Sci. 101: 329.

Wu, M.T. and Salunkhe, D.K. 1977. Influence of temporary anoxia by submerging in water on light-induced greening and glycoalkaloid formation of potato tubers. J. Food Biochem. 1: 275.

Ms received 9/17/77; revised 11/21/77; accepted 12/1/77.

This research was supported by Grant FD-00683-03 from the Office of Research Grants, Food and Drug Administration.

A Research Note

ISOLATION OF NONPATHOGENIC CLOSTRIDIA FROM LIQUID CYCLONE PROCESSED COTTONSEED FLOUR

STEPHEN D. GOODWIN and JOHN P. CHERRY

ABSTRACT

Liquid cyclone processed cottonseed flour was shown not to be contaminated with *Clostridium perfringens*, but did contain two culturally similar nonpathogenic microorganisms. Data show that, if cottonseed products are positive for *Clostridium perfringens* using screening procedures recommended by the Food & Drug Administration, they should be tested further for lecithinase activity, hemolysis of blood agar, gelatin liquefaction, and fermentation of arabinose, mannitol, and xylose to accurately identify the microorganisms.

INTRODUCTION

THE ROLE of *Clostridium perfringens* as an etiologic agent of food poisoning is well documented (Hobbs et al., 1953; McClung, 1945). One of the presently accepted methods for the enumeration of *C. perfringens* is outlined in a publication of the Food and Drug Administration (FDA, 1976). According to this publication, any organism that is a nonmotile, gram-positive, obligately anaerobic, spore-forming bacillus, capable of reducing nitrate to nitrite, produces acid and gas from lactose, and liquefies gelatin, is provisionally identified as *C. perfringens*.

The liquid cyclone process (LCP) has made it possible to produce edible grade high protein cottonseed flour (Gardner et al., 1976). Each lot of LCP cottonseed flour is routinely examined for microbial contamination with emphasis on food associated pathogens and organisms that indicate improper sanitation, such as *C. perfringens, Salmonella*, and fecal coliforms. During these analyses, organisms that did not reduce nitrate to nitrite but resembled *C. perfringens* in all other respects according to the FDA (1976) recommended procedures were found in some samples. In order to more completely characterize these bacteria, samples of flour that yielded nitrate-negative clostridia during routine screening were re-examined. This communication describes the evaluation of LCP cottonseed flour for contamination by *C. perfringens*, and suggests that additional tests should be employed along with presently recommended methods (FDA, 1976) when screening for the presence of *C. perfringens* in foods that contain cottonseed products.

EXPERIMENTAL

THREE SAMPLES of LCP cottonseed flour, prepared aseptically under pilot plant conditions at Southern Regional Research Center (SRRC), were screened for *Clostridium* contamination. A nitrate-negative *Clostridium* sp., isolated during routine examination of an earlier processed SRRC LCP cottonseed flour (SRRC isolate) and maintained in thioglycollate medium, was also evaluated. Twenty-five gram samples were aseptically removed from each of the LCP cottonseed flours and placed in sterile pint-size mason jars containing 225 ml of 0.3 mM phosphate buffer. Colony counts (Table 1) were obtained by mixing a 5.0 ml aliquot with 25 ml of molten (50°C) Trypticase Sulfate Neomycin (TSN) agar which was then poured into a petri plate and allowed to solidify. Prior to incubation, all plates were overlaid with an additional 5.0 ml of TSN agar.

Authors Goodwin and Cherry are with the USDA Oilseed & Food Laboratory, Southern Regional Research Center, SEA, P.O. Box 19687, New Orleans, LA 70179.

Table 1—Anaerobic organisms[a] from LCP cottonseed flours[b]

Sample	Black colonies[c]	Total colonies[c]
1	38	65
2	114	145
3	30	95

[a] The SRRC isolate produced only black colonies on TSN agar under anaerobic conditions.
[b] Colony count data listed as per gram of LCP cottonseed flour.
[c] Represents the average of three separate determinations.

Two additional aliquots from each flour suspension and 1.0 ml of thioglycollate stock-culture of the SRRC isolate were inoculated into duplicate tubes containing 15.0 ml of cooked meat medium (Difco). All incubations were carried out in a Gas Pak Anaerobic Jar for 24 hr at 45°C. Broth from tubes of cooked meat medium showing vigorous gas production was streaked onto TSN medium. Pure cultures of possible *C. perfringens* were obtained by repeated restreaking selected black colonies onto new TSN agar plates. After seven successive serial platings, homogenous cultures of small black colonies, approximately 1.5–3.0 mm in diameter, were obtained. Two colonies were selected from each plate representing an LCP cottonseed flour (stock cultures 1 and 2, Table 2), and a single colony picked from a plate inoculated with the SRRC isolate. These colonies were placed into cooked meat medium, incubated at 45°C for 24 hr in the Gas Pak Anaerobe Jar, then stored at room temperature as stock cultures. Differential media used in this study was prepared by methods listed in the Difco manual (*Difco Manual*, 1953); egg yolk agar was prepared according to the procedure of the Food & Drug Administration (1976).

RESULTS & DISCUSSION

MICROSCOPIC EXAMINATION of the stock cultures used in this study revealed homogenous populations of gram-positive rods, most of which contained subterminal spores. The results summarized in Table 1 indicate that each flour sample yielded similar levels of contamination on anaerobic TSN agar, with the number of black colonies (presumptive *C. perfringens*; Marshall et al., 1965) representing from 31% to 79% of the total colonies.

Inoculations from each stock-culture were made into the differential media listed in Table 2. In addition to the results shown, each isolate produced stormy fermentation in iron milk, was negative for indole production, and grew well in cooked meat medium but failed to hydrolyze the meat particles. The presumptive *C. perfringens* organism isolated from LCP cottonseed flour did not reduce nitrate to nitrite (Table 2). However, this test alone should be considered inadequate to rule out the presence of *C. perfringens* since reference manuals have shown that nitrate reduction by this organism is variable (Buchanan and Gibbons, 1974; Holdeman and Moore, 1972). Further testing showed that the organisms isolated from LCP cottonseed flour were motile, did not produce β-hemolysis on anaerobic blood agar, and slowly developed lecithinase activity on egg yolk agar, unlike that of *C. perfringens* (Table 2).

The gelatin liquefication and carbohydrate fermentation patterns shown in Table 2 indicate that the organisms isolated from LCP cottonseed flours 2 and 3 and the SRRC isolate were similar to those for *C. acetobutylicum* (Buchanan and Gibbons, 1974). This nonpathogenic organism is typically found in soil and is associated with most plant material. The

Table 2—Culture[a] test characteristics of microorganisms

Tests[b]	LCP cottonseed flours						SRRC Isolate[c]	*C. perfringens*[d]	*C. acetobutylicum*[d]	*C. fallax*[d]
	1		2		3					
	Stock cultures									
	1	2	1	2	1	2				
Motility	+	+	+	+	+	+	+	–	+	+
Nitrate reduction	–	–	–	–	–	–	–	V	–	–
Gelatin liquefaction	–	–	+	+	+	+	+	+	+	–
Blood agar reaction:										
Anaerobic	α	α	–	α	–	α	–	β	–	β
Aerobic	–	–	–	–	–	–	–	–	–	–
Lecithinase activity										
on egg yolk agar	+	+	+	+	–	+	+	+	–	–
Carbohydrate fermentation:										
Arabinose	AG	A	AG	A	A	A	A	–	AG	–
Esculin	A	A	A	A	A	A	A	–	V	AG
Fructose	AG	AG	AG	AG	AG	A	AG	AG	AG	AG
Glucose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Inositol	–	–	A	A	A	A	A	AG	V	–
Lactose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Maltose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Mannitol	–	–	A	A	A	AG	A	–	V	–
Mannose	A	AG	AG	AG	AG	A	A	AG	AG	AG
Sucrose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Xylose	A	A	A	A	A	A	A	A	A	A

[a]Cultures inoculated with two drops of stock culture and incubated in the Gas Pak Anaerobic Jar for 24 hr at 45°C.
[b] Abbreviations for activites: A = acid; AG = acid and gas; + = positive; – = negative; β = beta hemolysis; α = alpha hemolysis; V = variable activity.
[c] Clostridium species isolated from LCP cottonseed flour produced at SRRC prior to this study and maintained in thioglycollate medium.
[d] Compilation of data from references: Breed et al., 1957; Buchanan and Gibbons, 1974; Holdeman and Moore, 1972.

two isolates from LCP cottonseed flour did not liquify gelatin, and produced fermentation patterns similar to those of the nonpathogenic soil organism *C. fallax* (Buchanan and Gibbons, 1974; Table 2).

While the origin of these organisms from cottonseed flour remain obscure, and although the microbial isolates reported herein grossly resemble *C. perfringens*, they instead should be regarded as nonpathogenic organisms based on additional testing. Thus, they do not present a hazard when LCP cottonseed flour derivatives are used as food. The data suggest, therefore, that when a cottonseed protein product is positive for *C. perfringens* according to methods recommended by the FDA (1976), the selective and differential tests described in this study should also be used to ascertain accurate description of the clostridia. The additional tests should include lecithinase activity, hemolysis of blood agar, gelatin liquefaction, and fermentation of arabinose, mannitol and xylose.

REFERENCES

Breed, R.S., Murray, E.G.D. and Smith, R.N., (Ed.). 1957. "Bergey's Manual of Determinative Bacteriology," 7th ed, p 634. The Williams and Wilkins Co., Baltimore, MD.

Buchanan, R.E. and Gibbons, N.E. (Ed.). 1974. "Bergey's Manual of Determinative Bacteriology," 8th ed, p 551. The Williams and Wilkins Co., Baltimore, MD.

Difco Manual. 1953. 9th ed. Difco Laboratories Inc., Detroit, MI.

FDA. 1976. "Bacteriological Analytical Manual for Foods," p xv-1. Association of Official Analytical Chemists, Washington, DC.

Gardner, H.K. Jr., Hron, R.J. Sr. and Vix, H.L.E. 1976. Removal of pigment glands (gossypol) from cottonseed. Cereal Chem. 53: 549.

Hobbs, B.C., Smith, M.E., Oakley, C.L., Warrack, G.H. and Cruickshank, J.F. 1953. Clostridium welchii food poisoning. J. Hyg. 51: 72.

Holdeman, L.V. and Moore, W.E.C. 1972. "Anaerobe Laboratory Manual, VPI Anaerobe Laboratory." Southern Printing Co., Blacksburg, VA.

Marshall, R.S., Sttenberger, J.F. and McClung, L.S. 1965. Rapid technique for the enumeration of Clostridium perfringens. J. Appl. Environ. Microbiol. 13: 559.

McClung, L.S. 1945. Human food poisoning due to growth of Clostridium perfringens (C. welchii) in freshly cooked chicken. J. Bacteriol. 50: 229.

Ms received 10/18/77; revised 1/11/78; accepted 1/18/77.

This work was funded by cooperative agreement #12-14-7001-505 between the National Cottonseed Products Association, Cotton Incorporated, and Southern Regional Research Center.

A Research Note

BREAD MAKING PROPERTIES OF PEANUT FLOUR PRODUCED BY DIRECT SOLVENT EXTRACTION PROCEDURE

M. N. KHAN, D. MULSOW, K. C. RHEE and L. W. ROONEY

ABSTRACT

Bread making properties of four experimental peanut flours (PNF-1, PNF-2, PNF-3 and PNF-4) processed with direct solvent extraction technique were evaluated and compared with commercial defatted peanut and defatted soy flours. PNF-1, PNF-2 and PNF-3 (1.0, 6.0 and 19.2% fat, respectively) received no heat treatment; while PNF-4 (1.0% fat) received a mild heat treatment during processing. Commercial wheat flour (15% protein d.w.b.) was replaced with 10, 15 and 20% of each of the oilseed flour. There were no significant differences in bread making properties of PNF-1, PNF-2, PNF-3 and the commercial peanut flour. Bread with 15–20% level of PNF-4 had better baking properties than the ones containing the other peanut flours. Bread made with the commercial soy flour had highly acceptable baking properties, however it had unacceptable flavor, which was also noticed in fresh bread containing PNF-1, PNF-2 or PNF-3. Bread with PNF-4 had acceptable taste and aroma. Fat content of the experimental peanut flours did not affect their baking performance and a mild heat treatment was essential during the processing of the experimental peanut flour to produce a high protein and a better flavor bread.

INTRODUCTION

SEVERAL PAPERS AND REVIEWS have been published in the past 10 years on the use of an oilseed protein product in white pan bread (Pomeranz, 1969; Martinez, 1969; Matthews et al., 1970; Tsen and Hoover, 1973; Tsen and Tang, 1971). Most of these emphasize that the compatability of an oilseed protein product with wheat flour depends on (1) the type of oilseed protein product (Rooney et al., 1972); (2) method of processing an oilseed protein product (Lawhon et al., 1972; Khan et al., 1975); (3) composition of an oilseed protein product (Tsen and Hoover, 1973); (4) the type of baking system used (Tsen and Tang, 1971; Khan and Rooney, 1977), and (5) the quality and quantity of wheat flour protein (Tsen and Tang, 1971). Most of the work has been reported on the use of soy protein products in bread making. However, other high protein sources such as cottonseed, peanut, sesame, sunflower and safflower had also been evaluated in bread systems (Rooney et al., 1972; Matthews et al., 1970).

Heat treatment is usually used in oilseed flour processing. This is to either improve the flavor of a finished product or to enhance the compatibility of an oilseed protein product with wheat flour. Heat also destroys antinutritional factors present in an oilseed protein. However the main disadvantage of heat is the denaturation of the protein and reduction in availability of lysine. This information is on the evaluation of bread making properties of four experimental peanut flours processed with direct solvent extraction technique. The experimental peanut flours varied in their fat contents and three of them did not receive any heat treatment. Defatted commercial soy and peanut flours were included for comparative purposes.

Authors Khan, Mulsow, Rhee and Rooney are with the Cereal Quality Lab and Food Protein R & D Center, Texas A&M University, College Station, TX 77843.

0022-1147/78/0004-1334$02.25/0
© 1978 Institute of Food Technologists

Table 1—Protein, fat and NSI of the protein sources

Protein source	Heat treatment	Protein[a] % (d.w.b.)	Fat % (d.w.b.)	NSI %
Low fat peanut flour (PNF-1)	No	66.4	1.0	90.0
Med. fat peanut flour (PNF-2)	No	63.7	6.2	91.5
Full fat peanut flour (PNF-3)	No	54.1	19.7	89.6
Low fat peanut flour (PNF-4)	Yes	67.4	0.8	77.2
Commercial defatted peanut flour		66.5	1.6	58.0
Commercial defatted soy flour[b]		56.5	1.1	60.0
Commercial wheat flour[c]		15.0		

[a] N × 6.25 for oilseed protein and N × 5.7 for wheat protein
[b] Values were supplied by GoldKist Inc.
[c] Values were supplied by ADMCo.

MATERIALS & METHODS

EXPERIMENTAL PEANUT FLOURS were processed according to the pilot plant procedure reported by Mulsow et al. (1976). Briefly the procedure consists of the mechanical blanching of peanut kernels which removed germ and skin. Mechanical means were used to slice the peanut cotyledons. The full fat peanut slices were then solvent extracted to produce the three experimental peanut flours with different fat contants (1.0, 6.0 and 19.0%). The slices were then ground to produce fine flour. The fourth experimental peanut flour was also processed with direct solvent extraction; however, it was heated at 110°C for 15 min. The experimental peanut flours were analyzed for protein, ash, moisture, crude fiber and nitrogen solubility index (NSI) according to the standard AOCS (1971) procedure. The commercial defatted peanut flour called Gold Nut flour was supplied by the Gold Kist Inc. and the defatted bakers Nutrisoy flour was obtained from ADM Co. Commercial wheat flour was used in the experiment. It was a mixture of hard red spring and winter wheats. It was malted and bromated. It was replaced with each of the oilseed flours at 10, 15 and 20% levels. Altogether 19 treatments were baked in a randomized complete block design.

Pound loaves were baked using each flour blend with a short-time dough system as reported by Khan et al. (1975). 0.5% of sodium-stearoyl-2 lactytate was used. Crumb grain score, crumb and crust colors were measured according to the procedures described by Khan et al. (1975). All the flour blends were baked on the same day and the whole bake was replicated on each of the three different days.

RESULTS & DISCUSSION

THE PROXIMATE ANALYSES of all the oilseed and wheat flours presented in Table 1, indicated that protein content of the experimental peanut flours varied from 54–67% and their fat content ranged from 0–8 to 19.7%. NSI of PNF-4, commercial peanut and commercial soyflours were significantly less than that of the experimental peanut flours. However, NSI of the oilseed flours and their bread making potential were not correlated with each other. Color of PNF-1, PNF-2 and PNF-3 was off white compared to light brown of PNF-4 and the commercial peanut flour. Baking properties of the bread made with the different protein sources are presented in Table 2. Baking absorptions of the doughs containing the nonheated experimental peanut flours were significantly less than their respective farinograph absorptions and the doughs had characteristic green peanut flavor and poor handling properties. This

Table 2—Baking properties of the experimental and the commercial oilseed flours

Protein source	Amount of oilseed flour added											
	10%				15%				20%			
	Baking absorp %	Loaf vol cc	Crust color[a]	Crumb color[a]	Baking absorp %	Loaf vol cc	Crust color[a]	Crumb color[a]	Baking absorp %	Loaf vol cc	Crust color[a]	Crumb color[a]
Low fat peanut fl.	64.2	3050	29.0	73.4	64.7	2775	25.4	70.3	62.4	2625	23.3	68.2
Med. fat peanut fl.	61.6	2988	28.4	74.5	61.2	2800	24.1	72.1	62.5	2600	24.5	67.5
High fat peanut fl.	62.3	3000	27.5	74.5	62.3	2875	25.6	72.4	63.5	2650	24.3	68.4
Heat treated defatted peanut fl.	70.4	3021	29.0	74.6	70.0	2938	26.0	70.1	71.5	2863	23.0	69.5
Commercial defatted peanut fl.	68.3	2950	26.8	74.8	71.0	2800	26.3	72.6	73.7	2675	25.4	70.2
Commercial defatted soy fl.	75.7	3175	28.4	75.2	81.2	3138	25.3	72.6	85.7	2688	25.1	71.3
Wheat flour (control)	67.2	3250	33.0	78.6								

[a] 'L' value of Hunter Lab colormeter

may be due to no heat treatment used in the processing of these peanut flours. The dough containing PNF-4 had acceptable dough handling properties and its baking absorption was 3% higher than its farinograph absorption.

Baking properties of the breads made with PNF-1, PNF-2, PNF-3 and commercial peanut flour were not significantly different from each other. Higher fat level of PNF-3 did not have any significant effect on the loaf volume of the bread. Bread made with 20% of PNF-4 had significantly higher loaf volume than the one containing the same levels of the other experimental peanut flours. The fresh bread baked with PNF-1, PNF-2 and PNF-3 had a raw peanut odor and taste which was significantly improved after toasting. Fresh and toasted bread made with PNF-4 had better taste and aroma than the one made with the other peanut and commercial soy flours. The aroma of the toasted bread made with the commercial soy flour was unacceptable.

On an average bread made with PNF-1, PNF-2, PNF-3, commercial peanut and commercial soy flour had 44, 42, 31, 36 and 35% more protein than the regular white pan bread. Oil content of the bread containing full fat experimental peanut flour was three times more than that of the white bread.

The process used in preparation of the experimental peanut flours was developed in our lab and is different from the regular hexane extraction procedure used in preparation of a defatted oilseed flour. The data obtained in the study suggested that the direct solvent extraction technique can be used successfully to make an acceptable peanut flour for use in bakery products. This type of peanut flour may have more available lysine than present in hexane extracted, heat-treated peanut flour.

REFERENCES

AACC. 1970. "Approved Methods," American Association of Cereal Chemists, St. Paul, MN.

AOCS. 1971. "Official and Tentative Methods," 3rd ed. Americal Oil Chemists Society, Chicago, IL.

Khan, M.N. and Rooney, L.W. 1977. Baking properties of five oilseed flours evaluated with a short-time dough system. Bakers Digest (3): 43.

Khan, M.N., Rhee, K.C., Rooney, L.W. and Cater, C.M. 1975. Bread baking properties of aqueous processed peanut protein concentrates. J. Food Sci. 40: 580.

Khan, M.N., Lawhon, J.T., Rooney, L.W. and Cater, C.M. 1976. Bread baking properties of cottonseed protein concentrates prepared from solvent extracted glandless cottonseed flour. Cereal Chem. 53: 388.

Martinez, W.H. 1969. Glandless cottonseed protein products in bread. Cereal Sci. Today 14: 110 (Abst.)

Matthews, R.H., Sharpe, E.J. and Clark, W.M. 1970. The use of some oilseed flours in bread. Cereal Chem. 47: 181.

Mulsow, D., Cater, C.M. and Mattil, K.F. 1976. Investigation of some parameters involved in a direct solvent extraction process for producing defatted peanut meal. Presented at the 36th Annual meeting of the Institute of Food Technologists, Anaheim, CA, June 6—9.

Pomeranz, Y. 1969. High protein supplements in bread making. Bakers Digest. 43(5): 39.

Rooney, L.W., Gustafson, C.B., Clark, S.P. and Cater, C.M. 1972. Comparison of the baking properties of several oilseed flours. J. Food Sci. 37: 14.

Tsen, C.C. and Hoover, W.J. 1973. High-protein bread from wheat flour fortified with full-fat soy flour. Cereal Chem. 50: 7.

Tsen, C.C. and Tang, R.T. 1971. K-State process for making high-protein breads. 1. Soy flour bread. Bakers Digest 45(5): 26.

Ms received 11/3/77; revised 1/7/78; accepted 1/10/78.

A Research Note

SHEAR CREEP TESTING OF DEMETHYLATED PECTIN GEL

V. N. M. RAO, M. O. GROSS and C. J. B. SMIT

ABSTRACT

An apparatus to measure the shear modulus (rigidity) of low-methoxyl pectin gels was constructed and successfully tested. The shear modulus of the gels were found to be dependent upon the methoxyl content of the pectin sample and the normal load during shear testing. The results also indicate that shear modulus provides a measure of differences in the gel characteristics.

INTRODUCTION

THE RIDIGITY of a dessert-type pectin gel plays an important role in it's acceptability. However, due to the delicate nature of the pectin gel, very little is known about the shear modulus (also referred to as modulus of rigidity) of the gel. The shear modulus is defined as the ratio of shear stress to shear strain and is indicative of the rigidity of a material. Many methods using test instruments have been developed for defining the texture of pectin gels. These have been classified into two major groups: one in which elastic limits are exceeded and the gel is ruptured whereas the other involves measurement of deformation without exceeding elastic limits. Although there are considerable data in the literature concerning the effect of different factors upon the strength of low-methoxyl pectin gels, they are confusing because so many different empirical measurements were used.

Some of the common instruments used in grading pectin gels are the Delaware jelly tester and its modified versions (Hills et al., 1942; Speiser and Eddy, 1946; Christensen, 1954) and the ridgelimeter (Christensen, 1954). Owens et al. (1949) measured the amount of sag by the Cox and Higby sag tester to find the effect of calcium to pectinate ratio, pH, temperature and the amount of sugar on gelation characteristics. Mitchell and Blanshard (1976) determined the creep compliance of pectin gels with 4% esterification using parallel plate viscoelastometer of Shama and Sherman (1968). However, commerically available low-methoxyl pectins have an esterification of 20–40% (Black and Smit, 1972), and gels made from these pectins are too sensitive to be tested in the parallel plate viscoelastometer of Shama and Sherman (1968). The shear modulus of low-methoxyl dessert-type pectin gels vary from 0.7–0.07 kPa, whereas the shear modulus of pectin gels with 4% esterification is about 20 kPa (Mitchell and Blanshard, 1976) and for gelatin and rubber the values are 20 kPa and 29 kPa, respectively (Muller, 1973). The objective of this investigation was to design a shear creep apparatus to determine shear modulus of pectin gels and to find whether shear modulus could be used as a quality factor in pectin gels.

MATERIALS & METHODS

ALL THE PECTIN used in this study were commercially prepared by Sunkist Growers, Inc. (California). Four different low-ester pectin samples labelled A through D having different methoxyl levels were used for gel making. The objective of using the above four samples of pectin was to determine whether shear modulus would be an appropriate measure of differences in the gel characteristics.

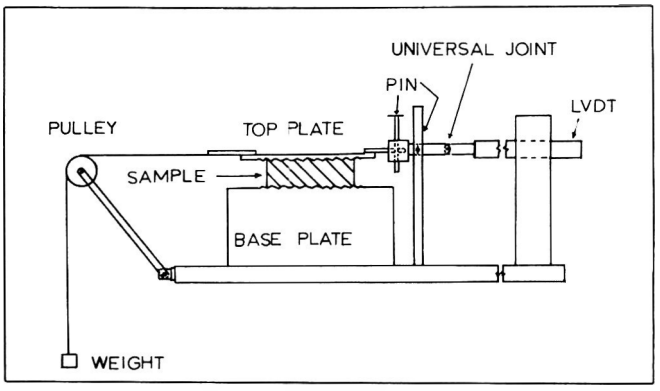


Fig. 1—Schematic diagram of shear creep apparatus.

The preparation of gels for all the pectin samples for the shear creep test was in accordance with LM Pectin Exchange Manual (1947). Four hundred twenty-five ml of distilled water, 10 ml of sodium citrate solution (8.4g in 140 ml of water) and 5 ml of citric acid solution (42g in 70 ml of water) were mixed thoroughly in a sauce pan. The solution was heated with a bunsen burner, and a mixture of 30g sucrose with 6g pectin was slowly stirred in. The mixture was brought to a boil (approximately 360 sec), and an additional 150g of sucrose was added with intermittent stirring. After approximately 150 sec the mixture was brought to a boil, and 25 ml of calcium chloride solution (3.5g in 275 ml of water) was added. The mixture was heated until the final weight reached 600g. Then, the pan was removed from the flame; the mixture was skimmed, poured into cylindrical Plexiglas containers (6.9 cm diameter × 1.5 cm height), cooled for 20 min at room temperature, refrigerated at 4.5°C overnight, and allowed to equilibrate to room temperature prior to testing.

The apparatus for measuring shear creep was fabricated by the Instrument Shop at the University of Georgia. The apparatus was modified successively after several trial runs with gels to eliminate errors from loading techniques and to insure application of uniform shear stress over the gel surface. A schematic diagram of the apparatus is shown in Figure 1. The application of force was by means of a weight over a frictionless pulley (with needle bearings). The deformation was measured continuously with a LVDT Model 500 (Daytronic Inc., Ohio) connected to a strip chart recorder via an amplifier. The gel was placed on a Plexiglas loading platform (serrated and center line aligned), and another thin Plexiglas top plate (14g weight and serrated) was placed over the gel. One end of the top plate was connected to a string, which was attached to a weight over the frictionless pulley. The other end of the top plate was connected to the LVDT via 2 pin keys and a universal joint. The first pin was to connect the top plate to LVDT without disturbing the gel whereas the second pin provided a lock mechanism. When it was removed the top plate was free to move and a load was applied to the sample.

All samples for the shear creep test were of the following dimensions: 6.9 cm in diameter and 1.5 cm high. A weight of 4g was applied to produce shear stress across the gel surface and to hold the resulting shear strain within 5% at zero applied normal load (other than the weight of the top plate). Eight replications were performed for each of the 4 pectin samples. The test was repeated using a normal load of 0.196N by placing a 20g weight on the center of top plate. Six replications were performed for each of the 4 pectin samples. The shear modulus (G) of the gels was determined after 5 sec of the application of load.

Authors Rao, Gross and Smit are with the Dept. of Food Science, University of Georgia, Athens, GA 30602.

0022-1147/78/0004-1336$02.25/0
© 1978 Institute of Food Technologists

Table 1—Means and analysis of variance of shear modulus (G) in Pascal (Pa) for pectin gels without normal stress

Replications	Pectin samples A	B	C	D
1	579	139	228	108
2	850	138	216	112
3	981	172	255	169
4	777	176	208	163
5	579	153	277	70
6	885	126	319	68
7	685	145	245	238
8	797	130	255	168
Means[a]	767a	147c	250b	137c
F-value	111.4 with 3/28 degrees of freedom			
Prob > F	0.01			

[a] Means with different letters are significantly different at the 1% level.

Table 2—Means and analysis of variance of shear modulus (G) in Pascal (Pa) for pectin gels with 0.196 N normal stress

Replications	Pectin samples A	B	C	D
1	2541	143	335	130
2	1677	118	277	155
3	3035	152	335	182
4	2897	145	271	155
5	1821	177	375	89
6	1275	168	266	83
Means[a]	2374a	150b	310b	132b
F-value	34.9 with 3/20 degrees of freedom			
Prob > F	0.01			

[a] Means with different letters are significantly different at the 1% level.

$$\text{Shear modulus, } G = \frac{\text{shear stress}}{\text{shear strain}}$$

The reason for not determining the long time response of gels was because it took nearly 6 hr to obtain reasonable creep behavior data and hence would not be practical in rapid quality evaluation. Moreover, the sag of the gel with time would affect the creep and the analysis would be complicated for quality control purposes.

RESULTS & DISCUSSION

THE VALUES of shear modulus for the 4 pectin samples with and without normal stress were subjected to an analysis of variance (Tables 1 and 2). Differences in shear modulus values for the 4 pectin samples were significant. The shear modulus varied from 981 Pa for pectin A to 68 Pa for pectin D at zero applied normal load. At 0.196N applied normal load, the shear modulus values ranged from 3541 Pa for pectin A to 83 Pa for pectin D (Table 2). The coefficient of variation within the same pectin sample ranged from 11–42%. Large variability results because of the sensitivity of the gel to very slight differences in positioning or loading techniques. This probably is the greatest drawback in using the shear creep test with gels.

In spite of the experimental errors, the analysis of variance indicated significant differences between the gels made from the 4 pectin samples at zero applied normal load (Table 1). Pectin A (lowest in methoxyl amongst the 4) yielded the highest shear modulus and was significantly different (1% level) from B, C and D. The gels made from pectin B and D yielded shear moduli that were not significantly different (1% level). Gels made from pectin C were more rigid than pectin samples B and D but less rigid than pectin A.

Results of this study indicate that the pectin sample containing the lowest methoxyl level produced the most rigid gel. The analysis of variance done on the shear modulus values of the gels with an applied normal load of 0.196N (Table 2) also showed significant differences (1% level). However, the differences were not as pronounced as before. The gels from pectin A were significantly different from other gels made from pectin B, C and D. But, the rigidity of the gels from pectin samples B, C and D were not significantly different from each other.

In conclusion, an apparatus to measure the rigidity (or shear modulus) of pectin gels was constructed and tested successfully. The shear modulus of the gels was found to be a discriminating parameter in determining gel quality and the pectin sample at zero applied normal load. The normal load during shear creep increased the rigidity of the pectin gels but was not as effective in differentiating the gels made from different pectin samples. Further work on measuring the influence of methoxyl content (percent esterification) on pectin gel rigidity is needed and should provide valuable information in maintenance of gel quality.

REFERENCES

Black, S.A. and Smit, C.J.B. 1972. The effect of demethylation procedure on the quality of low-ester pectin for use in dessert gels. J. Food Sci. 37: 730.
California Fruit Growers Exchange. 1947. "LM Pectin Exchange Manual." Ontario, CA.
Christensen, P.E. 1954. Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. Food Res. 19: 163.
Hills, C.H., White, J.W. and Baker, G.L. 1942. Low-sugar jelling pectinates. Proc. Inst. Food Technol. 47.
Mitchell, J.R. and Blanshard, J.M.V. 1976. Rheological properties of pectate gels. J. Texture Studies 7: 341.
Muller, H.G. 1973. "An Introduction to Food Rheology." Grane, Russack and Company, N.Y.
Owens, H.S., McCready, R.M. and MacClay, W.D. 1949. Gelation characteristics of acid-precipitated pectinates. Food Technol. 3: 77.
Shama, F. and Sherman, P. 1968. An automated parallel plate viscoelastometer for studying the rheological properties of solid food materials. SCI Monograph 27: 77.
Speiser, R. and Eddy, C.R. 1946. Effect of molecular weight and method of de-esterification on the gelling behavior of pectin. J. Am. Chem. Soc. 68: 287.
Ms received 11/18/77; revised 1/20/78; accepted 1/30/78.

The authors appreciate the help of Mr. Michael G. Fitch in conducting experiments for this study and Sunkist Growers, Inc. (California) for the pectin samples.

A Research Note

CHANGE IN REGULATION OF MYOFIBRILLAR ATPase ACTIVITY BY CALCIUM DURING POSTMORTEM STORAGE OF MUSCLE

Y. IKEUCHI, T. ITO and T. FUKAZAWA

ABSTRACT

Postmortem change of rabbit myofibrillar ATPase activity was investigated at varying concentrations of free calcium ions. At concentrations of free calcium ions higher than 10^{-7} M the ATPase activity increased with increasing postmortem time, while at concentrations of free calcium ions lower than 10^{-7} M no significant change of the ATPase activity was found during postmortem storage of muscle. A slight shift of the myofibrillar pCa-dependent ATPase activity curve toward lower calcium ion concentration occurred during postmortem storage: the affinity of myofibrils for calcium ions increased during postmortem storage.

INTRODUCTION

GOLL AND ROBSON (1967) have reported that Mg^{++}- and Ca^{++}-ATPase activities of myofibrils prepared from bovine muscle after 24 hr postmortem storage are 20–80% higher than those of myofibrils prepared from at-death muscle. As to the real cause for the increased ATPase activity during postmortem storage, Goll et al. (1970) have speculated the following three possibilities: (1) alteration of actin-myosin interaction which increases actomyosin ATPase; (2) desensitization of the troponin-tropomyosin system which regulates the actin-myosin interaction; and (3) acceleration of the actin-myosin interaction due to the function of free α-actinin released from the Z-line structure. Arakawa et al. (1970) have shown that the actomyosin ATPase-accelerating ability of α-actinin gradually decreases during postmortem storage, while the troponin-tropomyosin complex still has Ca^{++}-sensitivity even after 14 days postmortem storage. Thus, it has been considered that the troponin-tropomyosin system and α-actinin, which probably undergo some postmortem changes, have no significant effect on the actin-myosin interaction in postmortem muscle. Also, Suzuki and Goll (1974) have reported that digestion of myofibrils by CASF (calcium activated-sarcoplasmic factor) is very similar to the changes caused by postmortem storage.

In the present study, we have investigated the effect of free calcium ion concentrations [pCa = $-\log (Ca^{++})$] on myofibrillar ATPase activity of rabbit muscles during postmortem storage at 0°C in order to elucidate the relation between myofibrillar ATPase activity and the function of regulatory proteins, troponin and tropomyosin.

MATERIALS & METHODS

RABBITS were anesthesized with sodium pentobarbital (90 mg) and d-tubocurrarine chloride (15 mg) just prior to exsanguination. The carcasses were soaked in 10 mM NaN_3 solution for a few moments to prevent bacterial growth and then wrapped in a polyethylene bag. Prior to the preparation of myofibrils, longissimus thoracis muscle was dissected from the carcass. Myofibrils were prepared from the muscle of at-death and after 24- and 168-hr postmortem storage at 0°C according to the procedure described by Briskey and Fukazawa (1971), except that myofibrils were washed with 100 mM borate-KCl solution (100 mM KCl, 39 mM borate, 5 mM ethylenediamine-tetraacetic acid (EDTA), pH 7.1) containing 0.02% Triton X-100 at the first washing step to eliminate the ATPases of sarcoplasmic reticulum and other organelles. Each myofibril in myofibrillar preparations from at-death and postmortem muscle had approximately 5–10 sarcomeres.

Authors Ikeuchi, Ito and Fukazawa are with the Lab. of Chemistry & Technology of Animal Products, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

0022-1147/78/0004-1338$02.25/0
© 1978 Institute of Food Technologists

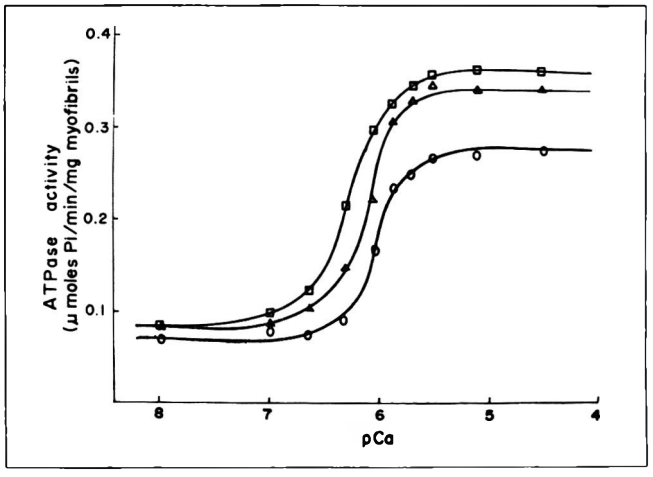


Fig. 1—pCa-dependent ATPase activity of myofibrils prepared from at-death, 24- and 168-hr postmortem rabbit muscles. ATPase activity was determined in a reaction mixture containing 0.35 mg/ml protein, 50 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 20 mM Tris-maleate, pH 6.8 and varying concentrations of free calcium ions at 25° C. The concentrations of free calcium ions in the medium (pCa) were calculated as described in MATERIALS & METHODS. ○, *Myofibrils prepared from at-death muscle;* △, *Myofibrils prepared from 24-hr postmortem muscle;* □, *Myofibrils prepared from 168-hr postmortem muscle.*

Myofibrils were washed five times with 30 volume of 100 mM KCl solution prior to measuring ATPase activity in order to eliminate EDTA from myofibrils. The steady-state ATPase activity of isolated myofibrils was measured in a 2 ml reaction mixture containing 50 or 100 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 20 mM Tris-maleate, pH 6.8, varying concentrations of free calcium ions and 0.35 mg/ml protein at 25°C. The concentrations of free calcium ions (pCa) in the reaction mixtures were determined by Ca^{++}-EGTA buffers: a solution of Ca^{++}-EGTA buffer was obtained by adding a definite amount of $CaCl_2$ to EGTA (1 mM), followed by neutralization to pH 6.8. The concentrations of free calcium ions (pCa) was calculated by assuming the apparent binding constant of ethyleneglycol-*bis*-(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) for Ca^{++} as 5×10^5 M^{-1} at pH 6.8 (Ogawa, 1968). The reaction was evaluated with 1-min intervals for 4 min and stopped by adding an equal volume of ice cold 10% trichloroacetic acid (TCA) solution. After centrifugation (1,500 × G, 10 min), the liberated phosphorus in the supernatant was determined by the method of Fiske and Subbarow (1925).

Protein concentration was determined by the biuret reaction of Gornall et al. (1949), which had been standardized by bovine serum albumin.

RESULT & DISCUSSION

FIGURE 1 shows ATPase activity of myofibrils prepared from at-death, 24- and 168-hr postmortem muscles as a function of pCa in 50 mM KCl. As shown in this figure, myofibrillar ATPase activity at higher concentrations of free calcium ions (less than pCa 7) increased with increasing postmortem time: ATPase activity of myofibrils prepared from 168-hr postmortem muscle was about 30% higher than that of myofibrils from at-death muscle. On the other hand, there was no significant difference in myofibrillar ATPase activity among at-death, 24- and 168-hr postmortem muscles at lower concentra-

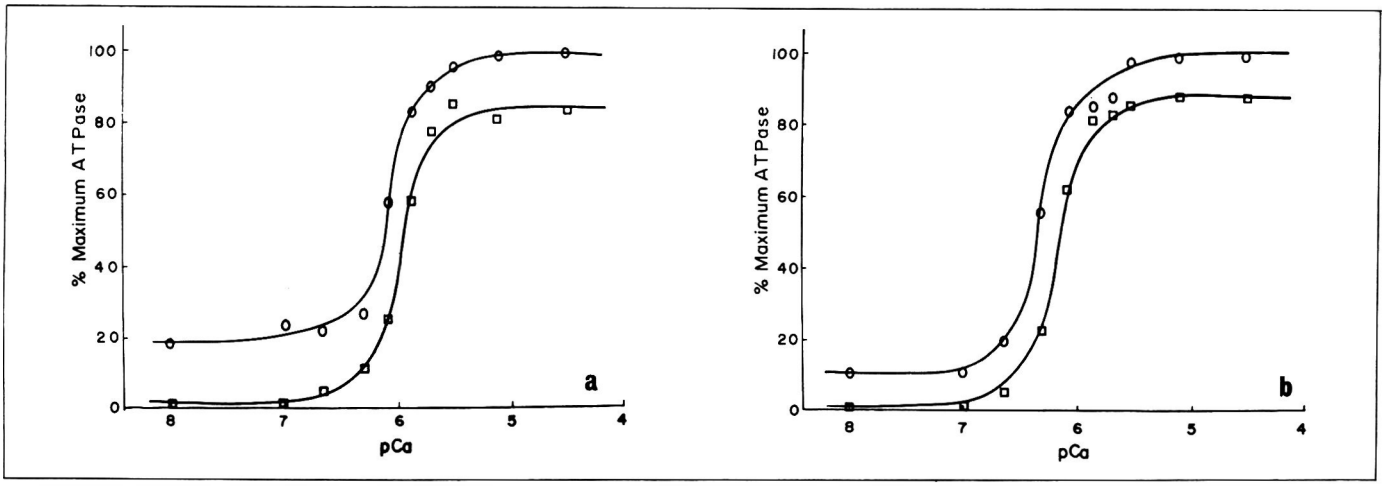


Fig. 2—Relative pCa-dependent ATPase activity of myofibrils at different concentration of KCl. ATPase activity was determined as in Fig. 1, except that the concentration of KCl was 50 and 100 mM. (a) Myofibrils prepared from at-death muscle; (b) Myofibrils prepared from 168-hr postmortem muscle. O, *ATPase activity at 50 mM KCl;* □, *ATPase activity at 100 mM KCl.*

tions of calcium ions (more than pCa 7). The curve of ATPase activity versus pCa was shifted to slightly higher pCa values as postmortem storage time increased. In addition, the pCa-dependent ATPase activity curve showed a sigmoidal one, suggesting that in all myofibrils tested the regulatory system of myofibrils effectively works. This experiment was carried out three times and the results showed a similar tendency.

Figures 2a and b show relative ATPase activities of myofibrils as a function of pCa at two different concentrations of KCl (50 and 100 mM). It was clearly shown that at every concentration of Ca^{++} ATPase activities of myofibrils prepared from at-death and 168-hr postmortem muscles were lower at high salt concentration than at low salt concentration. In addition, the affinity of both myofibril preparations for Ca^{++} was higher at 50 mM KCl than at 100 mM KCl. Thus, the pCa-dependent ATPase activity curve was shifted by changing the ionic strength. However, there was no significant difference in the degree of the shift between myofibrils prepared from at-death muscle and that from 168-hr postmortem muscle. The present results (Fig. 2a and b) also showed a similar pCa-dependent ATPase activity curve to that of Weber and Herz (1963).

Figure 1 demonstrates that myofibrillar ATPase activity increases with increasing postmortem time and the regulatory function of the troponin and tropomyosin system is slightly altered during postmortem storage of muscle. Moos (1972) have reported that a decrease of acto-heavy meromyosin ATPase activity in a high ionic strength salt solution is due to an increased apparent dissociation constant of actin for HMM. In addition, Ito et al. (1978) have shown from the results of double reciprocal plots of acto-heavy meromyosin (HMM) ATPase activity of postmortem muscle that the affinity of actin for HMM is much higher in postmortem muscles than in at-death muscle and have suggested that postmortem change of acto-HMM ATPase activity is due to changes in property of both actin and myosin. Therefore, it appears that the increase of myofibrillar ATPase activity during postmortem storage of muscle is due to the increase of the actin-myosin interaction. The results shown in Figures 2a and b which denote that myofibrillar ATPase activity and Ca^{++}-sensitivity of myofibrils are higher under low ionic strength also suggest that the actin-myosin interaction is greater at lower ionic strengths than at higher ionic strengths (Moos, 1972).

We have also found that Ca^{++} sensitivity of myofibrils increases with increasing postmortem time (Fig. 1). This suggests that (1) the increase of the actin-myosin interaction increases the affinity of myofibrils for Ca^{++}, or (2) postmortem change of the regulatory proteins alters the actin-myosin interaction. The former consideration has already been suggested by Bremel and Weber (1972). The result shown in Figure 2 also supports this consideration. Also, the latter can be supported by the interpretation that the increase in activity of myofibril may be due to CASF degradation of troponin (Suzuki and Goll, 1974). However, differences in SDS gel electrophoretograms of the regulatory proteins in myofibrils were not significant among at-death, 24-hr and 168-hr postmortem muscle (data not shown).

Although the regulatory proteins maintain their function and structure, there are considerable differences in the pCa-dependent ATPase activity curve among myofibrils prepared from at-death, 24- and 168-hr postmortem muscles (Fig. 1). Therefore, we cannot deny the possibility that a desensitization of the regulatory proteins takes place during aging. We are presently investigating changes in the properties of the regulatory proteins as a function of postmortem storage.

REFERENCES

Arakawa, N., Goll, D.E. and Temple, J. 1970. Molecular properties of postmortem muscle. 8. Effect of postmortem storage on α-actinin and the Tropomyosin-Troponin complex. J. Food Sci. 35: 703.

Bremel, R.D. and Weber, A. 1972. Cooperation within actin filament in vertebrate skeletal muscle. Nature 238: 97.

Briskey, E.J. and Fukazawa, T. 1971. Myofibrillar proteins of skeletal muscle. Adv. Food Res. 19: 279.

Fiske, C.H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375.

Goll, D.E. and Robson, R.M. 1967. Molecular properties of postmortem muscle. 1. Myofibrillar nucleoside-triphosphatase activity of bovine muscle. J. Food Sci. 32: 323.

Goll, D.E., Arakawa, N., Stromer, M.H., Busch, W.A. and Robson, R.M. 1970. Chemistry of muscle proteins as a food. In "The Physiology and Biochemistry of Muscle as a Food," Ed. E.J. Briskey, R.G. Cassens and B.B. Marsh, Vol. 2, p. 755. University of Wisconsin Press.

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

Ito, T., Sung, S.K. and Fukazawa, T. 1978. The change of acto-heavy meromyosin ATPase of rabbit skeletal muscle during postmortem storage. J. Agric. Food Chem. In press.

Moos, C. 1972. Actin activation of heavy meromyosin and subfragment-1 ATPase; steady state kinetics studies. Cold Spring Harbor Symposia Quant. Biol. 37: 137.

Ogawa, Y. 1968. The apparent binding constant of glycoletherdiaminetetraacetic acid for calcium at neutral pH. J. Biochem. 64: 255.

Suzuki, A. and Goll, D.E. 1974. Quantitative assay for CASF (Ca^{++}-activated sarcoplasmic factor) activity, and effect of CASF treatment on ATPase activities of rabbit myofibrils. Agr. Biol. Chem. 38: 2167.

Weber, A. and Herz, R. 1963. The binding of calcium to actomyosin systems in relation to their biological activity. J. Biol. Chem. 238: 599.

Ms received 12/9/77; revised 3/2/78; accepted 3/8/78.

We thank Dr. R.G. Cassens, University of Wisconsin, for his valuable suggestions, and also for his reading this manuscript.

A Research Note

IN VITRO STUDIES ON SAPONIN–VITAMIN COMPLEXATION

L. G. WEST and J. L. GREGER

ABSTRACT

The possibility that certain saponins produce growth depression by complexing fat soluble vitamins with a mechanism similar to saponin-cholesterol complexation was studied. An in vitro procedure was developed to quantitate the formation of addition complexes in aqueous solutions. Saponins studied included digitonin, ammoniated glycyrrhizin, quillaja bark saponin, and saponin-containing extracts from alfalfa leaves and roots and soybeans. Vitamins tested included provitamin D_3 and vitamins E, A and D_3. The study demonstrated that the saponins did not complex fat soluble vitamins in a manner similar to the complexation of cholesterol.

INTRODUCTION

THE COMPLEXATION of vitamins by saponins to form non-absorbable saponin-vitamin complexes has been suggested as a possible mechanism for producing saponin-induced growth depression (Cheeke, 1976). Brune and Gunther (1960) have reported that the loss in weight and osteomalacia seen in rats fed saponins was the result of saponin antagonism toward vitamin D with the formation of an intestinal saponin-vitamin D addition compound. Cheeke (1971) has proposed that saponin-vitamin D antagonism could be the result of forming complexes similar to saponin-cholesterol complexes.

The complexation of digitonin to form addition compounds possessing varying degrees of insolubility with steroids, phenols, thiophenols and higher alcohols in alcoholic solutions is well recognized (Merck, 1968). It seems reasonable that certain vitamins could also be bound by saponins possessing digitonin-like properties because many vitamins belong to these chemical classes. However, digitonin will not form insoluble addition complexes with steroids having a 3-α-hydroxyl substitution, such as vitamin D, nor will it precipitate vitamin E (Merck, 1968), but these observations were obtained under largely nonaqueous conditions (50–80% alcohol).

We were interested in developing an in vitro technique to study saponin-vitamin complexation in aqueous systems and determining if various vitamins form complexes with saponins similar to those observed with cholesterol.

EXPERIMENTAL

Source of saponins and test compounds

Dried alfalfa leaf meal, alfalfa roots and fresh soybeans were pulverized in a large Wiley mill. Samples were defatted in Soxhlet extractors with petroleum ether (30–60°C) and acetone. Saponin extracts were obtained by exhaustively percolating the defatted plant material with 50% ethanol. The ethanolic extracts were concentrated under rotary vacuum evaporation at temperatures not exceeding 40°C and the aqueous residues were freeze dried. The presence of saponins in all extracts was confirmed with the hemolysis, froth and Liebermann-Burchard tests (Farnsworth, 1966).

Authors West and Greger are with the Dept. of Foods & Nutrition, Purdue University, West Lafayette, IN 47907.

0022-1147/78/0004-1340$02.25/0
© 1978 Institute of Food Technologists

Digitonin, provitamin D_3, vitamin D_3, retinol and DL-α-tocopherol were obtained from Sigma Chemical Company (St. Louis, MO), quillaja saponin was obtained from Fischer Scientific Company (Chicago, IL), ammoniated glycyrrhizin was obtained from Dr. Madis Laboratories Inc. (South Hackensack, NJ). and cholesterol was obtained from Aldrich Chemical Company (Milwaukee, WI).

Formation of addition complexes

The saponin or saponin-containing extract was mixed with 5 ml of deionized water and the pH was recorded. Weights of the various saponins as well as saponin-vitamin ratios were chosen after doing preliminary studies with cholesterol, reviewing previously established maximum saponin-cholesterol binding ratios (Tschesche and Wulff, 1964), and calculating approximate saponin composition of crude extracts as based on reported purity (Birk, 1969). Controls consisted of 5 ml deionized water. A 5 ml aliquot of the vitamin to be tested dissolved in A.C.S. grade hexanes was added to the saponin sample and the flask was securely stoppered. All flasks were shaken on a wrist-action shaker at constant speed for 15 min. After this period, the contents of each flask were transferred and centrifuged for 5 min to completely break any emulsions. A portion of the hexane layer was removed and placed in a tightly sealed vial before analysis. Light sensitive compounds were protected throughout the study.

Method of analysis

A Constametric II (Laboratory Data Control, Riviera Beach, FL) liquid chromatograph coupled with a variable wavelength UV detector (Varian Vari-Chrom, Palo Alto, CA) was used throughout the study. The column was 25 cm × 4.6 mm i.d. stainless steel and packed with 3.5g of Partisil-10 ODS (Whatman Inc., Clifton, NJ). Samples were introduced onto the column employing a syringe loading sample injector (Rheodyne Inc., Berkeley, CA) containing a 20 μl sample loop.

The mobile phase was prepared with A.C.S. grade methanol and deionized water (9:1) and degassed prior to use. A flow rate of 2.0 ml/min was used for all compounds except retinol where 1.5 ml/min was employed.

Compounds were detected at the following wavelengths: cholesterol, 215 nm; provitamin D_3 and vitamin D_3, 265 nm; DL-α-tocopherol, 294 nm; and retinol, 325 nm. An absorbance range was chosen to obtain a tracing utilizing 40–70% of full-scale deflection.

Peak areas were calculated using the height × width at half-height technique and represent the average of three determinations. The relative standard deviation for three injections was consistently ± 2% to 3% for all test compounds except vitamin A where a poorer precision of ± 5% to 6% was experienced. A one-way analysis of variance and Duncan new multiple range test were applied to all data (Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE TRANSFERENCE of otherwise water insoluble compounds into the aqueous saponin-containing phase was considered to be the result of complex formation. Most complexes formed were clearly visible as precipitates in the aqueous phase, but water soluble addition compounds, if formed, could also exclude the bound species from the organic phase. The possibility, although unlikely, of forming hexane soluble saponin complexes was considered. Cholesterol digitonide was prepared and found not to partition into the hexane phase as analyzed by liquid chromatography. Other saponin complexes were assumed to exhibit similar behavior.

The procedure as developed gave predictable results in those cases where complexation had previously been documented. Results are presented in Table 1. Clearly, digitonin formed addition complexes with cholesterol and provitamin D_3. Digitonin complexed 97% and 100% of the cholesterol

Table 1–Percentage of test sample not complexed by saponin[a]

Test Sample	Saponin					
	Digitonin	Ammon. Glycyrrhizin	Quillaja Bark	Alfalfa Leaf	Alfalfa Root	Soybean
Cholesterol	3a	99d	102d	79c	65b	99d
Provitamin D_3	0a	98d	61b	78c	80c	98d
Vitamin D_3	102a	100a	104a	104a	98a	97a
Vitamin E	98a	103a	103a	96a	98a	105a
Vitamin A	109a	97a	99a	112a	106a	105a

[a] % test sample not complexed = $\frac{\text{test sample chromatographic peak area}}{\text{control chromatographic peak area}}$ X 100. Means in the same row bearing a common letter do not differ ($p < 0.01$).

and provitamin D_3, respectively, yet had no capacity to complex with vitamins D_3, E or A. The inability of digitonin to complex with several of these compounds is in agreement with previous results (Merck, 1968). A composite chromatogram illustrating the capacity of digitonin, as well as other saponins, to complex cholesterol is presented in Figure 1.

Alfalfa leaf and root saponins demonstrated a "digitonin-like" pattern of complexation, but were considerably less efficient with regard to the total amount of compound complexed. This was probably the result of the dilution of saponins responsible for complexation in other 50% ethanol extractables. Due to this heterogeneity, the total capacity of the extracts to complex cholesterol and provitamin D_3 cannot unequivocally be attributed only to saponins. However, in those alfalfa extract experiments where complexation did not occur, such as with vitamins D_3, E and A, all possible 50% ethanol soluble compounds can also be disregarded as significant binders of these vitamins.

There was no significant difference between the ability of alfalfa root and leaf extracts to complex provitamin D_3. This is in contrast to the cholesterol complexation results where the root extract bound 14% more cholesterol. This particular observation was consistent with reported higher root tissue concentrations of medicagenic acid-containing saponins, which are primarily responsible for cholesterol binding in alfalfa (Reshef et al., 1976). The results obtained with provitamin D_3 might be explained by proposing that the major component complexing this compound is in similar concentrations in both roots and leaves. The cholesterol results do not conflict with this suggestion since saponins differ greatly with regard to specificity of complexation (Tschesche and Wulff, 1964). The major alfalfa saponins complexing cholesterol may not be the same ones complexing provitamin D_3.

This difference in binding specificity was also illustrated by quillaja bark saponins. The saponin mixture did not form an addition complex with cholesterol, yet bound 39% of the provitamin D_3. Quillaja bark saponins did not complex any other compounds in the organic phase.

Ammoniated glycyrrhizin and soybean saponins did not form addition complexes with any of the test compounds. Soybean saponins are known not to complex with cholesterol (Ishaaya et al., 1969).

Since the influence of pH upon vitamin complexes formed with saponins has not been reported, the pH of the aqueous systems was not modified. However, with the exception of digitonin and alfalfa leaf extract (pH values of 4.0 and 2.8, respectively), the pH of all remaining samples fell within a range of 5.5–5.9. Therefore, the possibility must be considered that some of the difference in binding capacity between digitonin and alfalfa leaf saponins, when compared to the values obtained for the other saponins, could be attributed to the variation in pH.

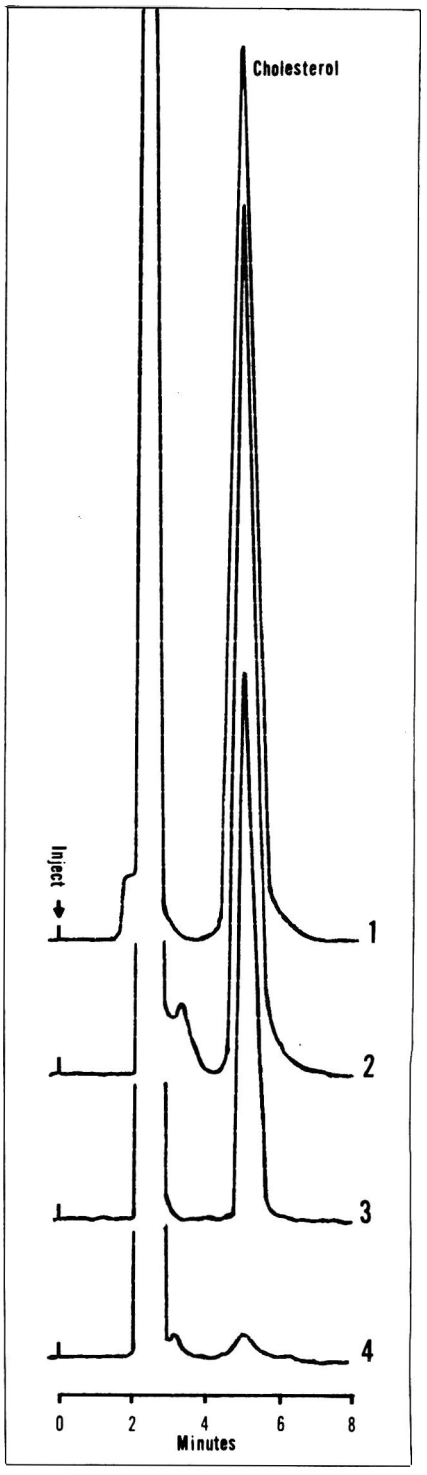


Fig. 1–Composite chromatogram illustrating the noncomplexed cholesterol remaining after saponin-cholesterol complexation (1–Control; 2–Soybean saponin extract; 3–Alfalfa root saponin extract; 4–Digitonin).

Digitonin, quillaja bark saponins, and alfalfa leaf and root saponins have been demonstrated to depress growth in test animals (Birk, 1969; Reshef et al., 1976; Morgan et al., 1972; Peterson, 1950; Coulson and Evans, 1960). Various authors have suggested that this growth depression might be partially due to binding of fat-soluble vitamins by saponins in a manner similar to saponin-cholesterol complexation (Cheeke, 1976, 1971; Brune and Gunther, 1960). Our results indicate none of the saponins tested complexed fat-soluble vitamins A, D_3 or E.

–Continued on page 1343

A Research Note

IN VITRO STUDIES ON SAPONIN-MINERAL COMPLEXATION

L. G. WEST, J. L. GREGER, A. WHITE and B.J. NONNAMAKER

ABSTRACT

The possibility that certain saponins produce growth depression by complexing minerals was studied. Saponins tested included digitonin, ammoniated glycyrrhizin, quillaja saponin and saponin-containing extracts from alfalfa leaves and roots. Minerals studied were zinc, iron and magnesium. Alfalfa root saponins and ammoniated glycyrrhizin, but not digitonin, quillaja and alfalfa leaf saponins, complexed zinc and iron, but not magnesium. It is proposed that one mechanism of action whereby saponins suppress growth is by the formation of insoluble saponin-mineral complexes. Such complexes could reduce the availability of minerals necessary for growth.

INTRODUCTION

GROWTH DEPRESSION in animals as a result of ingesting certain saponins has been well established (Peterson, 1950; Coulson and Evans, 1960; Reshef et al., 1976). However, the mechanism(s) by which these naturally occurring compounds exert this activity are not well understood. Possibilities include: decreased feed intake because of poor palatability or irritation of the gastrointestinal tract, changes in intestinal transit time, inhibition of cellular metabolic enzymes, inhibition of digestive enzymes, and decreased availability of nutrients due to saponin-nutrient complexation (Cheeke, 1976). Paired-animal feeding studies have not supported the suggestion that depression of food intake accounts for all the growth depression (Coulson and Evans, 1960; West and Greger, 1977). Also, other investigators have questioned the proposals that inhibitory effects on digestive and cellular processes are significantly involved in saponin-induced growth depression (Birk, 1969).

Evidence does exist that several saponins may complex with cations to form insoluble and possibly nonabsorbable addition compounds (Eis et al., 1952; Basu and Rastogi, 1967; West, 1977). The complexation of minerals by saponins, resulting in decreased mineral utilization, could suggest a mechanism by which certain saponins suppress growth. However, the study of interactions between saponins and minerals has received only limited past attention. Ewart (1931) reported, but did not reference, that saponins may enhance absorption of minerals. Although possible, such activity seems unlikely. Other naturally occurring mineral complexing agents, such as phytate (Oberleas, 1973; Davies and Nightingale, 1975), tannins (Disler et al., 1975), and pectins (Branch et al., 1975), can depress growth in animals by impairing mineral absorption in the gastrointestinal tract.

We were interested in determining if several saponins known to depress growth in animals formed insoluble complexes with minerals, such as zinc, magnesium and iron.

Authors West, Greger, White and Nonnamaker are with the Dept. of Foods & Nutrition, Purdue University, West Lafayette, IN 47907.

0022-1147/78/0004-1342$02.25/0
© 1978 Institute of Food Technologists

Table 1—Percent of minerals complexed by saponins

Saponin	% Mineral complexed[a]		
	Zinc	Iron	Magnesium
Sucrose (control)	0.0	0.0	0.0
Ammoniated glycyrrhizin	17.5[b]	18.2[b]	0.0
Alfalfa root	22.5[b]	22.7[b]	0.0
Digitonin	0.0	0.0	0.0
Quillaja	0.0	0.0	0.0
Alfalfa leaf	0.0	0.0	0.0

[a] % mineral complexed = $\frac{\text{(mineral in control} - \text{mineral in supernatant tested)}}{\text{mineral in control}} \times 100$

[b] Samples containing precipitates

EXPERIMENTAL

Source of saponins

Dried alfalfa leaf meal and alfalfa roots were pulverized in a large Wiley mill. Samples were defatted in Soxhlet extractors with petroleum ether (30–60°C) and acetone. Saponin extracts were obtained by exhaustively percolating the defatted plant material with 50% ethanol. The ethanolic extracts were concentrated under rotary vacuum evaporation at temperatures not exceeding 40°C and the aqueous residues were freeze-dried. The presence of saponins in all extracts was confirmed with the hemolysis, froth, and Liebermann-Burchard tests (Farnsworth, 1966).

Digitonin was obtained from Sigma Chemical Company (St. Louis, MO), quillaja saponin was obtained from Fisher Scientific Company (Chicago, IL), and ammoniated glycyrrhizin was obtained from Dr. Madis Laboratories, Inc. (South Hackensack, NJ).

Preparation of saponin-mineral complexes and determination of the percent of mineral complexed

Weighed samples of saponin were shaken for several hours with deionized water to yield 0.01M solutions (assuming average saponin molecular weight of 1000) and then centrifuged. Aliquots of the supernatant liquids were mixed with equal portions of 0.01M zinc, iron, and magnesium sulfate solutions. Controls consisted of identical concentrations of mineral in water and of saponin in water. A sucrose solution was prepared as described for saponins and served as a noncomplexing control. The pH of the controls was adjusted to that of the experimental samples and the mixtures were allowed to stand undisturbed overnight.

After standing, the samples were again centrifuged. All precipitates were washed twice by suspending in water and centrifuging. The presence of both mineral and saponin in a precipitate was determined by qualitative inorganic analysis (Searcy et al., 1967) and the froth and hemolysis tests.

The amount of mineral remaining in solution after complexation by saponin was quantitatively determined on diluted aliquots of the supernatant by atomic absorption spectrophotometry (Perkin-Elmer Model 360 atomic absorption spectrophotometer, Norwalk, CT).

RESULTS & DISCUSSION

ZINC AND IRON were complexed and precipitated by ammoniated glycyrrhizin and alfalfa root saponins. The results are presented in Table 1. This observation may explain the mechanism of growth depression reported for alfalfa root saponins (Birk, 1969; Bondi et al., 1973), since a deficiency of minerals could result in poor growth and performance (Davies and Nightingale, 1975; Oberleas and Prasad, 1969; Bowering et al., 1976). The absence of precipitate, when magnesium was

tested, may reflect a lack of complexation or the formation of soluble saponin-magnesium complexes.

Digitonin did not complex any of the minerals. Therefore, the growth depression reported for this saponin (Morgan et al., 1972) cannot be attributed to a decrease in mineral availability involving this mechanism. It is quite possible that saponins exert growth depression by several mechanisms as suggested by the diverse pharmacologic activities of these compounds (Basu and Rastogi, 1967; Vogel, 1963).

Quillaja saponin also did not form insoluble complexes with any of the minerals tested. However, the ability of this saponin mixture to depress growth is extremely variable and depends upon the commercial source of the saponin (Peterson, 1950).

None of the minerals were precipitated by alfalfa leaf saponins. Alfalfa leaf meal will depress growth in chickens (Brune and Gunther, 1960), but even concentrated leaf extracts must be purified substantially to produce growth depression in other test animals (Reshef et al., 1976). Alfalfa root saponins are considerably more effective in depressing growth (Reshef et al., 1976; Shaney et al., 1970).

These results strongly suggest that certain saponins can form insoluble complexes with minerals. The potential significance of this binding to mineral utilization is currently being investigated with animal studies as well as determining the chemical composition of several saponin-mineral complexes.

REFERENCES

Basu, N. and Rastogi, R.P. 1967. Triterpenoid saponins and sapogenins. Phytochemistry. 6: 1249.

Birk, Y. 1969. Saponins. In "Toxic Constituents of Plant Foodstuffs," p. 169. Academic Press, New York, NY.

Bondi, A., Birk, Y. and Gestetner, B. 1973. Forage saponins. In "Chemistry and Biochemistry of Herbage," p. 511. Academic Press, New York, NY.

Bowering, J., Sanchez, A.M. and Irwin, M.I. 1976. A conspectus of research on iron requirements of man. J. Nutr. 106: 985.

Branch, W.J., Southgate, D.A.T. and James, W.P.T. 1975. Binding of calcium by dietary fiber: its relationship to unsubstituted uronic acids. Proc. Nutr. Soc. 34: 120A.

Brune, H. and Gunther, K. 1960. "Zur Frage der Wirksamkeit der Saponineals Antivitamin D." Z. Ernahrungs 2: 45.

Cheeke, P.R. 1976. Nutritional and physiological properties of saponins. Nutr. Rep. Int. 13: 315.

Coulson, C.B. and Evans, R.A. 1960. The effect of saponin, sterols and linoleic acid on the weight increase of growing rats. Br. J. Nutr. 14: 121.

Davies, N.T. and Nightingale, R. 1975. The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of zinc, copper, iron and manganese in rats. Br. J. Nutr. 34: 243.

Disler, P.B., Lynch, S.R., Torrance, J.D., Sayers, M.H., Bothwell, T.H. and Charlton. R.W. 1975. The mechanism of the inhibition of iron absorption by tea. S. Afr. J. Med. Sci. 40: 109. [In Chem. Abstr. 1976, 84(19) 101.]

Eis, F.G., Clark, L.W., McGinnis, R.A. and Alston, P.W. 1952. Floc in carbonated beverages. Ind. Eng. Chem. 44: 2844.

Ewart, A.J. 1931. The poisonous action of ingested saponins. Council Sci. Industr. Res. (Australia) Bull. No. 50.

Fransworth, N. 1966. Biological and phytochemical screening of plants. J. Pharm. Sci. 55: 225.

Morgan, B., Heald, M., Brooks, S., Tee, J. and Green, J. 1972. The interactions between dietary saponin, cholesterol, and related sterols in the chick. Poult. Sci. 51: 677.

Oberleas, D. 1973. In "Toxicants Occurring Naturally in Foods," 2nd ed, p. 363. National Academy of Sciences, Washington, DC.

Oberleas, D. and Prasad, A.S. 1969. Growth as affected by zinc and protein nutrition. Am. J. Clin. Nutr. 22: 1304.

Peterson, D.W. 1950. Effect of sterols on the growth of chicks fed high alfalfa diets or a diet containing quillaja saponin. J. Nutr. 42: 597.

Reshef, G., Gestetner, B., Birk, Y. and Bondi, A. 1976. Effect of alfalfa saponins on the growth and some aspects of lipid metabolism of mice and quails. J. Sci. Fd. Agric. 27: 63.

Searcy, V.V., Daub, G.H. and Riebsomer, J.L. 1967. "Introductory Qualitative Analysis." William C. Brown Co., Dubuque, IA.

Shaney, S., Birk, Y., Gestetner, B. and Bondi, A. 1970. Preparation, characterization and some properties of saponins from lucerne tops and roots. J. Sci. Fd. Agric. 21: 131.

Vogel, G. 1963. "Zur pharmakologie von saponinen," Planta Medica 12: 362.

West, L. 1977. Part 1. Toxic principles from higher plants: Epithelantha micromeris and Ilex opaca. Part 2. Studies on the hydrolysis of sinigrin in cole slaw and cabbage. Ph.D. thesis, Purdue University, West Lafayette, IN.

West, L. and Greger, J. 1977. Unpublished data.

Ms received 11/27/77; revised 2/11/78; accepted 2/15/78.

This work was supported by the Purdue Agricultural Experiment Station and is paper no. 6978. B.J. Nonnamaker acknowledges support of the AES Research Assistantship Grant Program.

Appreciation is expressed to Dr. Madis Laboratories, South Hackensack, NJ for the sample of ammoniated glycyrrhizin.

SAPONIN–VITAMIN COMPLEXATION . . . From page 1341

It is doubtful that growth-depression, reported in animals fed digitonin, quillaja bark saponins, and alfalfa leaf and root saponins, is due to poor absorption of fat-soluble vitamins as a result of the formation of saponin-vitamin addition complexes.

REFERENCES

Birk, Y. 1969. Saponins. In "Toxic Constituents of Plant Foodstuffs," p. 169. Academic Press, New York, NY.

Brune, H. and Gunther, K. 1960. "Zur Frage der Wirksamkeit der Saponineals Antivitamin D." Z. Ernahrungs 2: 45.

Cheeke, P.R. 1971. Nutritional and physiological implications of saponins: A review. Can. J. Anim. Sci. 51: 621.

Cheeke, P.R. 1976. Nutritional and physiological properties of saponins. Nutr. Rep. Int. 13: 315.

Coulson, C.B. and Evans, R.A. 1960. The effect of saponin, sterols and linoleic acid on the weight increase of growing rats. Br. J. Nutr. 14: 121.

Farnsworth, N. 1966. Biological and phytochemical screening of plants. J. Pharm. Sci. 55: 225.

Ishaaya, I., Birk, Y., Bondi, A. and Tencer, Y. 1969. Soybean saponins. Studies of their effect on birds, mammals and cold-blooded organisms. J. Sci. Fd. Agric. 20: 433.

Merck. 1968. "The Merck Index," 8th ed. Merck and Co., Rahway, NJ.

Morgan, B., Heald, M., Brooks, S., Tee, J. and Green, J. 1972. The interactions between dietary saponin, cholesterol, and related sterols in the chick. Poult. Sci. 51: 677.

Peterson, D.W. 1950. Effect of sterols on the growth of chicks fed high alfalfa diets or a diet containing quillaja saponin. J. Nutr. 42: 597.

Reshef, G., Gestetner, B., Birk, Y. and Bondi, A. 1976. Effect of alfalfa saponins on the growth and some aspects of lipid metabolism of mice and quails. J. Sci. Fd. Agric. 27: 63.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics," McGraw-Hill Book Co., Inc., New York, NY.

Tschesche, R. and Wulff, G. 1964. Konstitution und Eigenschaften Der Saponine. Planta Medica 12: 272.

Ms received 11/18/77; revised 1/31/78; accepted 2/8/78.

Appreciation is expressed to Dr. Madis Laboratories, South Hackensack, NJ for the sample of ammoniated glycyrrhizin.

This work was supported by the Purdue Agricultural Experiment Station and is paper no. 6975.

A Research Note

EFFECT OF COOKING ON VEGETABLE FIBER

V. MATTHEE and H. APPLEDORF

ABSTRACT

Carrots, cabbage, broccoli and okra, raw, short cooked, and long cooked, were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose by the detergent systems of analysis and crude fiber (CF) by standard methodology. On a dry weight basis, NDF, ADF, CF and cellulose value tended to increase upon cooking. Lignin and hemicellulose value were not affected. Most change was due to cellulose alone. While cooking changes the amount of fiber value present in vegetables studied, the changes may not be of physiological significance considering the small amounts of fiber present in and consumed from these vegetables.

INTRODUCTION

THERE HAVE BEEN few reports regarding the effect of cooking on dietary fiber. Hawk and Rehfuss (1926) noted that cooked vegetables showed a greater disintegration in the stomach than raw vegetables and Wyman et al. (1976) found that cooked bran had a smaller effect on fecal weight than did raw bran. McConnell et al. (1974) found that cooking did not affect the water-holding capacity of vegetable fiber, but had a varied effect on the cation-exchange capacity. Hellendoorn et al. (1975), using the enzymatic method for fiber determination as well as the AOAC crude fiber procedure, could not detect any difference in fiber content of human food after cooking and claimed that the improved digestibility was more physical than chemical in nature. Van Soest and Robertson (1976) reported the formation of indigestible residues during cooking such as tannins and products of the Maillard reaction which were isolated with lignin, causing an inflation of lignin values. As yet no detailed studies on the effect of cooking on the various fibrous fractions in vegetables have been reported. This study demonstrates the effect of cooking on the following fibrous fractions of four vegetables: neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, hemicellulose, cellulose and crude fiber (CF).

EXPERIMENTAL

CARROTS, cabbage, broccoli and okra were divided into three groups, raw, short cooked in boiling water for 15–20 min, and long cooked in boiling water for 45–50 min simulating home preparation techniques. Following cooking, the vegetables were homogenized in a Waring Blendor, frozen overnight at −15°C, and freeze dried at 45°C for 72 hr. The freeze-dried samples were milled through a 1 mm (20 mesh) screen and packed airtight in polyethylene bags.

The detergent systems for fiber analysis developed by Goering and Van Soest (1970) were used to determine NDF, ADF, lignin, hemicellulose and cellulose values. Glass wool was used as a matting with the NDF procedure in order to facilitate filtration. The AOAC standard method for crude fiber determination was used to determine the crude fiber content of the vegetables (AOAC, 1970). Each procedure for analysis was replicated five times except for short cooked cabbage where only four replications were performed. Due to the hygroscopic nature of the dry samples, a moisture determination was made simultaneously with every fiber analysis.

Analysis of variance was performed on the data for NDF, ADF, lignin, cellulose and crude fiber, followed by a two-tailed t-test after significance at the 0.05 level (Steel et al., 1960). The data for hemicellulose were anlayzed by using Satterthwaite's approximation (Mendenhall, 1968). Missing data were estimated according to a method developed by Yates (Steel and Torrie, 1960).

Table 1—Cooking times and moisture content of vegetables samples

Vegetable and treatment	Cooking times (min)	Moisture (%)
Carrot		
Raw	0	88.4
Short cooked	20	91.6
Long cooked	50	92.5
Cabbage		
Raw	0	92.2
Short cooked	15	93.4
Long cooked	45	93.9
Broccoli		
Raw	0	91.8
Short cooked	15	93.9
Long cooked	45	93.6
Okra		
Raw	0	90.3
Short cooked	15	92.1
Long cooked	45	93.4

RESULTS & DISCUSSION

TABLE 1 shows the changes in moisture content during cooking as well as the individual cooking times. All vegetables except broccoli showed an increase in moisture content with lengthened cooking time. The moisture content of broccoli, however, decreased slightly after the longer cooking period. McCance et al. (1936) attributed weight gain during boiling to hydration and weight loss to the collapse of lignocellulose walls with subsequent extrusion of cellular fluids. Simpson and Halliday (1961) found that moisture content increased with short cooking periods and decreased with longer cooking. Values of moisture content found in this study resemble those previously reported by Munsell et al. (1949), Van Duyne et al. (1951) and Adams (1975).

The effect of cooking on vegetable fibrous fractions is shown on a dry weight basis in Table 2. In carrots, ADF and CF values increased significantly after each cooking period, NDF and cellulose values increased only after the long cooking period, whereas lignin and hemicellulose values were not affected by cooking.

In cabbage, cellulose and CF values increased significantly with both cooking periods, but NDF, lignin, and hemicellulose values did not change.

The results for broccoli tended to be more varied than

Author Appledorf is with the Dept. of Food Science & Human Nutrition, University of Florida, Gainesville, FL 32611. Author Matthee, formerly with the Univ. of Florida, is now with the Dept. of Home Economics, University of Stellenbosch, Stellenbosch, Republic of South Africa.

0022-1147/78/0004-1344$02.25/0
© 1978 Institute of Food Technologists

those observed with carrots and cabbage. NDF and cellulose values both increased with cooking, but the length of the cooking period had not influence on the increases. ADF and CF values however increased with each cooking period, whereas hemicellulose values were not affected. Lignin values increased only after the long cooking period. This could be due to the formation of other indigestible residues during cooking which are isolated together with lignin. Products such as tannin-protein condensations and products of nonenzymatic browning have been reported by Van Soest and Robertson (1976).

In okra, NDF, cellulose and CF values increased only after the long cooking period whereas the ADF values increased at all levels. Lignin and hemicellulose values were not affected by cooking.

Values for the various detergent fiber fractions for carrot, cabbage and broccoli (Table 2) were higher than those reported by Van Soest and Robertson (1976). It was noted the crucibles lost weight during the procedures and could result in inflated values. A statistical analysis showed that the weight loss variance did not significantly influence the treatment variance and would not substantially affect the significance or nonsignificance of the various treatments.

Comparison between NDF and CF on a dry weight basis for the various vegetables were all significant at the 0.05 level. The NDF values were all higher than the CF values and confirms previous observations that CF does not give a good estimation of the total amount of fiber present (Williams and Olmsted, 1935; Van Soest and McQueen, 1973). Losses of 20–50% cellulose, 80% hemicellulose and 50–90% lignin have been reported (Van Soest and Robertson, 1976) and it has been suggested that the NDF values should replace the existing CF values of food tables (Spiller and Amen, 1975; Watt and Merrill, (1963).

Since the NDF fraction consists of cellulose, hemicellulose, and lignin, and the ADF fraction consists of cellulose and lignin, the changes noted in NDF and ADF in this study are due to changes in cellulose alone. Hemicellulose and lignin fractions were generally unaffected by cooking.

Simpson and Halliday (1941) performed chemical and histological experiments on fresh carrots and found that cellulose and pectin disintegrated during cooking. Histological studies showed that cell walls in raw carrots were thick and continuous, but as cooking increased from 15 to 45 minutes, the cell walls became thinner and more fragile. It was postulated that the cellulose might either have been liberated from the surrounding material or hydrolyzed during the cooking process. The increase in cellulose value observed on a dry weight basis might be a result of the liberation of cellulose making it more available for analysis.

Table 2–Effect of cooking on vegetable fiber[a]

Vegetable	Fibrous fraction	% Dry weight		
		Raw	Short cooked	Long cooked
Carrots	NDF	12.06 ± 0.98a	14.02 ± 2.06ab	15.44 ± 2.81b
	ADF	11.02 ± 0.56a	12.36 ± 0.66b	13.95 ± 0.36c
	Lignin	1.32 ± 0.61a	1.52 ± 0.39a	1.36 ± 0.12a
	Hemicellulose	1.04 ± 1.13a	1.66 ± 2.16a	1.48 ± 2.82a
	Cellulose	9.70 ± 1.14a	10.84 ± 1.04ab	12.59 ± 0.25b
	CF	5.22 ± 0.30a	6.81 ± 0.35b	7.52 ± 0.19c
Cabbage	NDF	18.69 ± 1.47a	20.50 ± 2.60a	21.64 ± 2.26a
	ADF	12.30 ± 0.48a	15.46 ± 0.77b	18.54 ± 0.93c
	Lignin	1.26 ± 0.45a	1.27 ± 0.35a	1.51 ± 0.26a
	Hemicellulose	6.38 ± 3.46a	5.04 ± 2.71a	3.10 ± 2.44a
	Cellulose	11.04 ± 0.70a	14.35 ± 0.89b	17.04 ± 0.99c
	CF	6.61 ± 0.10a	8.46 ± 0.06b	10.70 ± 0.3lc
Broccoli	NDF	18.59 ± 1.47a	21.78 ± 1.70b	22.78 ± 3.01b
	ADF	15.96 ± 0.76a	18.32 ± 0.65b	19.21 ± 1.04c
	Lignin	1.73 ± 0.13a	2.26 ± 0.23a	3.03 ± 0.89b
	Hemicellulose	2.63 ± 1.65a	3.46 ± 1.82a	3.57 ± 3.18a
	Cellulose	14.23 ± 0.74a	16.06 ± 0.67b	16.17 ± 1.22b
	CF	8.90 ± 0.18a	11.14 ± 0.30b	11.99 ± 0.52c
Okra	NDF	14.79 ± 1.80a	16.48 ± 0.68a	18.87 ± 1.62b
	ADF	10.30 ± 0.45a	11.84 ± 0.31b	13.24 ± 0.83c
	Lignin	1.46 ± 0.23a	2.44 ± 1.53a	1.97 ± 0.44a
	Hemicellulose	4.49 ± 1.86a	4.64 ± 0.74a	5.63 ± 1.82a
	Cellulose	8.86 ± 0.61a	9.40 ± 1.61a	11.27 ± 0.50b
	CF	6.82 ± 0.62a	7.14 ± 0.72a	7.67 ± 0.47b

[a] Mean ± standard deviation values followed by the same letters are not different ($P < 0.05$).

REFERENCES

Adams, C.F. 1975. Nutritive value of American foods in common units. U.S.D.A. Agriculture Handbook No. 456. U.S. Gov't Print. Off., Washington, D.C.

AOAC. 1970. Crude fiber. "Official Methods of Analysis," 11th ed. 7.053, p. 129. Association of Official Agricultural Chemists, Washington, D.C.

Goering, H.K. and Van Soest, P.J. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications. Agr. Handbook No. 379. ARS/USDA, Washington, D.C.

Hawk, P.B. and Rehfuss, M.F. 1926. The response of the normal human stomach to various standard foods and a summary. Am. J. Med. Sci. 171: 359.

Hellendoorn, G.W., Noordhof, M.G. and Slagman, J. 1975. Enzymatic determination of the indigestible residue (dietary fiber) content of human food. J. Sci. Fd. Agric. 26: 1461.

McCance, R.A., Widdowson, E.M. and Shakelton, L.R.B. 1936. The nutritive value of fruits, nuts and vegetables. Med. Res. Coun. Spec. Rep. Ser. No. 213. HMSO, London.

McConnell, A.A., Eastwood, M.A. and Mitchell, W.D. 1974. Physical characteristics of vegetable foodstuffs that could influence bowel movement. J. Sci. Fd. Agric. 25: 1457.

Mendenhall, W. 1968. "Design and Analysis of Experiments." Duxbury Press, Belmont, CA.

Munsell, H.F., Streightoff, F., Bandor, B., Orr, M.L., Ezekiel, S.R., Leonard, M.H., Richardson, M.E. and Koch, F.G. 1949. Effect of large scale methods of preparation on the vitamin content of food. 3. Cabbage. J. Am. Dietet. Assn. 25: 420.

Simpson, J.I. and Halliday, E.G. 1941. Chemical and histological studies of the disintegration of cell-membrance materials in vegetables during cooking. Food Research 6: 189.

Spiller, G.A. and Amen, R.J. 1975. Dietary fiber in human nutrition. CRC Crit. Rev. Food Sci. Nutr. 7: 39.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., New York.

Van Duyne, F.O., Owen, R.F., Wolfe, J.C. and Charles, V.R. 1951. Effect of cooking vegetables in tightly covered and pressure saucepans. Retention of reduced ascorbic acid and palatability. J. Am. Diet. Assn. 27: 1059.

Van Soest, P.J. and McQueen, R.W. 1973. The chemistry and estimation of fibre. Proc. Nutr. Soc. 32: 123.

Van Soest, P.J. and Robertson, J.B. 1976. Analytical problems of fiber. Presented at the 36th Annual Meeting of the Institute of Food Technologists, June 6-9, 1976 at Anaheim, CA.

Watt, B.K. and Merrill, A.L. 1963. Composition of Foods - raw, processed, prepared. USDA Agriculture Handbook No. 8. U.S. Gov't Print. Off., Washington, DC.

Williams, R.D. and Olmsted. W.H. 1935. A biochemical method for determining indigestible residue (crude fiber) in feces: lignin, cellulose and non-water soluble hemicellulose. J. Biol. Chem. 108: 653.

Wyman, J.B., Heaton, K.W., Manning, A.P. and Wicks, A.C.B. 1976. The effect of intestinal transit and the feces of raw and cooked bran in different doses. Am. J. Clin. Nutr. 29: 1474.

Ms received 10/5/77; revised 12/23/77; accepted 12/30/77.

Florida Agricultural Experiment Stations Journal Series No. 741.

A Research Note

ODOR THRESHOLD OF 2,3,6-TRICHLOROANISOLE IN WATER

D. G. GUADAGNI and R. G. BUTTERY

ABSTRACT

The odor threshold of pure 2,3,6-trichloroanisole was determined in water solution using trained and untrained panelists. The values obtained with the trained and untrained panel were 7.4 and 24.5 ppt (parts per 10^{12} parts of solvent) respectively. Trichloroanisole samples prepared in two different laboratories did not result in significantly different threshold values. These values are about 4 orders of magnitude higher than those found in the literature for this compound.

INTRODUCTION

SINCE CURTIS et al. (1972) first reported the extremely low detection threshold of 3×10^{-4} ppt for 2,3,6-trichloroanisole (TCA) (3×10^{-16} parts of compound per part of water), this compound has been referred to as the most potent known odor compound (Griffiths, 1974). Recently, Frijters and Bemelmans (1977) reported on the flavor thresholds of a number of tri- and tetrachloroanisoles, including TCA in coagulated egg yolk. As expected, these authors found much higher values in egg yolk, and give a good discussion of the possible reasons for the higher values in egg yolk as compared to water solutions. They indicate their values were at least 10^3 times higher than those reported for water solutions. However, comparison of the threshold values for TCA in egg yolk and water (Tables 1 and 2 of their publication) gives a factor of $1.2 \times 10^{-7}/3.0 \times 10^{-16} = 4 \times 10^8$ which is obviously far greater than 10^3 times higher for egg yolk than water.

Flavor thresholds of various compounds in potato products (Guadagni et al., 1971) gave a factor ranging from 10^1 to 6×10^4 times higher for the threshold in potatoes as compared to the threshold in water. The highest factor of 6×10^4 was observed for the compound with the lowest threshold in water which was 2 ppt (2 parts in 10^{12} parts of water). However, this factor is still about 4 orders of magnitude lower than that calculated from the reported thresholds for TCA. In an attempt to clarify the large differences in these factors, the threshold of TCA from two different sources was determined in water solution.

MATERIALS & METHODS

Sample preparation

TCA was synthesized from a reliable commercial sample of 2,3,6-trichlorophenol (Aldrich No. 15,158-0) by methylation with diazomethane. The TCA so formed was purified first by recrystallization then by gas liquid chromatography (GLC) separation. Gas liquid chromatography and NMR analysis indicated purity was in excess of 99.9%. No trace of impurities was detected. Mass and NMR spectra of the purified sample were consistent with the 2,3,6-trichloroanisole structure. The second sample of TCA was supplied as a courtesy by a different laboratory.

The purified TCA samples were dissolved in ethanol. The ethanol solution was thoroughly mixed with triple distilled water to give TCA concentrations from 1×10^{-3} to 1×10^3 ppt. One hundred ml of the various dilutions was measured into clean odor free 8 oz. Teflon squeeze bottles equipped with Teflon tubes. The Teflon bottles were cleaned in 1% TSP and detergent solution in an ultrasonic cleaner for 5 minutes followed by thorough rinsing in odor free distilled water. No residual odors were detectable in the bottles after cleaning. Each of the bottles containing TCA was paired with another bottle containing the same volume of triple distilled water and alcohol equal to that in the TCA bottle (<50ppm).

Authors Guadagni and Buttery are with the USDA Western Regional Research Laboratory, ARS, 800 Buchanan St., Berkeley, CA 94710.

Table 1—Thresholds of aqueous solutions of 2,3,6-Trichloroanisole obtained with trained and untrained panels

TCA sample	Panel	N'[b]	Threshold (ppt)[a]	95% confidence limits
Laboratory A	trained	164	7.4	5.5–9.9
Laboratory A	trained	352	7.2	6.1–8.5
Laboratory A	untrained	172	24.5	19.2–31.3
Laboratory B	untrained	340	27.0	22.0–33.1

[a] Parts of TCA per 10^{12} parts of solvent

[b] N' represents the number of judgments falling between 16 and 84% in the Litchfield and Wilcoxon procedure.

Sensory evaluation

Two groups of panelists were used and both individual and group thresholds were obtained for both groups. The first group consisted of 22 members with extensive odor evaluation experience and training, and the second group consisted of 23 randomly selected untrained individuals.

Each panelist was presented with one pair of bottles in midmorning and midafternoon to avoid the possibility of adaptation to TCA between samples. Position of the TCA samples in each pair was balanced and concentration order was randomized. Each concentration was replicated at least 3 times over a range of 5 concentrations. The task for each panelist was to determine which of the coded bottles contained the odorant. The sensory data obtained by this procedure was corrected for chance, and the thresholds and 95% confidence limits were determined by the Litchfield and Wilcoxon (1949) procedure as described previously (Guadagni et al., 1973).

RESULTS & DISCUSSION

THE GROUP THRESHOLD results shown in Table 1 indicate a threshold of 7.2–7.4 for the trained panel with a 95% confidence interval of 5.5–9.9 ppt (parts of TCA per 10^{12} parts of solvent). The untrained panel was significantly less sensitive in the detection of this compound with a threshold value of 24.5 and confidence limits of 19.2–31.3 ppt. These values were all obtained from the same TCA sample synthesized in laboratory A, and thus the difference in thresholds can be ascribed to panel differences rather than sample differences. The sample from laboratory B gave a threshold of 27.0 and confidence limits of 22.0–33.1 ppt with the untrained panel, indicating no significant difference between TCA samples. It is also apparent that increasing N' approximately twofold did not significantly alter the threshold values.

As a further check on the range of sensitivity to TCA, individual thresholds for each of the trained and untrained panelists are shown in Figure 1. While the data for the untrained group is somewhat more scattered, the individual thresholds of TCA generally follow a log-normal distribution for both groups. The most sensitive 10% of the panelists could detect about 1.5 ppt and the least sensitive 5–20% required 50 ppt for significant detection. However, this indicates that a concentration of TCA from 1–50 ppt would encompass the threshold for practically all of this sample population.

The group threshold of 7.2 ppt for trained panelists is $7.2 \times 10^{-12}/2.0 \times 10^{-16} = 2.4 \times 10^{4}$ times higher than the value reported by Curtis et al. (1972). This difference is far greater than the differences found between trained and untrained panelists or between the most and least sensitive panelist of either group. It is difficult to explain this unusually large difference in threshold, especially since compound purity and panel procedures were similar. Our figure of 7.2 ppt for TCA with the trained panel is about 3–4 times higher than the threshold of 2 ppt which we reported for 2 methoxy-3-isobutylpyrazine (Buttery et al., 1969) and 2 methoxy-3-isopropylpyrazine (Seifert et al., 1970). Using Frijters and Bemelmans (1977) figure for TCA in egg yolk and our values in water with the untrained panel, we obtain factors of roughly 4 to 5×10^{3} times higher thresholds in egg yolks than in water. This is well within the range of values obtained for potato products. It is also interesting to note that the threshold of TCA in egg yolks (0.12 ppm) compares rather well with thresholds of the methoxypyrazines in potato products (0.03 to 0.12 ppm). In view of this evidence, it appears unlikely that TCA is as potent an odorant as was originally claimed.

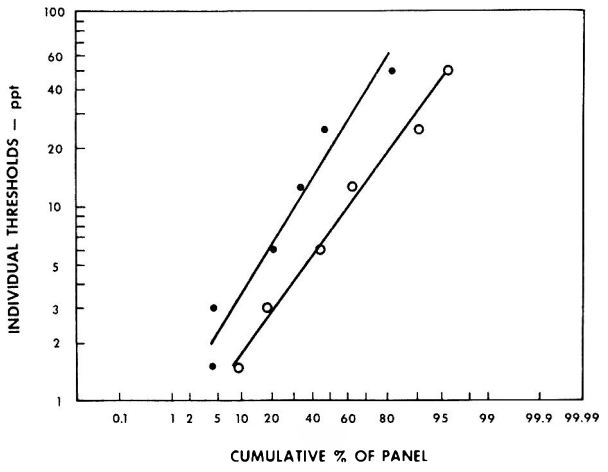


Fig. 1—Distribution of individual thresholds for TCA using trained and untrained panelists. ○-trained, N = 22. ●-untrained, N = 23.

REFERENCES

Buttery, R.G., Seifert, R.M., Guadagni, D.G. and Ling, L.C. 1969. Characterization of some volatile constituents of Bell Peppers. J. Agr. Food Chem. 17: 1322.

Curtis, R.F., Land, D.G., Griffiths, N.M., Gee, M., Robinson, D., Peel, J.L., Dennis, C. and Gee, J.M. 1972. 2,3,4,6-Tetrachloroanisole association with musty taint in chickens and microbiological formation. Nature 235: 223.

Frijters, J.E.R. and Bemelmans, J.M.H. 1977. Flavor sensitivity for chloroanisoles in coagulated egg yolk. J. Food Sci. 42: 1122.

Griffiths, N.M. 1974. Sensory properties of the chloroanisoles. Chem. Senses Flavor 1: 187.

Guadagni, D.G., Buttery, R.G., Seifert, R.M. and Venstrom, D.W. 1971. Flavor enhancement of potato products. J. Food Sci. 36: 363.

Guadagni, D.G., Maier, V.P. and Turnbaugh, J.G. 1973. Effect of some citrus juice constituents on taste thresholds for limonin and naringin bitterness. J. Sci. Fd Agric. 24: 1277.

Litchfield, J.T. Jr. and Wilcoxon, F. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96: 99.

Seifert, R.M., Buttery, R.G., Guadagni, D.G., Black, D.R. and Harris, J.G. 1970. Synthesis of some 2-methoxy-3-alkylpyrazines with strong Bell Pepper-like odors. J. Agr. Food Chem. 18: 246.

Ms received 11/11/77; revised 1/16/78; accepted 1/30/78.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

A Research Note

CHANGES IN THE FREE FATTY ACID AND AMMONIA CONTENT OF BEEF DURING AGING AND PROCESSING

G. M. BROOKS and G. R. AMMERMAN

ABSTRACT

This study was to evaluate the effect of equal F_o's at 104.4°, 115.6°, and 126.7°C on the free fatty acid and ammonia of aged and nonaged beef. The process of 126.7°C of the 5-day product resulted in a significantly lower free fatty acid. Processing reduced free fatty acids in the 1-day product. There was no significant difference in free fatty acid due to age between the 1- and 5-day products except for the 104.4°C process. The ammonia-in-water extracts of beef were not significantly different among the control and the processes. The longer process at 104.4°C resulted in significantly higher ammonia in the cooked beef extract.

INTRODUCTION

IT IS WELL KNOWN that retorted uncured canned meats do not taste the same as a freshly broiled meat.

According to Landmann and Batzer (1966), meat flavor consisted of four basic components, the nonvolatile and volatile fractions from both raw and cooked meat. They also stated that canning altered the quantitative relationships of the flavor systems by the addition of extraneous chemical substances resulting from unavoidable chemical changes produced by processing. These workers concluded that cooked meat flavor was far more complex than raw flavor.

Luh et al. (1964) concluded that aseptically canned foods were subjected to less heat damage than the retorted products.

Many investigators found that the elementary meaty flavor was associated with water-soluble components. Hornstein et al. (1960) found that the flavor precursors of beef were water soluble.

Macy et al. (1964, 1970) in a series of articles outlined the importance of water-soluble constituents as precursors of cooked meat flavor. Kramlich and Pearson (1954) indicated that many flavor contributors were water soluble.

In earlier work Olson et al. (1959) found that samples with high food acceptance scores by a taste panel generally contained relatively low concentrations of hydrogen sulfide and ammonia.

Ziemba and Malkki (1971) found an increase in hydrogen sulfide, methyl mercaptan and dimethyl sulfide in canned beef with increasing processing times.

The purpose of this study was to examine the level of free fatty acids and ammonia produced from 1-day and 5-day old beef subjected to equivalent processes at 104.4°, 115.6° and 126.7°C as compared to an unprocessed control.

EXPERIMENTAL

THE EXPERIMENTAL DESIGN was a 2 × 2 × 4 completely randomized factorial replicated four times. The treatments were 1-day and 5-day meat, beef chunks and a water extract of the beef, and control and heat processes yielding equivalent lethal heat treatments (F_o = 6.0) at 104.4°, 115.6° and 126.7°C. The data were evaluated by analysis of variance and, where necessary, the Duncan New Multiple Range Test.

Preparation of samples of beef

Approximately 200 lb of lean beef were secured from a local packing house. One hundred pounds of the beef (24-hr postmortem) was boned, trimmed and cut into 3-in. chunks. This lot of beef was labeled "1-day beef" and was put into the freezer at -29°C within 24 hr after slaughter. It was held at -18°C after freezing until needed for heat penetration studies or processing, at which time it was partially thawed at room temperature and used.

The other 100-lb lot of lean beef, after being boned, trimmed and cut into 3-in. chunks, was held at 3.3°C for five days. This beef was then frozen at -29°C and stored at -18°C until used as needed. This lot of beef was called "5-day beef."

Closing and filling

Selected amounts of "1-day" and "5-day beef" were partially thawed. It was cut into 1-in. chunks, and cans were filled to 95% of capacity with 431g of meat. Approximately 80 ml of water were added to each 303 × 406 can, and they were closed by use of an American Can Steam-Flo closing machine. Twenty-four cans were immediately processed. All cans of beef were treated in an identical manner with the exception of cans used for the heat penetration study; in these cans thermocouples were placed in the cold-spot of the cans before closing.

Process times and heat penetration data

Process temperatures of 104.4°C and 126.7°C and nonprocessed controls were used. These temperatures were selected to provide a great degree of variation in process time while yielding equivalent lethal cooks in terms of sterilizing values (F_o's). Using a z value of 18, a process time was calculated for each temperature so as to result in equivalent F_o's.

Six heat penetration curves were obtained for each temperature and each product, and the slowest heating and most rapid cooling curve was used in each to compute the process. The processes calculated were 303 min at 104.4°C, 51 min at 115.6° and 37 min at 126.7°C.

Preparation of liquid extracts

Because many of the flavor precursor components of beef were thought to be water soluble, water extracts of both lots of beef were prepared (Kramlich and Pearson, 1954; Hornstein et al. 1960; Zaika, 1969; Macy et al. 1970). One kilogram of partially thawed 1-in. chunks of raw beef was blended with 4L of distilled water in a commercial blender for 2 min. It was then filtered through cheesecloth, and centrifuged 20 min at 2,000 rpm. This process yielded about 2.6L of clear extract.

Processing and storage of test cans

Cans of the beef chunks, and cans of water extracts were exposed to the calculated process. All cans were quick-frozen and held at -18°C until needed for analysis.

Treatment of raw product

Beef was taken from storage at -18°C and allowed to partially thaw. Four hundred thirty-one grams of beef and 80 ml of water were placed in the blender and blended for 2 min. after which it was placed in ½-pt jars and frozen at -18°C. The unprocessed product served as the control for the lots processed at 104.4°, 115.6° and 126.7°C.

Free fatty acid

Ground samples of beef were formed into patties about 3–4 in. in diameter and ½–1 in. thick. This material was frozen at -18°C for 48 hr, placed in a freeze dehydration unit and held for 48 hr at a pressure of 5–10 microns. After lyophilization was complete, samples were crumbled by hand and placed into fat extraction thimbles 20 × 80 mm in size. They were extracted with Skelly Solvent F for 6 hr into 100 ml soxhlet flasks. Excess solvent was removed by holding the samples on

Author Brooks is a Food Technologist, Food & Fiber Center, Mississippi Cooperative Extension Service, Mississippi State University, Mississippi State, MI 39762. Author Ammerman is Professor of Food Technology and Coordinator, Food Science Institute, Mississippi State University, Mississippi State, MI 39762.

0022-1147/78/0004-1348$02.25/0
© 1978 Institute of Food Technologists

Table 1—Effect of processing on the percent of free fatty acid in the fat of 1- and 5-day beef[a]

	Free fatty acids (% Oleic)			
		Process °C		
Age	Unprocessed	104.4	115.6	126.7
1-Day	2.27 aA	1.53 bB	1.70 bA	1.54 bA
5-Day	1.92 aA	2.11 aA	1.78 aA	1.19 bA

[a] Means within a row not followed by the same lower case letter differ significantly ($P < 0.01$) according to Duncan's New Multiple Range Test. Means within a column not followed by the same capital letter differ significantly ($P < 0.01$) according to Duncan's New Multiple Range Test.

Table 2—Effect of processing on the mean ammonia content of 1-day and 5-day beef product values[a]

	Ammonia content (ppm)			
		Process °C		
Product	Unprocessed	104.4	115.6	126.7
Extract	125 aA	118 aB	96 aB	92 aB
Beef	2'1 cA	859 aA	422 bA	441 bA

[a] Means within a row not followed by the same lower case letter differ significantly ($P < 0.01$) according to Duncan's New Multiple Range Test. Means within a column not followed by the same capital letter differ significantly ($P < 0.01$) according to Duncan's New Multiple Range Test. The standard error of the means = 36.63 ppm.

the steam bath just long enough to remove the solvent. Samples were then placed in 100°C forced draft over 10 min for moisture removal. Samples were removed, allowed to cool in a desiccator and weighed. Lipids were calculated and expressed as percent by weight on dry weight basis.

The free fatty acids were determined by the American Oil Chemist Society method Ca 5a-40 (1971).

Ammonia

Five grams of sample were prepared in a blender and placed in a 600 ml Kjeldahl flask with 200 ml of distilled water, 5g of carbonate-free magnesium oxide, and several drops of Dow antifoam A were added. One hundred milliliters were diluted into 0.1N HCl and titrated with 0.1N NaOH using methyl red as the indicator. The procedure used was a modified AOAC procedure (1970).

RESULTS & DISCUSSION

Free fatty acid

The free fatty acid content of the 1-day beef was significantly lower in the three processes as compared with the unprocessed control, but there were no significant differences among processes when $F_0 = 6.0$ was given at 104.4°, 115.6° and 126.7°C (Table 1). No significant differences in free fatty acid content were observed in the 5-day beef among the control and the processes $F_0 = 6.0$ at 104.4° and 115.6°C. However, the process $F_0 = 6.0$ at 126.7°C, resulted in a significantly lower mean free fatty acid content in the 5-day beef.

No change in the free fatty acid content of the unprocessed control occurred during storage. The fact that no significant changes in free fatty acid content occurred in the unprocessed control and 104.4° and 115.6°C processes of the 5-day beef indicated some stabilization of free fatty acids during storage. However, it appeared that the aged beef was more susceptible to conversion to carbonyl compounds at 126.7°C than at the other two processes. The levels of free fatty acids in the fat extracted from the beef compared very favorably with the mean values found by Luh et al. (1964). These workers found 1.77% FFA as oleic.

Ammonia

The ammonia content of the extracts was not significantly changed by processing. The process which gave an $F_0 = 6.0$ at 104.4°C resulted in significantly larger ammonia values than were found in the unprocessed beef control. No significant difference in ammonia occurred due to the equivalent processes at 115.6° and 126.7°C in the canned beef. There was no significant difference between extract and beef ammonia content of the unprocessed control; but for all three processes, the ammonia content of the beef was significantly higher than in the extract. No significant difference was noted in ammonia content due to age.

It has been shown that ammonia was produced by hydrolysis of the dicarboxylic amino acids in protein (Schultz et al., 1967). Ammonia in the beef sample processed at 104.4°C as compared with the unprocessed beef control and the samples processed at 115.6° and 126.7°C was expected to be higher because more than 5 hr at 104.4° were required to accumulate a lethality of $F_0 = 6.0$.

It was expected that the unprocessed beef control would be lower in ammonia than the three processed samples due to the effect of the heat treatments. Values of ammonia were slightly higher in this study than values observed on various canned products by Olson et al. (1959).

REFERENCES

AOAC 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.
AOCS. 1971. "Official and Tentative Methods of the American Oil Chemists Society," 3rd ed. American Oil Chem. Society, Champaign, IL.
Hornstein, I., Crowe, P.F. and Sulzbacher, W.L. 1960. Constituents of meat flavor: Beef. Agric. & Food Chem. 8(1): 65.
Kramlich, W.E. and Pearson, A.M. 1954. Some preliminary studies of meat flavor. Food Res. 23: 567.
Landmann, W.A. and Batzer, O.F. 1966. Influence of processing procedures on the chemistry of meat flavor. J. Agric. Food Chem. 14: 210.
Luh, B.S., Gonzales-Acuna, C.G., Leonard, S. and Simone, M. 1964. Aseptic canning of foods, Food Technol. 18: 212.
Macy, R.L. Jr., Naumann, H.D. and Bailey, M.E. 1970. Water-soluble flavor and odor precursors of meat. 3. Changes in nucleotides, total nucleosides and bases of beef, pork and lamb during heating. J. Food Sci. 35: 78.
Macy, R.L. Jr., Naumann, H.D. and Bailey, M.E. 1964. Water-soluble flavor and odor precursors of meat. J. Food Sci. 29: 136.
Macy, R.L. Jr., Naumann, H.D. and Bailey, M.E. 1970. Water soluble flavor and odor precursors of meat. 4. Influence of cooking on nucleosides and bases of beef steaks and roasts and their relationship to flavor, aroma and juiciness. J. Food Sci. 35: 81.
Olson, L.E., Greenwood, D.A., Nielson, H.M. and Wilcox, E.B. 1959. Some techniques for evaluating the flavor of processed meat. Food Res. 24: 696.
Schultz, H.S., Day, E.A. and Libbey, L.M. 1967. Flavor of red meats. In "The Chemistry and Physiology of Flavors," p. 241. Avi Publishing Company, Inc, Westport, CT.
Zaika, L.L. 1969. Meat flavor. Method of rapid prepartation of the water-soluble low molecular weigh-fraction of meat tissue extracts. J. Agric. Food Chem. 17(4): 893.
Ziemba, Z. and Malkki, Y. 1971. Changes in odour components of canned beef due to processing. Lebensm-Wiss. u. Technol. 4: 118.
Ms received 10/8/77; revised 12/24/77; accepted 12/30/77.

A Research Note

NUTRITIONAL EVALUATION OF FULL-FAT SOY FLOUR PRODUCED BY DRY HEAT ROASTING

G. RICHARD JANSEN, J. M. HARPER and LOUISE A. O'DEEN

ABSTRACT

Full fat soy flour was made by roasting whole soybeans in a heated agitated salt bed followed by dehulling and grinding. PER's of 2.11–2.31 compared to 2.50 for reference casein were achieved with roasting bed temperatures of 206–234°C and treatment times of 15–24 sec. The process reduced the level of anti-trypsin activity 75–90% under these roasting conditions. The results demonstrate the utility of this dry heat process in making full-fat soy flour with a high protein quality. The potential advantages of the process in developing countries are discussed.

INTRODUCTION

IT IS KNOWN that soy proteins have a high nutritional quality and that satisfactory infant growth is obtained by feeding a soy based formula, even without supplemental methionine (Fomon, 1959). In connection with nutritional problems in less developed countries, emphasis has recently shifted from protein quality to energy but in reality, both protein and energy are of considerable importance in feeding young children (Jansen, 1977).

Because food energy is generally deficient in the developing world and is probably the most serious problem, utilizing both the oil and the protein content of the soybean in human nutrition would be of obvious nutritional advantage. Mustakas et al., (1964, 1970) described an extrusion process for making a full-fat soy flour with a protein efficiency ratio (PER) of 2.15 compared to 2.50 for casein. This product was reported to be comparable to casein in growth studies in human infants (Mustakas, 1971).

In contrast to the extrusion process, Harper and Lorenz (1974) have described a dry roasting process for the production of full-fat soy flour with excellent bread baking qualities, utilizing a bed of hot agitated salt. In the present paper, data are presented on the nutritional evaluation of full-fat soy flour produced by this dry heat process. In addition, the potential advantages and applications of this type of "intermediate technology" in less developed countries are discussed.

EXPERIMENTAL

WHOLE #1 SOYBEANS, 60 lb test weight with an initial moisture <12% were used in the roasting studies. A double barrelled horizonal agitated granular bed roaster manufactured by Food Processes, Inc., Frisco, CO, was used to roast the soybeans. One bed was used for heating the salt bed to temperature and the other to roast the soybeans. Residence time in the contacting drum was maintained by an internal helix within the drum whose speed of rotation was precisely controlled. Salt bed temperatures, measured with thermocouples, were varied from 178–234°C resulting in roasted bean temperatures, also measured with thermocouples, from 115–130°C. Dehauling was accomplished by cracking the bean with a roll mill whose gap was slightly less than the smallest bean dimension. Winnowing removed the cracked hulls from the bean halves. Grinding was done in a Fitzmill #0 hammer mill to approximately 80 mesh or less. The resulting full-fat soy flours were fortified with 0.1% vitamin premix and 2.7% mineral premix furnishing levels of these nutrients recommended for Title II blended foods by the USDA (1973). A Hobart blender was used to mix the flour with the premixes.

Proximate analysis, including moisture, fat, nitrogen, ash and crude fiber, was carried out using standard procedures (AACC, 1969). The amount of anti-trypsin activity was determined by the official AOCS procedure (AOCS, 1975) and protein efficiency ratios (PER's) were measured using the AOAC method in a four week assay (AOAC, 1975). In the PER studies, corn oil was substituted for cottonseed oil, and vitamins and minerals were supplied by Vitamin Diet Fortification Mixture and salt mixture USP XIV respectively, both obtained from ICN Pharmaceuticals Inc., Cleveland, OH. All PER's were corrected relative to an assigned value of 2.50 for Animal Nutrition Research Council (ANRC) reference casein obtained from Sheffield Chemical Co., Norwich, NY. Significant differences were determined by application of Duncan's multiple range and multiple F test (Duncan, 1955).

RESULTS & DISCUSSION

THE VITAMIN and mineral fortified full-fat soy flours averaged 5.1 ± 0.3% moisture, 20.5 ± 0.3% fat, 36.6 ± 0.2% protein (N × 6.25), 6.9 ± 0.1% ash and 2.5% fiber. The mineral supplement supplied 40% of the ash recovered.

The results of the nutritional evaluation of the roasted beans are presented in Table 1. Roasting soybeans at 178°C for 15–24 sec only slightly increased the corrected PER from 1.49 for raw beans to 1.62–1.67 for the roasted beans. However, roasting at 206°C for 22 sec gave a corrected PER of 2.20 and roasting at 234°C for 15 sec gave a corrected PER of 2.31. A reasonable relationship was observed between PER and anti-trypsin activity in the roasted soybeans. For example, flours with low PER's (1.62–1.67) were associated with 36–47% losses of anti-trypsin activity, and flours with high PER's (2.11–2.31) were associated with enzyme inhibitor losses of 75–90%. This work is generally consistent with the earlier work of Rackis et al. (1975) in which it was observed that if at least 55% destruction of the anti-trypsin activity was achieved, no pancreatic hypertophy occurred. The relationship between protein utilization and enzyme inhibition is not completely clearcut, however. We previously have shown that full-fat soy flour made on a low cost extruder cooker gave a corrected PER of 2.0 with only a 52% loss of anti-trypsin activity (Jansen et al., 1978). It is well known that anti-trypsin activity does not account for all the anti-nutritional properties of legumes. More work is needed to better understand the differences between the effectiveness of the roasting and extrusion processes in making full-fat soy flour.

Our data demonstrate that it is possible to produce full-fat soy flour with a high protein quality using a dry heat process and beans with initial moisture levels of approximately 5%. Smith et al. (1964) reported that the protein quality of autoclaved defatted soybean meal was lower for low moisture than for high moisture beans. Klose et al. (1948) had previously observed that dry heat was less effective than moist heat in improving the nutritive value of raw soybean meal. Our results suggest that it is the effectiveness of heat penetration that is important in the inactiviation of anti-growth factors in soybeans, rather than the moisture level. We have previously reported that a hot salt bed roasting process is effective in destroying anti-trypsin activity in raw navy beans (Carvalho et al., 1977).

The potential applicability of the roasting process in deve-

Authors Jansen and O'Deen are with the Dept. of Food Science & Nutrition, and Author Harper is with the Agricultural & Chemical Engineering Dept., Colorado State University, Fort Collins, CO 80523.

0022-1147/78/0004-1350$02.25/0
© 1978 Institute of Food Technologists

Table 1—Nutritional evaluation of roasted soy flours

Roasting conditions[a]	Wt gain (g)[b]	PER[c]	Corrected[d] PER	TUI[e]/mg	% Loss of TUI
Raw	54.1 ± 4.3[f]	1.88 ± 0.07	1.49 ± 0.06	75.5	–
Roasted 178°C, 15 sec	59.8 ± 3.8	2.11 ± 0.09	1.67 ± 0.07	48.5	36
Roasted 178°C, 24 sec	66.0 ± 5.9	2.05 ± 0.06	1.62 ± 0.13	40.2	47
Roasted 206°C, 22 sec	102.6 ± 4.2	2.79 ± 0.07	2.20 ± 0.03	19.0	75
Roasted 220°C, 20 sec	113.1 ± 4.6	2.75 ± 0.06	2.17 ± 0.05	9.2	88
Roasted 234°C, 15 sec	108.4 ± 3.3	2.93 ± 0.04	2.31 ± 0.03	7.2	90
Roasted 234°C, 24 sec	104.5 ± 4.7	2.67 ± 0.06	2.11 ± 0.05	9.6	87
Casein control	119.0 ± 4.8	3.16 ± 0.09	2.50	–	–

[a] Temperature of salt bed and exposure time
[b] Least significant difference $P < 0.05$, 14.3; $P < 0.01$, 18.7
[c] g wt gained/g protein consumed; Least significant difference $P < 0.05$, 0.22; $P < 0.01$, 0.29
[d] Corrected relative to an assigned value of 2.50 for ANRC casein; Least significant difference $P < 0.05$, 0.19; $P < 0.01$, 0.25
[e] Trypsin units of inhibition
[f] Mean ± standard error

loping countries is related to its simplicy and low cost. Since no water is required in processing, no redrying is requried. In addition, full-fat soy flour made by the roasting process is much easier to mill to a particle size suitable for bread baking than is the case with the extrusion process, because of a considerable difference in the distribution of the oil within the product. The roasting process greatly simplified the dehulling process since roasting partially cracks the hulls which, after light rolling, are easily winnowed away in a stream of air.

We currently are evaluating the potential of the roasting process in making not only full-fat soy flour but also full-fat cottonseed flour and blended foods using corn, sorghum, cottonseed and soybeans.

REFERENCES

AACC. 1969. Methods 08-03, 30-25, 32-15, 44-15, 46-12. "Approved Methods," 7th ed. American Association of Cereal Chemists. St. Paul, MN.

AOAC. 1975. "Official Methods of Analysis," 12th ed., p. 857. Association of Official Analytical Chemists. Washington, DC.

AOCS. 1975. Methods Bal2-75. "Official and Tentative Methods," American Oil Chemists Society, Champaign, IL.

Carvalho C.C.C., Jansen, G.R. and Harper, J.M. 1977. Protein quality evalutation of an instant bean powder produced by dry heat processing. J. Food Sci. 42: 553.

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

Fomon, S.J. 1959. Comparative study of human milk and a soya bean formula in promoting growth and nitrogen retention by infants. Pediatrics 24: 577.

Harper, J.M. and Lorenz, K. 1974. Production and evaluation of salt bed roasted full-fat soy flours. Lebensmitt-Wiss. u-Technol. 7: 268.

Jansen, G.R. 1977. Amino Acid fortification. In "Protein for Human Consumption," ed. Bodwell, C.E., Avi Press Westport, CT.

Jansen, G.R., Harper, J.M. and O'Deen, L. 1978. Nutritional evaluation of blended foods made with a low-cost extruder cooker. J. Food Sci. 43: 912.

Klose, A.A., Hill. B. and Fevald, H.L. 1948. Food value of soybean protein as related to processing. Food Technol. 9: 201.

Mustakas, G.C. 1971. Full-fat and defatted soy flours for human nutrition. JAOCS 48: 815.

Mustakas, G.C., Griffin, E.L. Jr. and Allen, L.E. 1964. Production and nutritional evaluation of extrusion-cooked full-fat soybean flour. JAOCS 41: 607.

Mustakas, G.C., Albrecht, W.J., Bookwalter, G.N., McGhee, J.E., Kwolek, W.F. and Griffin, E.L. Jr. 1970. Extruder-processing to improve nutritional quality, flavor and keeping quality of full-fat soy flour. Food Technol. 24: 102.

Rackis, J.J., McGhee, J.E. and Booth, A.N. 1975. Biological threshhold levels of soybean trypsin inhibitors by rat bioassay. Cereal Chem. 52: 85.

Smith, A.K., Rackis J.J., McKinney, L.L., Robbins, D.J. and Booth, A.N. 1964. Growth and pancreatic hypertrophy of rats fed commercial and laboratory soybean meals and hulls. Feedstuffs 36(7): 46.

USDA. 1973. Purchase of corn-soy blend for use in export programs. Terms and Conditions. Announcement CSB-1. United States Department of Agriculture, Agricultural Stabilization and Conservation Service. Minneapolis, MN Sept. 7.

Ms received 10/22/77; revised 12/13/77; accepted 12/20/77.

Paper presented at the Western Hemisphere Nutrition Conference V, Quebec, Canada, August 15-18, 1977.

Work supported by Agri-business Group, Economic Research Service, USDA.

The authors acknowledge the help of Messrs. Ron Tribelhorn and Marvin Stone, Department of Agricultural and Chemical Engineering, Colorado State University, in roasting the soybeans as described in this study. The authors thank Hoffman-LaRoche and the Mallinkrodt Chemical Co. for donating the vitamin and mineral pre-mixes respectively.

A Research Note

EFFECT OF TWO HOME CANNING PROCEDURES ON FISH AND SOY PROTEIN QUALITY

S. C. KEYES, I. D. WOLF, E. A. ZOTTOLA and P. V. J. HEGARTY

ABSTRACT

The effect of two home-canning pressures on the protein quality of fish and soy (10 psi and 15 psi), was examined using the protein efficiency ratio (PER) technique. The pressures and times used were: lactalbumin, 10 psi for 75 min and 15 psi for 25 min; Northern Pike, 10 psi for 100 min and 15 psi for 45 min; blanched, green soybeans, 10 psi for 60 min and 15 psi for 20 min. All canning procedures were determined to be safe microbiologically. In all proteins tested there was a decrease in protein quality between the unheated control and both heat treatments. No significant difference was observed between the two heat treatments. The use of the shorter 15 psi treatment can be recommended because it has no adverse effect on protein quality, and probably a lesser effect upon losses of other nutrients when compared with the more conventional 10 psi treatment.

INTRODUCTION

IN RECENT YEARS there has been a growing interest in home canning as a method of food preservation. The causes of the increase in home canning are primarily due to a desire to use "natural" preservation methods, and a need to preserve food produced in home gardens (Zottola et al., 1977). It is important that any losses in the nutritive value of these foods be fully understood. Vitamin losses during commercial canning are well documented (Harris and Karmas, 1975). There is no information on the effect of various home canning procedures on protein quality. In the 1940's and 1950's some researchers examined the effect of commercial canning procedures on protein quality. Rice and Robinson (1944) canned pork at 121°C for 3 hr and found no difference in protein efficiency ratio (PER) between the raw and canned product. Schweigert and Guthneck (1954) obtained no decrease in the availability of lysine and methionine when pork and beef were canned. Dunn et al. (1949) and Neillands et al. (1949) obtained no change in protein quality when fish and beef products were commercially canned.

The present study was undertaken to determine the effect on protein quality of a 10 psi canning procedure and also of a shorter 15 psi procedure.

MATERIALS & METHODS

THE THREE FOODS studied were lactalbumin, fish and green soybeans. Proteins of high quality were chosen because any possible detrimental effect of the home canning procedures would be detected more accurately with these foods.

Lactalbumin

A slurry was produced using the proportion by weight of 2:1, double distilled water to lactalbumin (Sigma Chemical Company, St. Louis, MO). Adequate heat penetration of this slurry is possible during the canning procedure. The unheated slurry was transferred into quart canning jars, and treated by two canning techniques used to replicate the procedure suggested for canning beef (Presto, 1974). One treatment involved 10 psi for 75 min, and the other 15 psi for 25 min in a pressure cooker. The canning jars were not rotated during the canning treatments. The canned material was then freeze dried (Virtis Research Equipment, Gardiner, N.Y.) for incorporation into rat diets.

Fish

Northern pike fillets, purchased locally, were used to test the heat stability of an animal protein. The unheated control was ground in a meat grinder with 2 mm holes, and freeze dried immediately. The two heat treatments were 10 psi for 100 min and 15 psi for 45 min in pint jars. Heat penetration through the fish was slower than through the lactalbumin or through the soybeans (Larson, 1977). Therefore, the heat treatment times were extended when the fish was canned. The fish was ground as described above for the unheated control, then freeze dried for incorporation into rat diets.

Green soybeans

Green soybeans, chosen one week before they would begin to turn brown, were blanched in the shell for 10 min in boiling water. They were then shelled and stored for no longer than 2 days at 3°C. The beans were loosely packed into pint canning jars with 1 teaspoon of salt per jar. Boiling water was then added to within 1 cm of the top of the jar. Canning procedure was according to Sowers (1974), with one treatment at 10 psi for 60 min and the other at 15 psi for 20 min. The unheated control group was the soybeans that were subjected to the preliminary blanching only. Another treatment involved autoclaving the soybeans in a shallow aluminum pan for 20 min at 15 psi. This group was included to insure that the soybean antitrypsin factor was denatured (Nitsan and Liener, 1976). After all preparation was completed, each group of soybeans was ground and freeze dried for incorporation into rat diets.

Protein quality determination

The protein efficiency ratio (PER) was determined (AOAC, 1965) by using male Sprague Dawley rats (ARS, Madison, WI), 21 days old with an initial body weight of 45–50g. All animals were fed a stock laboratory diet (Ralston Purina, St. Louis, MO) for 2 days, then weighed and randomized into groups of 10 rats that were all caged individually. Water and test diets were fed *ad libitum* for the 28-day test period. Body weight, food intake and food spillage were calculated twice a week. One supplementary feeding was included during the last week because of increased food intake by the animals.

Table 1—Protein efficiency ratio (PER) values for three proteins home canned at 10 psi and 15 psi

Protein source	Control	10 psi	15 psi	Autoclaved
Lactalbumin	3.28 ± 0.11a	3.03 ± 0.07b	3.13 ± 0.08b	–
Fish	3.47 ± 0.04a	3.20 ± 0.04b	3.27 ± 0.05b	–
Soybean	2.39 ± 0.04a	1.98 ± 0.03b	2.16 ± 0.05b	2.17 ± 0.06b

[a] All values represent the mean plus or minus the standard error of the mean for a group of ten rats. PER values with different letters (within each protein group) are significantly different ($p < 0.05$). PER value of the casein control was 2.85 ± 0.04.

RESULTS & DISCUSSION

SIMILAR CHANGES in protein quality were observed when the lactalbumin, fish and soybeans were heated (Table 1). The unheated controls for all three proteins are of a higher protein quality ($P < 0.05$) than the 10 psi heated samples. There was no significant difference in PER values between the 10 and 15 psi heat treatments. The autoclaved soybeans had similar protein quality to the 10 and 15 psi treated soy. This autoclave treatment at 121°C for 20 min denatures the antitrypsin factor in soybeans (Nitsan and Liener, 1976). Therefore, the

All authors are with the Dept. of Food Science & Nutrition, University of Minnesota, St. Paul, MN 55108.

0022-1147/78/0004-1352$02.25/0
© 1978 Institute of Food Technologists

similarity in PER values between the home-canned and the autoclaved soybeans indicates that the anti-trypsin factor was denatured during the home canning process.

The PER values obtained for the animal proteins were as expected. Mauron (1972) stated that only slight losses, if any, would be anticipated in protein quality during normal home preparation procedures. Oven heating fish at 232°C for at least 35 min produced no change in protein quality. Mauron (1972) implies that even at an oven temperature of 232°C, the decrease in protein quality after 35 min would be very slow. The amount of reducing sugar is low in the fish and soybeans studied in the present project. Therefore, since the duration of most home cooking or canning procedures are not long, no intra- or inter-molecular interactions would occur that would reduce protein digestibility.

The effect of the canning procedures on the PER values of the soybeans was somewhat unexpected. Heat treatment usually improves the quality of vegetable proteins, for example, cottonseed meal and soybean (Osborne and Mendel, 1917). Heat can release a protein from its starch matrix or destroy any anti-nutritional factors, thereby improving protein digestibility (Mauron, 1972). The anomaly of the decrease of protein quality when the soybeans were heated can be explained by the prior heat treatment at 100°C during the blanching process. Thus, there may have been an initial increase in nutritive value between the raw soybean and blanched control soybeans caused by the blanching that was subsequently decreased by further heating.

In conclusion, (1) home canning does not cause a substantial loss of protein quality, and (2) a shorter (15 psi) canning treatment can be recommended because this pressure and time is safe from a microbiological standpoint (Zottola et al., 1977), and because there is no difference in protein quality when 10 psi or 15 psi are the treatments.

REFERENCES

AOAC. 1965. "Official Methods of Analysis," 10th ed, p. 785. Association of Official Analytical Chemists, Washington, DC.

Beuk, J.F., Chornock, F.W. and Rice, E.E. 1948. The effect of severe heat treatment upon the amino acids of fresh and cured pork. J. Biol. Chem. 175: 291.

Dunn, M.S., Camien, M.N., Eiduson, S. and Malia, R.B. 1949. The nutritive value of canned foods. 1. Amino acid content of fish and meat products. J. Nutrition 39: 177.

Harris, R.S. and Karmes, E. 1975. "Nutritional Evaluation of Food Processing," 2nd ed. The Avi Publishing Company, Inc., Westport, CT.

Larson, K. 1977. Possible sources of variability in home canning of food. M.S. thesis, University of Minnesota.

Mauron, J. 1972. Influence of industrial and household handling of food protein quality. In: "International Encyclopedia of Food and Nutrition, Vol. 11—Proteins and Amino Acid Functions," Ed. E.J. Bigwood. Pergammon Press, Oxford.

Mayfield, H.L. and Hedrick, M.T. 1949. The effect of canning, roasting and corning on the biological value of the proteins of western beef finished on either grass or grain. J. Nutrition 37: 487.

Neillands, J.B., Sirny, R.J., Sohljell, I., Strong, F.M. and Elvehjem, C.A. 1949. The nutritive value of canned foods. 2. Amino acid content of fish and meat products. J. Nutrition 39: 187.

Nitsan, Z. and Liener, I.E. 1976. Studies of the digestibility and retention of nitrogen and amino acids in rats fed raw or heated soy blour. J. Nutrition 106: 292.

Osborne, T.B. and Mendel, L.B. 1917. The use of soybean as food. J. Biol. Chem. 32: 369.

Presto. 1974. "The Modern Guide to Pressure Canning and Cooking," National Presto Industries, Inc. Eau Claire, WI.

Rice, E.E. and Robinson, H.E. 1944. Nutritive value of canned and dehydrated meat and meat products. Am. J. Public Health 34: 587.

Schweigert, B.S. and Guthneck, B.T. 1954. Utilization of amino acids from foods by the rat. J. Nutrition 54: 333.

Sowers, M.F. 1974. Soybeans and their products. Family Living Topics, (Okalhoma State University, Stillwater, OK).

Zottola, E.A., Larson, K.C., Wolf, I.D., Wallace, M.J. and Thompson, D.R. 1977. Home canning of food: Evaluation of methods. Paper presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, June 6—8.

Ms received 12/9/77; revised 3/3/78; accepted 3/8/78.

Scientific Journal Series Paper No. 9959, Minnesota Agricultural Experiment Station.

We are grateful to Dr. Jean Lambert for the soybeans, and to the Ball Corporation, Muncie, IN 47302, for financial assistance.

A Research Note

IDENTIFICATION AND QUANTITATION OF TRYPTOPHAN AND TYROSINE METABOLITES IN THE BANANA BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

THOMAS M. KENYHERCZ and PETER T. KISSINGER

ABSTRACT

In order to understand the origin of numerous metabolites found in human urine following ingestion of bananas, a study of aromatic metabolism in the banana has been initiated. Thin-layer chromatography and reverse-phase ion-pair liquid chromatography with electrochemical detection have been combined to yield specific information about aromatic amino acid metabolites in the pulp and peel of the banana.

INTRODUCTION

THIN-LAYER CHROMATOGRAPHY (TLC) and liquid chromatography with electrochemical detection (LCEC) have been combined to determine several tryptophan and tyrosine metabolites in the banana. A previous communication from this laboratory demonstrated the utility of this approach for the quantitation of dihydroxyphenethylamines and identified salsolinol as a major metabolite of dopamine in the banana (Riggin et al., 1976). The present note describes the extension of this effort to characterize aromatic metabolites in the banana using a new technique generally applicable to the study of plant phenolics.

EXPERIMENTAL

Samples and sample preparation

Bananas were purchased locally while slightly green and allowed to ripen at room temperature to a full yellow color. Banana pulp and peel were homogenized with 0.1M $HClO_4$ (50 ml/g of material) in a blender and then centrifuged at 12,100 × G for 10 min at 2°C. The supernatant was removed to an amber bottle and refrigerated prior to analysis.

Extraction

A 2 ml aliquot of the acidic supernatant in a 12 ml glass centrifuge tube was extracted with 4 ml of ethyl acetate:acetone (2:1). After 10 min of mechanical shaking, the mixture was centrifuged briefly and the organic layer discarded. The remaining solution was adjusted to pH 10.3 with 58% NH_4OH, saturated with NaCl, and extracted with 2 ml of ethyl acetate:acetone (2:1). Following 10 min of mechanical shaking, the mixture was centrifuged briefly, and the organic layer transferred to a 6 inch culture tube using a disposable Pasteur pipette. The extraction was repeated thrice and the combined organic layers dried over anhydrous sodium sulfate (ca 1g). The combined extract was transferred to an acid washed 12 ml centrifuge tube. The residual sodium sulfate was washed with 1 ml of the ethyl acetate:acetone mixture which was combined with the original extract. The extract was evaporated to dryness under a stream of nitrogen at 20°C and taken up in either 200 µl of ethyl acetate for TLC analysis or 2 ml of the LCEC mobile phase.

Thin-Layer chromatography

Preliminary identification of the basic compounds was accomplished using prescored silica gel thin-layer plates (Quantum Industries LDQ, 5 × 20 cm) spotted with ethyl acetate extracts (50 µl/channel) using disposable 50 µl pipettes. The TLC plates were developed with a 10:17:1 chloroform:acetone:ammonium hydroxide (58%) mixture.

Authors Kenyhercz and Kissinger are with the Dept. of Chemistry, Purdue University, West Lafayette, IN 47907.

0022-1147/78/0004-1354$02.25/0
© 1978 Institute of Food Technologists

Table 1—Concentration of selected tryptophan and tyrosine metabolites of the banana[a]

Compound	µg/g Banana pulp	Banana peel
5-Hydroxytryptamine	25.6 ± 0.30[b]	2.71 ± 0.20
Metanephrine	1.01 ± 0.15	1.20 ± 0.15
5-Methoxytryptamine	1.17 ± 0.10	1.14 ± 0.10
Normetanephrine	17.6 ± 0.20	12.9 ± 0.26
Octopamine	3.71 ± 0.40	5.48 ± 0.50
Tyramine	94.5 ± 0.70	10.3 ± 0.60
Tryptamine	1.18 ± 0.10	0 691 ± 0.06

[a] Average whole banana weight 180 ± 8g
[b] All data averaged from at least 3 measurements

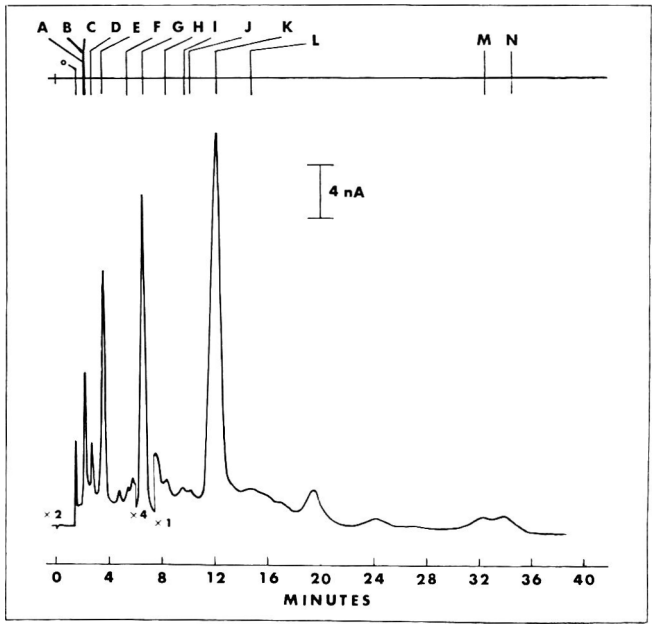


Fig. 1—Reverse-phase (C_{18}) liquid chromatogram of basic metabolites in an extract of banana pulp (o = void volume time). Oxidation current (nanoamperes) is plotted as a function of retention time. The retention times for reference compounds are indicated above the chromatogram as follows: A = Tyrosine; B = m-Tyrosine; C = o-Tyrosine; D = Octopamine; E = Normetanephrine; F = Metanephrine; G = Tyramine; H = m-Tyramine; I = o-Tyramine; J = 3-Methoxytyramine; K = 5-Hydroxytryptamine; L = Salsoline; M = 5-Methoxytryptamine; and N = Tryptamine.

The alkaloids were visualized under UV illumination by the serial overspraying of fluorescamine, dansyl chloride and tetrazotized benzidine (Ranieri and McLaughlin, 1975) or acetic ninhydrin (Macek, 1972). Identification of the basic components in unvisualized TLC channels was confirmed by reverse phase liquid chromatography with electrochemical detection, a technique with much greater specificity than TLC.

Reference compounds

Tyrosine, m-tyrosine, o-tyrosine, octopamine, normetanephrine, metanephrine, tyramine, 3-methoxytyramine, 5-hydroxytryptamine,

—Text continued on page 1356

A Research Note

ENZYMATIC ESTIMATION OF AVAILABLE LYSINE

L. E. McBEE and R. T. MARSHALL

ABSTRACT

The automated lysine decarboxylase method of determining lysine concentrations was integrated with a previously developed method of treating samples with 1-fluoro-2,4-dinitrobenzene to bind free epsilon-amino groups of lysine. Together these procedures provide an improved test for available lysine.

INTRODUCTION

ASSAYS WITH ANIMALS for available lysine are costly and time consuming. Microbiological methods are rapid and less costly, but both methods are influenced by bioavailability.

Uniformly lower values were obtained in assays with *Streptococcus zymogenes* when compared to assays with chickens (Ford, 1962; Miller et al., 1963). When *Tetrahymena pyriformis* was used to assay available lysine, results equivalent to assays with *S. zymogenes* were obtained by Stott and Smith (1966). Microbiological assay values were closer to those obtained with animal assays if samples were pretreated with papain (Ford, 1964).

Chemical assays of lysine may be divided into two types: total and available. Determination of total lysine usually begins with an acid hydrolysis followed by ion-exchange or gas chromatography. Higher lysine concentrations are usually obtained by chemical assays than by animal or microbiological assays.

In determinations of available lysine attempts usually are made to distinguish between lysine units with free and bound ϵ-NH_2 groups. The 1-fluoro-2,4-dinitrobenzene (FDNB) method of Carpenter (1960) distinguishes between such groups. The bond between FDNB and the ϵ-NH_2 group is not broken by acid hydrolysis. Alternate methods using methylacrylate and 2,4,6-trinitrobenzene sulfonic acid (TNBS) are available (Finley and Friedman, 1973). Kakade and Liener (1969) determined available lysine in proteins by measuring spectrophotometrically the ϵ-trinitrophenyl derivative of lysine.

Lysine may be quantitated by reaction with L-lysine decarboxylase followed by measurement of liberated CO_2. This method has been automated by Wall and Gehrke (1974). L-lysine decarboxylase is specific, utilizing either L-lysine or hydroxylysine, but not acetyl or methyl derivatives of L-lysine (Barman, 1969).

This paper reports the use of automated equipment of differentiate quantitatively between L-lysine and FDNB derivatives of L-lysine.

EXPERIMENTAL

THE METHOD DEVELOPED for estimating available lysine consisted of treating proteinaceous samples with FDNB according to Carpenter (1960) with subsequent determination of L-lysine by the method of Wall and Gehrke (1974). L-lysine, 2,4-dinitrophenyl (DNP)-L-lysine derivatives and FDNB were from Sigma Chemical Company.

Available lysine was estimated chemically by the difference between L-lysine concentrations of samples treated with FDNB and untreated samples. Samples containing various concentrations of lysine, lactose, thiamine-HCl and riboflavin were hydrolyzed 20 hr in 25 ml 6N HCl at 110°C and analyzed for L-lysine by the automated L-lysine decarboxylase method. Carbon dioxide produced was selectively dialyzed into a stream of sodium carbonate containing phenolphthalein. Change in indicator color was measured at 550 nm and was proportional to the concentration of lysine in the sample.

Available lysine was also estimated, using the protozoan *T. pyriformis*, by a modification of the method of Stott and Smith (1966). Sample aliquots containing 1 mg N/ml as lysine and/or lysine derivative, were fortified with a basal lysine-free medium, diluted to constant volume, autoclaved (121°C for 10 min), cooled and inoculated with the test organism. After 96 hr incubation at 25°C, growth response was measured by titration of acid produced with standard base. Lysine concentration was calculated using a standard curve.

RESULTS & DISCUSSION

THE FIRST EXPERIMENT was to determine if L-lysine decarboxylase would be inhibited by or react with FDNB derivatives of L-lysine. Samples containing 1.43 molar lysine equivalent were formulated with 2.5 mg L-lysine/ml alone or in combination with equimolar quantities of ϵ-DNP-lysine or di-DNP-lysine. Blanks containing no L-lysine were included.

Recovery of L-lysine from samples containing only L-lysine was 83 ± 2.8%. About one-half that amount, 42.5 ± 1.4%, was recovered from samples containing equimolar concentrations of L-lysine and ϵ-DNP-lysine or L-lysine and di-DNP-lysine. L-lysine was not detectable in samples containing only lysine-FDNB derivatives. Thus, consistent quantities of L-lysine were recovered by the lysine decarboxylase method and lysine derivatives did not react with lysine decarboxylase. This experiment confirmed the specificity of lysine decarboxylase, suggesting the method could be used in practical determinations of available lysine.

The modified test was evaluated by formulating aqueous samples containing 2.5 mg/ml of L-lysine alone and in combination with 0.52 g/ml lactose, 4 μg/ml thiamine-HCl and 18 μg/ml riboflavin. Samples were subjected to (1) no heating, (2) 72°C for 15 sec or (3) 137°C for 2 sec. Available lysine was determined by the FDNB-lysine decarboxylase method and by the response of *T. pyriformis* (Table 1).

Analysis of variance indicated that sample composition, heat treatment, or method of analysis did not significantly affect the data. Methods of analysis, however, were significantly ($P < 0.001$) different when evaluated using the T-test. The overall mean for the FDNB method was 10.7% higher than the mean for the *T. pyriformis* method, 2.86 vs 2.55 mg/ml.

The FDNB-lysine decarboxylase method of determining available lysine provides significant advantages. Interfering compounds are minimized due to two characteristics of the system. Firstly, the enzyme is extremely specific. There are no known interfering compounds except the amino acid hydroxylysine. Secondly, reactions between FDNB and other amino acids frequently created problems in other methods in which FDNB was used. Dialysis in the modified procedure excludes interfering substances. Additionally, the toxic compound methoxycarbonyl chloride is not required in this procedure as it is in other modifications for Carpenter's method.

Authors McBee and Marshall are with the Dept. of Food Science & Nutrition, University of Missouri-Columbia, Columbia, MO 65201.

0022-1147/78/0004-1355$02.25/0
© 1978 Institute of Food Technologists

While the final test of any nutrient assay method is actual bioavailability, this combination of methods provides rapid estimates of bioavailability at moderate cost. The automated enzymatic method of lysine analysis has been proved by use for the past 10 years. Integrating it with Carpenter's method of differentiating bound and free lysine permits rapid assays, allowing researchers to obtain greater quantities of reliable information.

Table 1—Available lysine content of heated samples assayed by FDNB and T. pyriformis methods

	Heat treatment					
	Control		72°C, 15 sec		137°C, 2 sec	
Sample[a]	FDNB	*T. pyri.*	FDNB	*T. pyri.*	FDNB	*T. pyri.*
	mg lysine/ml					
1	2.90	2.40	2.95	2.33	2.85	2.28
2	2.40	2.15	3.05	2.33	2.45	2.30
3	2.55	2.69	3.00	2.67	2.80	3.30
4	3.05	2.66	2.93	2.75	2.95	2.24
5	3.00	2.63	3.25	2.47	2.90	2.69
6	2.75	2.54	2.70	2.69	2.90	2.80
Mean	2.78	2.51	2.98	2.54	2.81	2.60

[a] All samples contained 2.5 mg/ml of lysine and in addition the following: (1) no additive; (2) 0.52 g/ml lactose; (3) 4 μg/ml thiamine-HCl; (4) 18 μg/ml riboflavin; (5) thiamine-HCl + riboflavin; and (6) thiamine-HCl + riboflavin + lactose.

REFERENCES

Barman, T.E. 1969. "Enzyme Handbook." Springer-Verlag, New York.
Carpenter, K.J. 1960. The estimation of the available lysine in animal-protein foods. Biochem. J. 77: 604.
Finley, J.W. and Friedman, M. 1973. Chemical methods for available lysine. Cereal Chem. 50: 101.
Ford, J.E. 1962. A microbiological method for assessing the nutritional value of protein. 2. The measurement of 'available' methionine, lysine, leucine, arginine, histidine, tryptophane, and valine. Brit. J. Nutr. 16: 409.
Ford, J.E. 1964. A microbiological method for assessing the nutritional value of protein. 3. Further studies on the measurement of available amino acids. Brit. J. Nutr. 18: 449.
Kakade, M.L. and Liener, I.E. 1969. Determination of available lysine in proteins. Anal. Biochem. 27(2): 273.
Mauron, J. and Mottu, F. 1958. Relationship between in vitro lysine availability and in vivo protein evaluation in milk powders. Arch. Biochem. Biophys. 77: 312.
Miller, E.L., Carpenter, K.J. and Morgan, C.B. 1963. International Congress Nutrition VI, Edinburg Programme, 166.
Stott, J.A. and Smith, H. 1966. Microbiological assay of protein quality with Tetrahymena pyriformis W. 4. Measurement of available lysine, methionine, arginine and histidine. Brit. J. Nutr. 20: 663.
Wall, L.L. and Gehrke, C.W. 1974. Automated enzymatic determination of L-lysine in grain. J. AOAC 57: 1098.

Ms received 12/13/77; revised 2/11/78; accepted 2/15/78.

Contribution from the University of Missouri Agricultural Experiment Station. Journal Series No. 8017.

This research was jointly supported by grants from the National Dairy Council and Dairy Research, Inc. and by the Agricultural Experiment Station, University of Missouri-Columbia.

METABIOLITES IN THE BANANA . . . From page 1354

5-methoxytryptamine and tryptamine were purchased as salts from Sigma Chemical Co. (St. Louis, MO), Tyramine, m-tyramine and o-tyramine were prepared from the parent tyrosines by the action of the decarboxylase enzyme while salsoline was formed by the Pictet-Spengler condensation of 3-methoxytyramine and acetaldehyde.

Liquid chromatography

The liquid chromatographic system was similar to that previously described (Felice and Kissinger, 1976) using an electrochemical detector (Bioanalytical Systems, Model LC-40, West Lafayette, IN). A microparticle reverse phase material (μBondapak C_{18}, Waters Associates, Inc.) was slurry packed (methanol) in a 15 cm × 4.1 mm i.d. smooth bore stainless steel column. Separations were carried out at ambient temperature (25 ± 2°C). The mobile phase consisted of a mixture of 400 ml of 0.1M ammonium acetate, 20 ml of spectrograde methanol ("Photorex") and 8 ml of 0.1M sodium octyl sulfate. The potential of the amperometric (electrochemical) detector was controlled at +0.95v vs SCE. Quantitation was achieved by comparison of peak heights of constituents with those of standard solutions processed through the entire TLC-LCEC procedure. In each case, the comparison was made under conditions where the absolute recovery was independent of concentration and the instrumentation was linear.

RESULTS & DISCUSSION

FIGURE 1 illustrates a typical reverse-phase ion-pair chromatogram obtained for a basic ethyl acetate extract of banana pulp. Table 1 presents the quantitative data for those compounds which could be identified with confidence by the combination of TLC, LCEC, and electrochemical information (oxidation potential). The quantitative results were obtained by injection of filtered extracts of individual TLC regions conforming to the R_f values (± 0.05 units) of standard compounds. Chromatograms obtained in this way are, of course, much less complex than that illustrated in Figure 1. In a previous publication from this laboratory, data were presented for the catecholamine fraction in pulp and peel (dopamine, norepinephrine, and salsolinol) (Riggin et al., 1976).

While the use of two modern chromatographic techniques and electrochemical information can not be considered unequivocal for the identification of trace metabolites, the specificity is quite good and the sensitivity is superior to most alternate approaches. Important advantages of this scheme for trace determination of phenolics in food include its relatively low cost and the fact that derivatization is unnecessary, in contrast with most GC and GCMS techniques.

REFERENCES

Felice, L.J. and Kissinger, P.T. 1976. Determination of homovanillic acid in urine by liquid chromatography with electrochemical detection. Anal. Chem 48(6): 794.
Macek, K. 1972. "Pharmaceutical Applications of Thin-Layer and Paper Chromatography." American Elsevier Publishing Company, Inc., New York.
Ranieri, R.L. and McLaughlin, J.L. 1975. Cactus alkaloids. 27. Use of fluorescamine as a thin-layer chromatographic visualization reagent for alkaloids. J. Chromatogr. 111: 234.
Riggin, R.M., McCarthy, M.J. and Kissinger, P.T. 1976. Identification of salsolinol as a major dopamine metabolite in the banana. J. Agr. Food Chem. 24(1): 189.

Ms received 9/3/77; revised 1/31/78; accepted 2/8/78.

A Research Note

EFFECT OF pH ON VOLATILE RETENTION IN FREEZE-DRIED MODEL SOLUTIONS

T. G. SMYRL and MARC LeMAGUER

ABSTRACT

The retention level of several essential oil components in freeze-dried solutions of sucrose and gum arabic was found to be dependent on the initial pH of the solution. In such carbohydrate solutions the presence of an acid or a base was found to enhance volatile retention. Retention values were found to be lowest near neutral pH values.

INTRODUCTION

THE ORGANOLEPTIC acceptability of a rehydrated freeze-dried food is largely determined by the concentration of volatile flavoring compounds. Thus it is essential that the concentration of the flavoring compounds in the rehydrated sample closely matches the concentration levels in the original foods, i.e. the flavoring compounds should be retained during the freeze-drying process. Flink (1975) has discussed many of the important process parameters associated with volatile retention during the freeze drying of liquid foods. One parameter that has received very little attention is that of solution pH prior to freeze drying. Saravacos and Moyer (1968) concluded that flavor retention in freeze-dried apple was influenced by the acidity of the fruit. Chalmers and Watts (1972) investigated the retention of several metabolically important acids during freeze drying of solutions of variable pH. These workers, however, did not incorporate any dissolved solids within the freeze-dried solutions. Chirife and Karel (1974) noted that 2-propanol retention varied slightly in freeze-dried protein solutions adjusted to different pH levels. Voilley (1975) recently monitored octanoic acid retentions in acidified sugar solutions.

We have been investigating the retentions of several sparingly soluble essential oil components in freeze-dried solutions containing sucrose or gum arabic. We wish to report preliminary results showing the influence that solution pH has on volatile retentions during freeze drying.

EXPERIMENTAL

SUCROSE AND GUM arabic were employed as dissolved solids. The essential oil components, carvone (K and K) and piperitone and eugenol (ICN) were shown to be at least 95% pure by GLC analysis and were used as received.

A Model 42 RePP Sublimator was used to freeze dry the samples. 5.0 ml aliquots of the volatile containing sample were placed in 50 ml Erlenmeyer flasks and cooled to −40°C on the shelf of the freeze dryer. The samples were subjected to a vacuum of 10μ for 48 hr without the input of heat. Immediately after drying, the samples were rehydrated to their original weight and the volatile content was determined with a Unicam SP 1800 Ultraviolet Spectrophotometer which was equipped with a deuterium lamp for ultraviolet analysis. All of the essential oil components employed in this study possessed very strong absorptions in the ultraviolet region of the spectrum. Hence, percent volatile retention was determined from the ratio of the absorbance of the rehydrated samples to the absorbance of the sample prior to freeze drying. All volatiles were proven to follow the Lambert-Beer law in the concentration ranges employed. All freeze-drying experiments were conducted in triplicate.

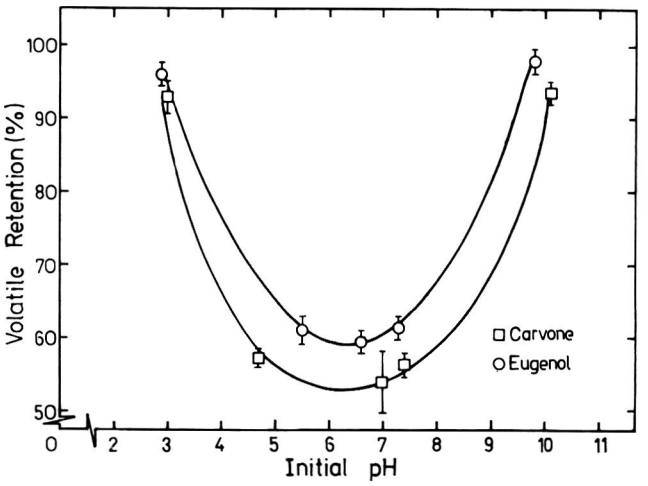


Fig. 1—Variation of carvone and eugenol retention in a 10% sucrose solution as a function of solution pH.

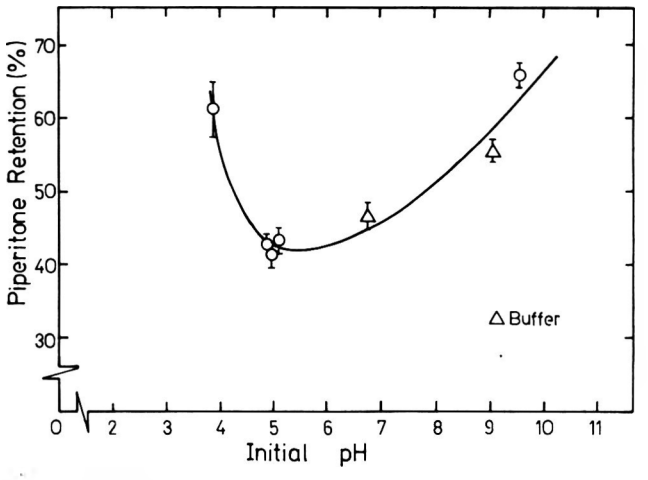


Fig. 2—Piperitone retention in a 1% gum arabic solution as a function of solution pH.

RESULTS & DISCUSSION

RETENTION VALUES for carvone (500 ppm) and eugenol (500 ppm) in 10% (w/w) sucrose solution, as a function of solution pH, are shown in Figure 1. The pH values shown in Figure 1 correspond to the pH of solution immediately before freeze drying. These pH values were obtained by the addition of hydrochloric acid or sodium hydroxide. The vertical bars represent standard deviations calculated on the basis of triplicate measurements. Retention values for carvone and eugenol

Author LeMaguer is with the Dept. of Food Science, University of Alberta, Edmonton, Alberta, Canada T6G 2N2. Author Smyrl is with the School of Food Science, Macdonald College of McGill University, Quebec, Canada. H0A 1C0.

0022-1147/78/0004-1357$02.25/0
© 1978 Institute of Food Technologists

are seen to reach a minimum near neutrality and increase significantly at the upper and lower ends of the pH scale.

Figure 2 illustrates piperitone (500 ppm) retention in 1% (w/w) gum arabic solutions. Piperitone retentions in the gum arabic solution, in which the pH had been adjusted by hydrochloric acid or sodium hydroxide, are represented by ○. To investigate piperitone retentions in the pH range 5–10, gum arabic solutions were prepared using buffer solutions of pH 6.8 and 9.1. The retention levels of piperitone at these pH values are denoted by Δ in Figure 2. Volatile retention is again seen to be highest at the upper and lower ends of the pH scale with a minimum occurring in the more neutral region.

Ultraviolet spectrophotometry has shown that the essential oil components employed in this study are chemically stable in sucrose solutions of pH 3–11. Carvone (Schultz et al., 1977) has recently been shown to be chemically stable in the pH range 3.5–8. This information, together with the fact that the ultraviolet absorbances of the rehydrated samples exhibit maximum absorption at the expected wavelength, e.g. carvone 241.5 nm, supports the conclusion that the essential oil components do retain their chemical integrity during the freeze-drying process under acidic or basic conditions.

It is also expected that sucrose (Stadler, 1932) and gum arabic (Glicksman, 1969) are chemically stable to acid and base under the mild conditions used for the freeze-drying treatment. To investigate the possibility that the dissolved solid was not reacting in any way during freeze drying to perhaps give products which might interfere with the ultraviolet analysis of the essential oil components, 10% sucrose solutions of pH 3–11 were freeze dried without an essential oil component. Ultraviolet analysis of these rehydrated samples showed no absorptions in the ultraviolet region of the spectrum which could interfere with essential oil absorption.

At the present time the reason why volatile retention should be influenced by pH must remain somewhat speculative. It is well known that during the freezing of a food, water is transformed into ice crystals, while dissolved solutes are left in a more concentrated state. Taborsky (1970) has shown that the degree of redistribution of glucose in an acidic glucose solution during freezing is dependent upon the solution pH. According to Karel and Flink (1974) the degree of solutes concentration is a very important parameter in determining overall volatile retention as demonstrated by freezing rate experiments. During the concentration process, many solution properties including pH are known to change as well (Fennema and Powrie, 1964). Taborsky (1970) has shown that in half-frozen acidic glucose solutions, the proton concentration of the liquid may reach a value ten times that of the frozen portion. Whether such an increase in pH within the unfrozen portion of the liquid could enhance interaction between dissolved carbohydrate remains open to question. We are currently investigating these and other possible explanations for this observed influence that pH has on volatile retention in freeze-dried carbohydrate solutions.

REFERENCES

Chalmers, R.A. and Watts, R.W.E. 1972. Studies on the quantitative freeze-drying of aqueous solutions of some metabolically important acids prior to gas-liquid chromatographic analysis. Analyst 97: 224.

Chirife, J. and Karel, M. 1974. Volatile retention during freeze drying of protein solutions. Cryobiology 11: 107.

Fennema, O. and Powrie, W.D. 1964. Fundamentals of low temperature food preservation. Adv. in Food Res. 13: 219. Academic Press, New York.

Flink, J.M. 1975. Process conditions for improved flavor quality of freeze-dried foods. J. Agr. Food Chem. 23: 1019.

Glicksman, M. 1969. "Gum Technology in the Food Industry." Academic Press, New York.

Karel, M. and Flink, J.M. 1974. Theoretical approach to choosing process conditions giving optimal flavor quality in freeze-dried foods. Proc. 4th Int. Congress Food Sci. & Technol. 4: 175.

Saravacos, G.D. and Moyer, J.C. 1968. Volatility of some flavor compounds during freeze-drying of foods. Chem. Eng. Symp. Ser. 64: 37.

Schultz, T.H., Flath, R.A., Mon, T.R., Eggling, S.D. and Teranishi, R. 1977. Isolation of volatile components from a model system. J. Agr. Food Chem. 25: 446.

Stadler, F.D. 1932. Inversion problems in sugar manufacture. Intern. Sugar J. 34: 273.

Taborsky, G. 1970. Solute redistribution in some multicomponent aqueous systems on freezing. J. Biol. Chem. 245: 1063.

Voilley, A. 1975. Contribution á l'étude de la rétention des composes volatils lors de la réshydration des produits alimentaires. Ph.D. Thesis, Université de Dijon, Dijon, France.

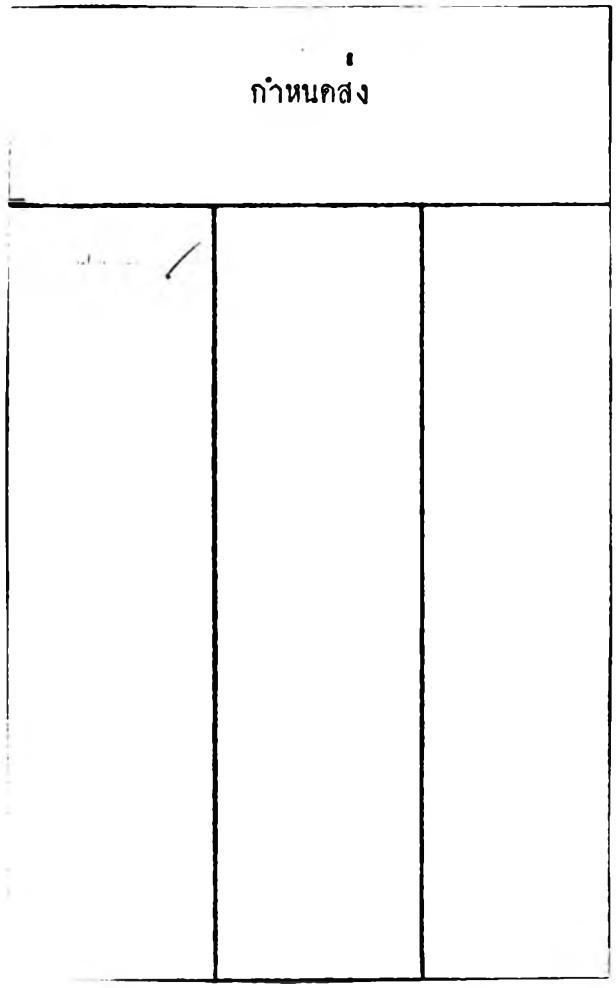
Ms received 12/20/77; accepted 1/18/78.

The authors are grateful to the National Research Council of Canada for their financial support of this work and to Miss Pat Olson for her technical assistance.

ERRATA NOTICE

J. Food Sci. 43(2): 304–306 (1978), R. L. Hostetler and T. R. Dutson: "Investigations of a rapid method for meat tenderness evaluations using microwave cookery." On page 305, Tables 2 and 3, the values for "Sensory panel tenderness $\bar{x}$ and SD" should be 4.7 ± 1.5 and 4.7 ± 1.8, respectively. Please change accordingly.

J. Food Sci. 43(2): 470–475 (1978). R. R. Willardsen, F. F. Busta, C. E. Allen and L. B. Smith: "Growth and survival of *Clostridium perfringens* during constantly rising temperatures." On page 470 under Materials and Methods, Test organisms, line 3, delete: "NCTC 8797 was obtained from M. Solberg, Cook College, New Brunswick, NJ" and insert: "NCTC 9851" after 8239, line 5.


กำหนดสง