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Research Notes

AUTHOR INDEX

Volume 47: Number 4

ERRATA NOTICE

• *J. Food Sci.* 47(1): 49–51 (1982). Relationship of mineral content and tenderness of meat from cattle differing in breed, sex, and age by A.C. Murray, H. Doornenbal, and A.H. Martin. On page 50, right column: change line 4 to read . . . 0.22 kg/year.

• *J. Food Sci.* 47(1): 207–209 (1982). Sensory attributes and Instron measurement of reduced-nitrite poultry frankfurters with sorbic acid or potassium sorbate by E. Chambers IV, J.A. Bowers, K. Prusa, and J. Craig, Table 1, page 208, first column: Change the next to last number from 8.3a to read 8.3e.

• *J. Food Sci.* 47(2): 402–404 (1982). Potato starch and flour in frankfurters: Effect on chemical and sensory properties, and total plate counts by A.A. Bushway, P.R. Belyea, R.H. True, T.M. Work, D.O. Russell, and D.F. McGann. Two items of data in TAble 4, page 404 are incorrect. The third line under "Preference" should read −0.25f and the first line under "Tenderness" should read +0.04g**.

• *J. Food Sci.* 47(2): 609–613 (1982). Bioavailability of iron produced by the corrosion of steel in apples by A. Rosanoff and B.M. Kennedy. The formula for relative biological value (page 610) was partially reversed. Correctly stated, the equation should read:

$$RBV = 100 \times \frac{\text{mg Fe intake from standard that gives the same change in Hb iron}}{\text{mg Fe intake from test dose}}$$

The correct equation was used in calculations presented in the paper.

• *J. Food Sci.* 47(2): 666–667. The nutrient composition of fresh fiddlehead greens by A.A. Bushway, A.M. Wilson, D.F. McGann, and R.J. Bushway. The eighth reference (page 667) is incomplete and should read as follows: Bushway, R.J. and Wilson, A.M. 1981. Determination of α- and β-carotene in vegetables and fruits by high performance liquid chromatography. Canadian Institute of Food Sci. and Technol. In press.

• *J. Food Sci.* 47(3): 1027 + 1029. Accelerated fermentation of milk by nitrosoguanidine induced mutants of Lactobacilli by J. Singh and A.K. Chopra. Correct "mutans" in the title to read "mutants."

• *J. Food Sci.* 47(3): 930–932. Comparison of four media for the enumeration of fungi in dairy products–A collaborative study by O.E. Henson, P.A. Hall, R.E. Arends, E.A. Arnold Jr., R.M. Knecht, C.A. Johnson, D.J. Pusch, and M.G. Johnson. The thirteenth line under the subhead "Media" in the Materials & Methods section omitted the concentration when a correction was made. Please correct the line to read: . . . final concentration of 5 μg/ml dichloran. This was accomplished by . . .

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Development and Use of A Proficiency Test Specimen for Paralytic Shellfish Poisoning

JOHN A. ARES and HENRY A. DYMSZA

ABSTRACT

A proficiency test specimen was developed for evaluating laboratory precision in paralytic shellfish poisoning bioassays. Such a specimen is needed since much of health and economic consequence depends on an analyst's precision in measuring the toxin. Therefore, after testing clams, mussels, and mashed potato matrices, a test specimen composed of saxitoxin dihydrochloride and hydrated potato flakes was selected. Samples containing two toxin levels were sent to 14 collaborating laboratories. Analysis of results identified errors arising from poor dilution techniques. Recovery percentages and variances of mashed potato were comparable to those previously reported with shellfish matrices. Use of the potato matrix appears to offer cost and convenience advantages.

INTRODUCTION

PARALYTIC SHELLFISH POISONING (PSP) has been a public health problem since 1793 (Prakash et al., 1971). During a 1972 red tide, losses of up to $29 million dollars were attributed to PSP (Jensen, 1975).

The standard method of testing for the presence of PSP toxins in shellfish is a mouse bioassay (American Public Health Association, 1970). In use since 1937, the mouse assay, for means of simplicity and reliability has not been replaced by newer, chemical methodologies (Shimizu, 1979).

From the public health and economic viewpoints, it is essential that Federal and State laboratories be precise in their analysis of PSP, since shellfish beds must be closed to harvesting at levels of 80μg/100g shellfish meat (American Public Health Association, 1970).

A proficiency test specimen should be used periodically to spot check for substandard laboratory technique in addition to the conversion factor check with saxitoxin dihydrochloride (STX) standards. Yet, there has been no means of testing a laboratory's proficiency in PSP analysis. The last collaborative study reported gave no detailed procedure for the preparation of split samples (McFarren, 1959).

The microbiology task force assembled at the 10th National Shellfish Sanitation Workshop in 1977 recommended the development of a proficiency test specimen (Hunt, 1978). In response to this need, this paper describes the development of an artificially toxic matrix for use in proficiency testing. To prove the utility of such a test specimen, a collaborative study was performed with 14 laboratories that regularly analyze shellfish for PSP.

MATERIALS & METHODS

Selection of matrix

Four matrices were chosen for study: the soft shell clam, *Mya arenaria*; the blue mussel, *Mytilus edulis*; the bay quahog, *Mercenaria mercenaria* and instant mashed potato. The concept of using mashed potato in split sampling has been recognized for several years (Hunt, 1972).

Enough shellfish from each species were shucked to produce 600g of meats. Each sample of shellfish meat was blended at high speed until a uniform paste was produced. Shellfish paste (100g), was dispensed into each of five beakers and acidified with 100 ml of 0.18N HCl with stirring. The pH was adjusted to be between 3.0 and 3.5. Two vials of STX, each containing 500 μg in 5.0 ml were combined, diluted and brought to a volume of 25.0 ml with pH 3.5 HCl and stirred. Aliquots of 5.0 ml were distributed into each flask and stirred. Thus, each flask contained 200 μg/100g of STX preceding extraction. The remaining procedure followed the standard AOAC method for analysis of PSP (American Public Health Association, 1970).

Commercial instant mashed potato flakes (200g) were added to 800 ml of boiling distilled water, producing 1000g of mashed potatoes. Then, five 100g portions were dispensed into 400 ml beakers, acidified to pH 3.0 and toxified using the method previously described with one deviation. A clinical centrifuge was used at high speed for ten minutes in order to produce a clear supernatant. The resulting liquid, along with the three prepared shellfish samples, were tested for toxicity by the mouse bioassay.

After analysis of the data from the first experiment, mashed potato was selected as the matrix for use in the collaborative study. The procedure was modified slightly from the method previously described to arrive at a thoroughly homogenized test specimen. Four 5.0 ml vials of STX, each containing a total of 2,000 μg of toxin, were added to 780 ml of hot water and stirred for several minutes. Then, 200g of potato flakes were added to produce a uniform paste. This preparation of potato matrix contained STX at the level of 200 μg/100g. For the 500 μg/100g level, 5,000 μg of toxin were added to the water. All other procedures were identical to preparation of the 200 μg/100g sample. This sample was used to test for homogeneity and percentage recovery.

Determination of shipping characteristics

Two insulated plastic foam packages were prepared prior to the collaborative study: one contained refrigerated samples, and the other contained frozen samples. Before storage, thermocouples were placed into the center of the matrices. Ice packs were frozen and packed in the boxes prior to addition of the cold sample bottles. After 33 days of cold storage, the frozen and refrigerated samples were allowed to warm at room temperature. The warming cycle was monitored using a Honeywell multi-point temperature recorder.

Conduct of the collaborative study

Fourteen laboratories that regularly perform PSP analysis participated in the study. These laboratories included the Massachusetts State Department of Health Laboratories at Amherst, Fairhaven, Jamaica Plain and Lawrence; Maine Department of Marine Resources Laboratory, Boothbay Harbor; the Dept. of Health, Hartford, CT; National Health and Welfare, Ottawa, Canada; The State Consumer Protection Laboratory, Concord, NH; Rhode Island Dept. of Health, Providence, RI; California State Dept. of Health, Berkeley, CA; Public Health Laboratory, Portland, OR; Alaska State Dept. of Health, Juneau, AL; FDA Northeast Technical Services Unit, Davisville, RI; Washington Dept. of Social and Health Services, Seattle, WA.

A new lot of STX was used in the collaborative study samples. When added to 780 ml of water and 200g of potato flakes, 110 μg/ml STX dihydrochloride produced 1000g of toxic mashed potato. Final concentration of this test sample was 200 μg/100g. For the 500 μg/100g level, 45 ml of toxin solution were added to 755 ml of water to produce another 1000g of toxic mashed potato. Then 250g of each level of toxic product were dispensed into 14 bottles and

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immediately refrigerated at 2°C. Two 250g coded samples of STX in mashed potato matrix (200 and 500 μg/100g) were shipped in the insulated containers to collaborators to perform duplicate analyses on each sample in accordance with provided instructions.

Effect of circadian rhythm

In order to determine if circadian rhythm of the mice was responsible for producing analytical variations, six mice were injected with 1.0 ml of 0.33 μg/ml STX every 4 hr during a 24-hr period. Seven sets of mice were injected, a total of 42 mice. Temperature in the mouse room was held at 20°C, and lighting was continuous. Food and water were provided ad libitum. Care was taken to avoid traumatizing the mice prior to injection.

Statistical analysis

The statistical techniques used were modifications of procedures outlined in Youden and Steiner's (1975) manual of statistical techniques for collaborative tests. Homogeniety of samples was determined by performing a t-test on the difference between means of replicate samples containing either the 200 or 500 μg/100g level of STX. Total variance (Sd^2) was considered as being the sum of within laboratory variance (Sr^2) and between laboratory variance (Sb^2). It was calculated by squaring the standard deviation of all determinations of a particular level of toxin. Within laboratory standard deviation was calculated at $Sr=\sqrt{\frac{\Sigma d^2}{2n}}$ where d is the difference between replicates. The notation Sb^2, the between laboratory variance, was calculated using $Sd^2 - Sr^2 = Sb^2$. Outliers were determined by procedures described in the National Bureau of Standards Handbook No. 91 (Natrella, 1963).

RESULTS

TABLE 1 compares the results of using the four different matrices. While *Mya marenaria* yielded the highest recovery, at the 200 μg/100g level, the coefficient of variation was least with mashed potatoes (6.0%). The results of testing the 500 μg/100g toxicity level are also shown in Table 1. Mean recoveries for the clam homogenate and mashed potato were very similar, but the standard deviation and coefficient of variation were, again, lower for the mashed potato matrix. The use of two different methods of toxifying mashed potato matrix showed that there was no difference between adding pre-measured toxin to each beaker of mashed potato or toxifying the water before addition of potato flakes. However, the water toxification procedure was the practical way to make large quantities of homogenous samples for shipment to collaborators.

Table 2 presents the values reported by the 14 laboratories. The rejection range for outliers in the low level sample was < 34 and > 122 μg/100g. On this basis, laboratories 9 and 11, had values outside this range. The rejection range for the outliers at the 500 μg/100g level < 161 and > 542 μg/100g. Laboratories 9 and 10 are outliers on the high side, while laboratory 3 had one outlier on the lower end.

In searching for possible causes of variation, the circadian rhythm of the mouse was considered. Results of the 24-hr study showed during any particular time of day. Response to STX was fairly uniform, and the variation produced was attributed to the variation inherent in the mouse test itself.

DISCUSSION

THE DATA PRESENTED in Table 1 shows that mashed potato or any of the shellfish matrices could serve as a carrier of pure STX for use in a collaborative study. Percentage recovery of STX ranged from 31–51% for the 200 μg/100g level of inoculation. At the 500 μg/100g level, percentage recovery of added STX was 70% for both *Mercenaria mercenaria* and mashed potatoes. The physiological salt effect reported by McFarren (1959) and Shantz et al. (1958) may explain the low yields. Instant mashed potatoes are known to contain as high or higher amounts of sodium as clams (Marsh et al., 1980). In spite of the expected low recovery, consistent recovery percentages were obtained.

Other than always having the smallest coefficient of variation, the mashed potatoes offered several unique advantages over the shellfish matrices. With clams or other shellfish, the meats must be shucked, blended and carefully toxified. This procedure is time-consuming and tedious. In the preparation of mashed potato, the simple procedure as outlined assures complete homogeneity. Since the supply of mashed potato is not seasonally dependent, it does not suffer from the logistic problems associated with shipping toxic clams. Clams would not have to be dredged; this results in a cost reduction as well as the elimination of a biological variable.

As long as the potato was not frozen, texture and toxicity remained stable. In the collaborative study, precise results were obtained up to 2 months after shipping. When frozen and thawed, there was a drip loss at the bottom of the sample container, and toxicity of the product was no longer found to be homogenous. Because of this, a refrigerated sample was used in the collaborative study.

A number of outlying values and laboratories are shown in Table 2. After examination of the original data presented

Table 1—Recovery of toxicity from different matrices. Saxitoxin dihydrochloride added at 200 μg/100g and 500 μg/100g

	200 μg/100g				500 μg/100g	
Replicate	*Mercenaria mercenaria* μg/100g	*Mya arenaria* μg/100g	*Mytilus edulis* μg/100g	Instant mashed potato μg/100g	*Mercenaria mercenaria* μg/100g	Mashed potato μg/100g
1	64	110	88	60	315	370
2	61	101	86	63	326	312
3	79	(153)[a]	74	58	335	387
4	56	94	76	61	376	374
5	79	102	70	67	334	338
6					442	314
7					308	—
Mean	67.8	102.0	78.8	61.8	348	349
Standard deviation	10.6	10.1	7.8	3.4	43.4	29.5
Coefficient of variation, %	16.0	9.0	10.0	6.0	13.0	9.0
Recovery of toxicity, %	33.9	51.0	39.4	30.9	69.6	69.8

[a] Eliminated as an outlier

Table 2—Saxitoxin levels reported by collaborating laboratories assaying duplicate mashed potato matrix samples

	200 μg/100g sample				500 μg/100g sample			
	First replicate		Second replicate		First replicate		Second replicate	
Laboratory	Toxin (μg/100g)	Dilution factor	Toxin (μg/100g)	Dilution factor	Toxin (μg/100g)	Diluton factor	Toxin (μg/100g)	Dilution factor
1	70	1.0	76	1.0	400	5.0	387	5.0
2	108	1.6	100	1.6	441	6.0	380	7.5
3	68	1.0	66	1.0	(157)[a]	3.0	176	3.0
4	80	1.0	78	1.0	386	4.5	345	4.5
5	74	1.0	74	1.0	291	3.0	283	3.0
6	70	1.0	74	1.0	306	4.0	287	4.0
7	98	1.5	95	1.5	338	5.0	336	5.0
8	76	1.0	65	1.0	412	5.0	395	5.0
9	(154)[a]	2.8	(190)	3.5	(550)	12.5	(594)	11.0
10	91	1.2	108	1.2	(546)	7.0	508	7.0
11	(127)	2.0	(135)	2.0	357	5.0	426	5.0
12	67	1.0	—	—	265	3.5	—	—
13	57	1.0	60	1.0	253	4.0	312	6.0
14	73	1.0	65	1.0	396	5.0	403	5.0
Mean	86.7		91.2		364		371	
Standard deviation	25.9		41.5		104.5		100.6	
Coefficient of variation, %	29.0		45.0		28.0		27.0	
Recovery of toxicity, %	44.0		46.0		73.0		75.0	
Overall mean		88.0				367		
Overall Standard Deviation		31.4				102.5		
Overall Coefficient of Variation, %		35.0				28.0		

[a] Numbers within parentheses designated as outliers.

by the laboratories that reported the outliers, a pattern became apparent in the method used to determine an appropriate dilution factor (DF). For Laboratory 10, their 500 μg/100g level value of 546 was classified as an outlier. In making their initial analyses, short death times were noted with three mice at a DF of 3.3. The following dilution was made to yield a factor of 7, which resulted in toxicities of over 500 μg/100g. Our own results indicated that a DF of 5 yielded death times in the 5–7 min range using a conversion factor of 0.22. Also, our experience indicates that if dilutions had been made in smaller, stepwise increments, a more conservative estimate of the toxin level would have been obtained.

Laboratories 9 and 11 arrived at abnormally high values for their 200 μg/100g samples using excessive dilution factors. In similar fashion for the 500 μg/100g level, Laboratory 9 reported results based on dilution factors of 11 and 12.5. With these factors, proportionally smaller differences in death times occur. Thus, three or four dilution factors might produce 5–7 min death times. To avoid an artificially high interpretation, the most conservative times should be chosen as correct. The high results obtained by Laboratories 9, 10, and 11 support the process of making dilutions by a stepwise progression, starting with one. Laboratory 3, on the other hand, produced the only outlier on the low side for either sample level. Calculations and methods of dilution appear correct; however, the cause is unknown.

Previously, it had been assumed that time of day of mouse injection contributed to variation in death times (Prakash et al., 1971). Halberg (1960), using endotoxin from *E. coli* found that percentage of death in mice varied from 20% to nearly 100% with the only variable being the time of day. Our tests with STX did not produce a response similar to endotoxin. No circadian rhythm effect was found. Endotoxins are lipopolysaccaride-protein complexes which are antigenetically active and produce cellular injury

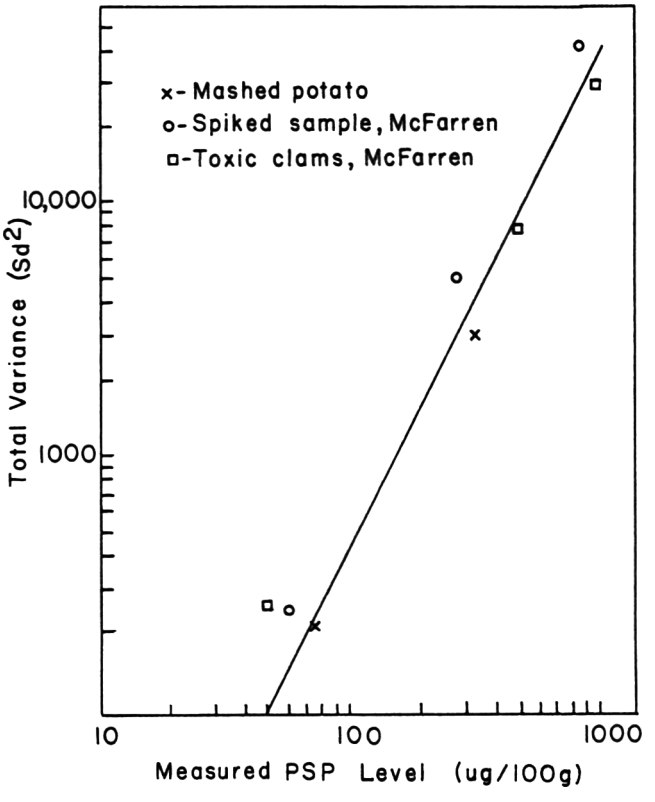

Fig. 1—Comparison of total variance obtained in this collaborative study with McFarren's 1959 collaborative study.

(Stanier et al., 1976). In contrast, STX selectively prevents sodium resorbtion in nerve cells.

—Continued on page 1079

Effects of Temperature on the Decomposition of Pacific Coast Shrimp (*Pandalus jordani*)

JACK R. MATCHES

ABSTRACT

Shrimp *(Pandalus jordani)* were allowed to decompose at five temperatures (0, 5.6, 11.1, 16.7 and 22.2°C). Mean aerobic plate counts on the shrimp as received at the laboratory were 1.6×10^6/g. The most rapid growth was obtained at the higher temperatures but the highest maximum population was obtained at 0°C. A total of 638 bacteria were isolated and tested for identification. The heterogenous population at day 0 was composed of 38% Gram positives. The population changed becoming predominantly Gram negative by day 13. At the higher temperatures the indole producing *Proteus* emerged by day 7 and became an important component of the flora. Volatile base (N) and indole increased both with increasing time and temperature during storage.

INTRODUCTION

NUMEROUS PAPERS on shrimp have appeared in the literature. In most of these studies which included microbiological and/or chemical changes, the shrimp were stored in ice for various lengths of time (Cann, 1974; Cann et al., 1971; Carroll et al., 1968; Cheuk et al., 1979; Cobb and Vanderzant, 1971; Cobb et al., 1977; Green, 1949; Iyengar, 1960; Jacob et al., 1962; Walker et al., 1970; Ward et al., 1979). The ice storage of shrimp, which is the method used by much of the fishing industry, is often done very poorly, the time from catch to icing is often extended, or the time in ice is excessive. In any case where shrimp and/or other marine animals are allowed to remain at warm ambient temperatures or are poorly iced, degradative changes begin to take place rapidly. The rate of these changes can be considered temperature dependent and any review of the effects of temperature on bacterial growth rates or enzyme activity will bear this out.

Most of the work reported has been done on species other than *Pandalus jordani* or *borealis* which are found in the cold waters off the Pacific Northwest coast of the United States. This industry is new (Harrison and Lee, 1969), expanding, and little technological data are available. Although studies have been done on microbiology (Harrison and Lee, 1969; Lee and Pfeifer, 1975). postmorten quality changes (Flores and Crawford, 1973) and processing and quality studies (Collins, 1960), little work has been done on spoilage changes at different temperatures. In this study *Pandalus jordani* were stored at five temperatures and held until the shrimp were decomposed. The changes in bacterial numbers and species were measured during storage. Chemical changes were monitored by measuring indole and total volatile nitrogen. These studies were done to determine the effects of temperature abuse on shrimp quality and decomposition.

Author Matches, formerly with the Seafood Products Research Center, FDA, 5003 Federal Office Bldg., 909 First Ave., Seattle, WA 98174, is now affiliated with the Institute for Food Science & Technology, College of Oceanography & Fishery Sciences, Univ. of Washington, Seattle, WA 98195. This work done while Dr. Matches was on sabbatical leave as Acting Laboratory Director.

EXPERIMENTAL

Sample preparation

Shrimp *(Pandalus jordani)* were caught off the coast of Washington and iced aboard the vessel. A period of five days elapsed between catching, landing and delivery to the laboratory. During this period the air temperature was in the mid to low 30's and the samples were kept in ice. Upon receipt at the laboratory, the shrimp were broken into five lots of approximately 10-lb each. Each lot was stored at a different temperature; those used were 0 and (0–1°C melting ice), 5.6, 11.1, 16.7 and 22.2°C (32, 42, 52, 62, and 72°F). At 0°C the shrimp were placed in crushed ice and the melt water allowed to drain off. At the other temperatures the shrimp were placed in enameled pans and the pans placed in a large, loose polyethylene bags to slow water evaporation. At sampling periods representative samples were removed with sterile forceps. These samples were randomly taken from all areas of the storage container with adhering drip. Fifty gram samples were removed for microbiological analyses and approximately 500g were removed and frozen for chemical and sensory analyses.

Microbiological analyses

All samples were analyzed for aerobic bacterial count by the spread plate method using standard methods agar (Gilliland et al., 1976). Duplicate 50-g samples were blended with 200 ml diluent (phosphate buffered water). The first dilution was prepared by adding 10 ml homogenate to 90 ml diluent; in subsequent dilutions 1 ml homogenate was added to 9.0 ml diluent. All plates were incubated at 22°C.

Approximately 30 colonies were picked from appropriate plates for each sample by a random number technique. Isolated colonies were streaked and picked from trypticase soy agar three times for purification. The isolates were identified using a modified scheme of Shewan et al. (1960). Each isolate was tested for Gram reaction, cellular morphology, colony morphology, pigment production, oxidase reaction, indole production, catalase and motility under phase contrast. Oxidase negative organisms were tested for urea production and oxidase positive motile rods were tested for oxidative or fermentative metabolism.

Oxidase test. The oxidase test was performed by rubbing the culture on a strip of filter paper impregnated with tetramethyl-p-phenylenediamine·2HCl. A platinum loop was used to pick and spread the colonies. Cultures turning pruple within 60 sec were considered positive.

Indole test. A medium containing the following ingredients was used: Tryptone (Difco), 10g; disodium phosphate, 0.2g; dextrose, 0.1g, and H_2O, 100 ml. Better growth of the isolates was obtained with this medium than with 1% tryptone.

Urea test. The urea test was run using 3 ml of broth prepared from Difco urea broth concentrate. Incubation was 24 hr at 30°C.

Oxidation-fermentation test medium. The test medium of Hugh and Leifson (1953) was used. Glucose was added as the test carbohydrate and the anaerobic tube was sealed with a layer of sterile mineral oil.

Chemical analyses

Total volatile base calculated as nitrogen was run using the Conway Microdiffusion Dish (Conway, 1958). A 1-ml sample of shrimp homogenate (1:5 dilution used for aerobic plate count) containing 0.2g shrimp was placed in the outer well. One ml of a 2% boric acid solution was used as the trapping agent in the center well. The rim of the dish was lightly coated with a starch glycerine cover adhesive (35g starch + 110 ml glycerine heated to boiling with stirring). The volatile base was released by adding 1.0 ml of saturated K_2CO_3 to the shrimp in the outer well. After

standing at room temperature overnight, the boric acid was titrated with standardized sulfuric acid and a mixed indicator (0.1% bromcresol green and 0.1% methyl red in 95 % ethanol).

Indole content of shrimp was measured by the colorimetric method AOAC 18: 063 (1975).

Sensory evaluation

Sensory evaluation of the shrimp was done by the analyst at the time of sampling. Samples of whole shrimp were also frozen at each takeoff time, later thawed and evaluated sensorily, again by the original analyst and another analyst specializing in marine products. The shrimp were classified as Class 1, 2 or 3. This classification is used by Food and Drug Administration analysts trained in decomposition workshops (Throm, 1980) and is as follows:

Class 1: Passable. This category includes fishery products that range from very fresh to those that contain fishy odors or other odors characteristic of the commercial product, not definitely identifiable as decomposition.

Class 2. Decomposed (Slight but Definite). The first stage of definitely identifiable decomposition. An odor is present that, while not really intense, is persistent and readily perceptible to the experienced examiner as that of decomposition.

Class 3: Decomposed (Advanced). The product possesses a strong odor of decomposition which is persistent, distinct and unmistakable.

RESULTS

MICROBIAL COUNTS on the shrimp held at five different temperatures are shown in Fig. 1. The sample listed as 0 day was actually 5 days in ice. This was used as the 0 day or starting sample for the samples stored at the five temperatures. The total number of bacteria from duplicate samples on 0 day was 1.6×10^6/gram.The effects of time and temperature can be seen in these data. Counts increased with time and at each temperature, and counts increased more rapidly during the first 3–5 days for the highest incubation temperatures. Counts at 0 and 5.6°C showed the fastest increase after 6 and 7 days, respectively. The highest maximum number was obtained at 0°C. Most of these samples were incubated beyond normal decomposition to show the effects of increased temperature or temperature abuse. As a result the counts obtained were very high and the sensory qualities were extremely low at the later storage times. Although the sensory method is a subjective evaluation, it is the most commonly used method in the seafood industry to determine the edibility of a product. The data in Table 1 show that shrimp held at 0°C remain in passable or Class 1 condition for 6 days. With the 5 additional days in ice prior to receipt at the laboratory, the acceptable life of the shrimp was a total of 11 days. As the storage temperatures are increased, the shrimp are acceptable (Class 1) for a total of only 6 days when stored at 5.6°C and 1 day at 11.1°C. At storage temperatures of 16.7 and 22.2°C a Class 1 shrimp is unacceptable (Class 2) after 1 additional day at the test temperature.

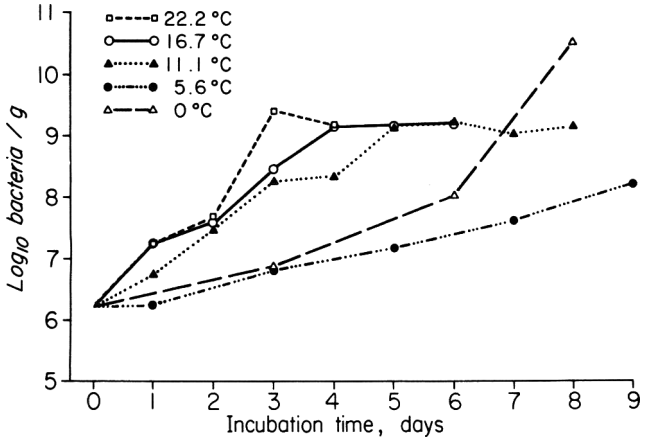

Fig. 1—Total bacterial counts on shrimp stored at five temperatures.

Isolation of bacteria

Although samples were stored at 30 different time-temperature combinations, bacteria were isolated from only a selected 21 different variables. A total of 650 organisms were isolated, 50 from 0 day sample and approximately 30 from each of the remaining 20 samples. After repeated transfers, 638 viable organisms remained. The numbers and percent of each genus are shown in Table 2. Although one can only speculate as to the populations at the time of landing aboard the vessel, it can be seen that the popula-

Table 1—Sensory evaluation of shrimp stored at five temperatures[a]

	Storage temp °C				
Days	0	5.6	11.1	16.7	22.2
0	1				
1		1	1	2	2
2			2	3	3
3	1	2	3	3	3
4			3	3	3
5			3	3	
6	1		3	3	
7		2	3		
8	1		3		
9		3			
10	2	3			
11					
12	3				

[a] Class 1—acceptable shrimp; Class 2—early decomposition; Class 3—decomposed

Table 2—Genera and numbers of bacteria isolated from shrimp during storage at five temperatures

	Days at 0°C						Days at 5.6°C						Days at 11.1°C					Days at 16.7°C				Days at 22.2°C			
Genera	0	3	6	8	10	13	0	1	3	5	7	9	0	1	3	5	7	0	1	3	5	0	1	2	3
Micrococcus	9	0	1	0	1	0	9	2	0	1	0	1	9	0	4	0	0	9	0	3	1	9	0	1	0
Flavobacterium	1	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0
Coryneform	10	6	4	0	0	1	10	3	3	2	2	2	10	1	2	2	2	10	0	2	0	10	2	0	3
Moraxella	21	13	13	3	10	7	21	13	10	26	18	26	21	11	14	23	15	21	18	9	4	21	17	8	2
Acinetobacter	5	3	1	0	0	0	5	1	0	1	0	1	5	1	5	1	0	5	1	3	3	5	1	2	0
Cytophaga	2	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	2	0	0	0	2	0	0	0
Pseudomonas	0	7	9	20	15	20	0	4	8	1	9	1	0	14	1	3	0	0	6	3	3	0	6	0	0
Vibrio	0	1	1	2	3	1	0	1	5	1	0	1	0	1	2	1	8	0	1	1	0	0	0	1	2
Proteus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	9	17	0	0	17	22
Unknown	2	0	0	4	1	1	2	0	1	0	1	0	2	0	2	0	4	2	1	0	1	2	2	2	2
TOTAL	50	30	30	30	30	30	50	25	27	32	30	30	50	28	30	30	30	50	27	30	29	50	28	31	31

Table 3—Indole concentration (μg/100g shrimp) produced in shrimp during storage at five temperatures

	Storage temp °C				
Days	0	5.6	11.1	16.7	22.2
0	1.08				
1		4.76	18.6	26.2	23.8
2			61.8	195	623
3	2.4	9.5	130.8	595	
4			120.8		
5		11.9	150		
6	3.3				
7		14.3	915		
8	4.8				
9		33.3			
10	65.7				
11					
12					
13	94.8				

Table 4—Total volatile nitrogen (mg/100g shrimp) produced in shrimp during storage at five temperatures

	Storage temp °C				
Days	0	5.6	11.1	16.7	22.2
0	40				
1		50.8	75.5	105.5	143.2
2			129.8	178.4	264.5
3	39.5	80.1	203.1	252.6	399.7
4			243.5	186.2	557.3
5		112.0	255.2	420.5	
6	47.8		373.7	420.5	
7		161.4	398.4		
8	45.6		629.5		
9		207			
10	70.1	223			
11					
12					
13	152.3				

tion at day 0 when received at the laboratory was heterogenous. Gram positives made up 38% of the isolates. This has also been shown in other data (Cann, 1974). The Gram negative rods normally found on other marine products, beef and poultry such as *Flavobacterium, Moraxella, Acinetobacter* and *Cytophaga* made up the remainder of the population. A shift in this population took place during storage. At 0°C in melting ice the population shifted to one composed of 67% *Pseudomonas*, 23% *Moraxella* and 3% each of coryneform, *Vibrio* and unknown by day 13. The population on samples stored at 5.6°C changed in a very similar manner except *Moraxella* made up 77% of the population while *Pseudomonas* made up only 1%. The remaining 6% were Gram positive. At 11.1°C the population became predominantly one of Gram negative rods by 7 days storage. In these samples *Moraxella* remained high throughout the storage period. *Pseudomonas* increased on day 1 and then decreased after day 5. *Vibrio*, on the other hand, increased from 4% on day 1 to 27% by day 7. *Acinetobacter* made up a significant portion of the population through day 3 and then dropped to 0 by day 7. The Gram positive *Micrococcus* and coryneforms contributed to the total population with the latter present in low numbers throughout the storage period. Of importance here is the emergence of *Proteus* by day 7. This indole producer became the major component of the population at the higher temperatures late in the storage period.

At 16.7 and 22.2°C the length of storage was reduced because of the very rapid bacterial growth. At these temperatures the Gram positive organisms contributed to the population but, as at the lower temperatures, the Gram negative organisms predominated at the time of spoilage. At both temperatures *Moraxella* made up the major portion of the Gram negative populations with low numbers of *Acinetobacter, Pseudomonas* and *Vibrio* also present. *Proteus* became a very important contributor to the populations by days 3 and 2 for 16.7 and 22.2°C respectively. By the end of storage this organism made up greater than 50% of the population.

Indole levels (micrograms indole/100g shrimp) obtained during incubation at the five temperatures are shown in Table 3. Indole levels increased with time and temperature. At day 0 (5 days in ice since catch) the shrimp contained 1.08 μg of indole. This level increased only to 4.8 μg by 8 days in ice and then increased rapidly to 65.7 and 94.8 after 10 and 13 days respectively. The rate of increase at 5.6°C was a little faster than at 0°C, which is to be expected. The rate of increase at the three higher temperatures of 11.1, 16.7 and 22.2°C was very rapid with extremely high levels of indole produced. These samples were in advanced stages of decomposition and bacterial counts were also high. The rapid increase in indole levels and the high levels obtained (day 7 at 11.1°C, day 3 at 16.7°C and day 2 at 22.2°C) correspond to the first isolations of *Proteus*, a rapid indole producing organism. From a regulatory standpoint, with actionable levels set at 25 μg/100g shrimp, the samples were considered decomposed after 8, 7 and 1 days storage at 0, 5.6 and 11.1°C respectively. The actionable level is the point at which FDA feels the fish is unfit for human consumption and initiates legal action. The levels obtained on day 1 at 22.2°C were only 21.4 μg. This is approximately 4 μg below the actionable level. However, it increased to 632 μg by day 2.

Total volatile bases calculated as nitrogen and obtained from the shrimp are shown in Table 4. The levels of nitrogen are very high and more than double values published by workers on other shrimp species (Cann, 1974; Cobb et al., 1973; Ward et al., 1979). Most volatile base data are obtained from headed and peeled shrimp. The shrimp used in these studies were stored whole and the high levels of nitrogen, also detected sensorily as ammonia, possibly result from bacterial action and enzymes in the animal's gut.

The total volatile nitrogen increases in the same manner as indole. The levels change slowly at 0°C for 8 days and then the rate of change increases. The rate of change and also the levels obtained increased with storage temperature.

DISCUSSION

THE SHRIMP used in these studies, *P. jordani*, are usually not held for long periods of time before being given a mild heat treatment and machine peeled. These studies were designed to show the effects of temperature abuse which can reasonably be assumed to be applicable to other shrimp species and in fact also to other fish.

The numbers of bacteria present on and in the shrimp as obtained at the laboratory were 1.6×10^6/gram. This is in close agreement with other data for *P. jordani* collected dockside (Harrison and Lee, 1969; Lee and Pfeifer, 1975) and higher than the levels of 1.4×10^4 and 3×10^4 reported by Liston (1974) for frozen Alaska shrimp. The shrimp used in these studies were held aboard the vessel in ice for 4 days. They were then unloaded at South Bend, Washington, separated from other fish species (mainly smelt), washed and reiced. The reiced shrimp were then placed in an ice chest for transport to the Seattle Laboratory, which required an additional day. Microbiological studies aboard

the fishing vessel and at the unloading dock were not done. Therefore, the reasons for the high count are only speculation. The increase to log 6.0 may have resulted from contamination with sediment during catch, poor handling after catch, during storage or after unloading, or the animals may inherently contain high numbers of bacteria. These bacteria subsequently increased to levels of approximately log 9 per gram during storage at the five temperatures.

The bacterial populations isolated from the shrimp are similar to those reported in the literature. Gram negative bacteria belonging to *Pseudomonas, Moraxella, Acinetobacter* and sometimes *Flavobacterium* dominate the microflora of most crustaceans caught in cold water (Cann, 1974, 1977). Temperate zone crustaceans sometimes also carry a high proportion of Gram positive bacteria such as coryneforms (Hobbs et al., 1971; Lee and Pfeifer, 1975). Other authors (Walker et al., 1970) reported spoilage patterns for shrimp in which *Achromobacter* and *Corynebacterium* strains dominated. In the studies reported here, *Moraxella (Achromobacter)* made up a very significant proportion of the total population in samples stored at all temperatures (there were changes in populations at each temperature showing the effects of temperature on population). It is surprising that *Pseudmonas* were below levels of detection at day 0 after the shrimp had been iced 5 days. *Pseudomonas,* absent from the isolates at day 0, were found later on samples at all storage temperatures, but the greatest numbers and persistence were most evident at the two lower temperatures tested. The Gram positive *Micrococcus* and coryneforms were found at all temperatures but numbers greatly diminished with storage after the 0 day count. These data on bacterial changes further substantiate the effects of temperature on bacterial populations and numbers.

The chemical changes taking place in fish and shellfish during spoilage are due primarily to bacterial action although studies have shown the contribution by endogenous enzymes (Cobb, 1977; Cheuk et al., 1979). In the studies reported here, the chemical changes measured were volatile base production calculated on nitrogen and indole. The production of indole was evident in all samples. The highest levels detected were in the samples from which *Proteus* were isolated. Indole producing bacteria were not isolated from all indole positive samples. This can be explained in two ways. First, the production of low levels of indole may be the result of endogenous enzymes and second, the number of indole producing bacteria may have been below the levels of detection. If the number of indole producers is 2 or more logs lower than the total count, their detection by random selection of colonies for identification is unlikely. The greatest jump in indole production corresponds to the detection of *Proteus*. Samples stored at 11.1°C showed an indole increase from 150 μg of indole/100g at 5 days to 915 μg at 8 days. The change between day 2 and day 3 at 16.7°C was from 195 to 595 μg and from day 1 to day 2 at 22.2°C the change was from 24 to 623 μg. These levels are unimportant from a regulatory standpoint because the actionable level is 25 μg/100g of shrimp. These data do, however, show the effects of temperature abuse and how selection by storage conditions for a given organism (*Proteus* in this study) can affect the chemical changes in the tissue during storage.

The volatile base calculated as nitrogen-produced in shrimp at the five temperatures tested was much higher than expected. During storage it was evident that the volatile base levels would be high because of the strong ammonia odor. As mentioned earlier, the method of packaging during storage may have selected for ammonia production. Perhaps more importantly, the shrimp were not beheaded which removes the gut and a high percentage of the total bacteria flora present. These organisms and the gut contents could possibly have contributed to the very high volatile nitrogen. Volatile bases were run on homogenates which had been stored 3 months at temperatures which fluctuated between 0 and −18°F, and although not measured, the breakdown of trimethylamine oxide (TMAO) may have contributed to the high levels.

These data show the effects of temperature on the decomposition of shrimp *Pandalus jordani*. Although the shrimp were caught in temperate waters and contained bacterial population composed primarily of Gram negative bacteria, the data may also apply to warm water shrimp containing a greater number of Gram positive bacteria. The higher storage temperatues used in these studies are the temperatures to which some of the import shrimp have possibly been subjected. These data, therefore, indicate some of the changes caused by temperature abuse of fresh shrimp and may even explain the low quality of some of the samples imported into the United States.

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Extending Shelf-Life of Fresh Wet Red Hake and Salmon Using CO_2-O_2 Modified Atmosphere and Potassium Sorbate Ice at 1°C

M. S. FEY and J. M. REGENSTEIN

ABSTRACT

The ability to safely extend the shelf-life of fish would open up the possibility of shipping fresh wet fish by boat. Red hake and salmon can be successfully held for almost 1 month and still be sensorally acceptable. A 60% carbon dioxide:20% oxygen:20% nitrogen-modified atmosphere with a 1% potassium sorbate ice was most satisfactory. CO_2 did not lower the pH of the fish. Low temperature (1°C) and presence of both oxygen and potassium sorbate served as protection against botulism development. The presence of oxygen, even with salmon, did not lead to rancidity (TBA) problems. The Torrymeter can be used to monitor red hake quality. Work incorporating potassium sorbate dips before modified atmosphere storage with or without potassium sorbate ice is needed.

INTRODUCTION

FISH is a highly perishable commodity. Despite this limitation, it is desirable to be able to transport high-quality fresh fish, particularly underutilized species of fish, to appropriate distant markets. To obtain the economic benefit (lower processing costs, higher sales prices) of marketing fresh, wet fish rather than frozen fish by containerized van or boat to distant markets may require a fresh fish shelf-life of 3–4 wk: 2–3 wk in transit and approximately 1 wk following transit.

Alaskan salmon are caught far from market. They are currently being shipped in modified atmosphere containers for up to about 1½ wk from Anchorage to Seattle (Veranth and Robe, 1979).

In the Northwest Atlantic a number of underutilized species have been identified, including the gadoid red hake. Because frozen storage of this (and other gadoids) leads to texutral problems, it might be preferable to market these as fresh wet fish.

A number of reviews of previous shelf-life extension work with fresh wet fish exist (Reay and Shewan, 1979; Windsor and Thoma, 1974; Wolfe, 1979). The work focuses on vacuum, nitrogen and/or CO_2 packs for both bulk transport and retail packages. Potassium sorbate (e.g. Robach, 1979; Robach and Ivy, 1978) is also being examined for its shelf-life extension properties. Our objectives were to:

1. Combine the effects of modified atmospheres and potassium sorbate.
2. To operate at the lowest practical temperature (~1°C).
3. To design a test system that will not require the experimental samples to be reused (or recharged with gas) after examination. The system should ideally permit various gas treatments within one refrigerated unit.

In conjunction with this work we have also examined the potential of the Torrymeter to serve as a rapid quality check of fresh wet fish quality.

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MATERIALS & METHODS

Fish samples

Whole-iced red hake (300–1200g) were obtained from commercial fish handlers in New York and Massachusetts and transported whole on ice to the laboratory in Ithaca, New York (approximately 3–5 days old). Gutted chinook salmon (~1500g), gills removed; and headed and gutted sockeye salmon (~2500g) (chlorine dipped) were air-freighted from Seattle. Both shipments, though ice-packed, had warmed up to room temperature by arrival (approximately 3 days old). Fish were re-iced on arrival. Fish samples with ice were stored in either drainage boxes with holes or with a raised screen.

Fish were gutted and rinsed to remove adhering blood and viscera along the backbone and debris from the mouth and gills.

Thick-walled storage Barrier Bags® (custom fabricated to 160 X 110 cm; Cryovac) were used in conjunction with the bulk storage of iced red hake. More flexible regular Barrier Bags® (100 X 65 cm; Cryovac) were used with the salmon. The oxygen transmission rate of the regular Barrier Bags® is about 30–50 cc per day at 23°C.

In bulk storage, the amount of ice was adjusted for fish weight, but enough ice was always added to completely cover the fish. No additional ice was added subsequently. Potassium sorbate and Fran-Kem® (a patented combination of sodium benzoate and fumaric acid) ices were made by modifying the input to an ice machine (Fey, 1980). Chemical ices were stored at −20°C in Barrier Bags®.

A septum was made by applying about 2 X 2 X 1 cm of clear silicone glue (G.E.) to the center of masking tape put on the bag and allowing it to cure. Fish were vacuum sealed with 2 "C" clips (Cryovac Model 6 Vacuumizing Unit with Model 6 clip applier). Note: It was helpful to insure that the septum collapsed on the fish in order to provide a site in which to insert the syringe into the bag without coming out the other side. Control bags without fish contained paper towels to give some bulk.

The small Barrier Bags® could hold about 6000 cc. Thus, the ratio of gas volume to fish volume ranged from about 7:1 to 20:1.

Modified atmospheres and charging

Modified atmospheres and pure gases (Airco Industrial Gases) were checked by gas chromatography (discussed later). To charge the bag a syringe attached to the gas line was run through the central thickened portion of the septum into the bag. Gas at about 10 psig took about 1 min to charge a bag to a pressure slightly above atmospheric. Since the bags were slightly permeable to gas, the pressure inside and outside the bag equilibrated after a few hours of storage.

To seal the larger bulk-storage bags, a strip of rubber gasket material was wrapped around the twisted end of the bag. A hose clamp was secured tightly around the gasket. A vacuum was pulled with a faucet-mounted aspirator.

The refrigerator temperature for all experiments was 0–1°C, except for one sample studied at 6°C to simulate abuse. An iced control was used with all experiments.

For dipping experiments, gutted red hake was dipped into 2000 ml of 0 (Control) 1, 3, or 5% w/v potassium sorbate (Monsanto) solutions. The sclution was changed after every five fish.

For chemically iced experiments, the gutted red hake were stored in ice containing 0 (Control), 0.1, 1, and 2% w/v potassium sorbate.

pH-adjusted potassium sorbate ice was prepared using 2.2 mM sodium phosphate buffer pH 6.5 or 7.3. The adjusted pH (6.5), equal approximately to the pH of the fish, was obtained with concentrated HCl.

Fran-Kem® ice was made according to the manufacturer's specifications (Washington Laboratories, Inc., Seattle, WA) and adjusted to a final pH of 3.6 with concentrated HCL

METHODS OF ANALYSIS

TORRYMETER (Nova and Co., Manchester-by-the Sea, MA) values (0–16, 16 = best value) were obtained in at least duplicate on newly-arrived fish set on a wooden cutting board. Subsequent Torrymeter readings were made immediately after swabbing the fish for the standard microbiological plate count.

With individual Barrier Bags®, drip was measured by collecting the exudate in a graduate cylinder. For the bulk storage experiments, fish were weighed before and after storage.

Standard Plate Count (SPC) and Psychrotroph Count (PC)

"Standard Methods of the Fish Inspection Laboratories of the Department of Fisheries of Canada, Section 5, the Swab Method" (Anderson, 1965) and "Standard Methods for the Examination of Dairy Products" (APHA, 1972) were used as guides for the microbiological procedures. Steel wire templates (~10 inch^2) were placed about mid-way on the lateral line of the fish. The area was swabbed three times. The cotton tip was broken off into 100 ml 0.1% sterile peptone water (Difco). Since each ml of the resulting suspension theoretically contained the bacteria removed from 0.1-inch^2 of swabbed surface, the initial suspension was treated as a one-in-ten dilution (Anderson, 1965).

Plates were prepared from plate count agar (Difco). For SPC they were stored inverted at 25 ± 1°C for 72 ± 4 hr. Plates for PC were stored inverted at 7 ± 1°C for 240 ± 4 hr.

Eight panelists (students, faculty and technicians from the laboratory) rated pairs of raw fish on a three point scale (1 = highest score) according to selected grading criteria of the "Fish Insepction Laboratories of the Department of Fisheries of Canada for Groundfish" (Anderson, 1965). Samples were presented in pairs and a single value obtained to minimize differences due to fish to fish variation.

Raw scores obtained from grading panels for each attribute were analyzed, assuming a normal distribution, by a Randomized Complete Block Analysis of Variance (AOV). All possible differences between the means of each treatment were computed and compared to the Least Significant Difference. Data analysis was performed by George Houghton, Dept. of Food Science, Cornell Univ.

Taste panel

After storage on ice for 1 day post-treatment, eight pieces of uniform size, about 6–8g, were cut from the fillet. (The remaining fillet piece was stored on ice in foil for pH, TMA and TBA measurements later the same day.) The taste panel samples were folded in a piece of aluminum foil (about 7 X 10 cm). Fish judged to be unfit for human consumption were not included in the panel, while fish of questionable quality were designated as "The last sample to be opened." Otherwise sample designation codes were randomized and servings were staggered. Samples were baked for 10 min in a tray at 177°C (350°F) in an electric oven (Hotpoint, 560W). Each panelist, separated by dividers, from the others, was provided with a list of descriptive terms for off-flavors and odors, crackers, celery or carrot, and ice water. Logistical problems prevented panelling of fish at day zero.

Panelists (who also did the raw evaluation) were oriented and trained by being shown fresh and spoiled samples and by discussing the sensory qualities of the fish.

—Continued on next page

Taste Panel Evaluation Sheet for Red Hake

Parameter	Score	Sample Designation
Color Unattractive.....1 2 3 4 5 6 7 8 9...(Attractive)		
Odor (Offensive).....1 2 3 4 5 6 7 8 9...(Appetizing)		
Texture (Flakiness) (Unappealing)....1 2 3 4 5 6 7 8 9...(Appealing)		
Juiciness (Dislike).....1 2 3 4 5 6 7 8 9...(Like)		
Tenderness (Undesireable)...1 2 3 4 5 6 7 8 9...(Desireable)		
Flavor (Offensive).....1 2 3 4 5 6 7 8 9...(Appetizing)		
Overall Acceptability (Unacceptable)...1 2 3 4 5 6 7 8 9...(Acceptable)		

WHICH SAMPLES WOULD YOU NOT CONSIDER PURCHASING FROM A FOOD STORE? _____

COMMENTS (PLEASE SPECIFY TO WHICH ITEMS COMMENTS ARE APPLICABLE):

USE OTHER SIDE IF NEEDED

a. IF ODOR OR FLAVOR IS 4 OR BELOW, YOU HAVE THE OPTION OF NOT EATING THE SAMPLES, BUT PLEASE COMMENT ON THE UNDESIREABLE CHARACTERISTIC.

Fig. 1—Taste Panel Score Sheet.

For each attribute, a partially structured scale was used with "1" representing the least desirable quality and "9" representing the most desirable quality (see Fig. 1). Panelists were informed that if they judged an odor score to be 4 or below they did not have to eat the sample.

Raw scores obtained from the taste panels were analyzed in the same manner as the grading panel data. However, if an F-value was found to be statistically significant, data were further analyzed using orthogonal comparisons (Snedecor and Cochran, 1976). Statistical significance was determined by comparing the differences between the averages of the duplicates, to the 95% probable maximum error (PME).

A Metrohm/Brinkman 103 pH meter Impulsomat E474 with an EA147 electrode that was put directly into the flesh was used to measure pH.

The method of Lemon (1975) was followed for the determination of TBA values using 15g of fish.

The average moisture content of red hake (82.5%) was determined in triplicate by drying at 105°C for 24 hr. The average moisture of chinook salmon from duplicate fillet samples was 79.1%. The moisture content of sockeye salmon was not determined, but was assumed to be about the same as the chinook salmon.

The method of Murray and Gibson (1972), was used for trimethylamine (TMA) with 10g of fish per 30 ml of 5% trichloroacetic acid rather than 100g of fish per 300 ml.

Three samples of the trimethylamine-HCl (0.1520–0.1720g) (Eastman) used for the standard curves were found to be 95.85% pure by the Kjeldahl nitrogen method (AOAC, 1965). (Data were not corrected for this impurity.) Values obtained as ug TMA-N were converted to umoles TMA/100g.

Gas chromatography (GC) (Fisher 4800 Gas Chromatography) was used to estimate the gas composition in both the premixed gases and Barrier Bags®. An 0.4 ml sample in a 1 ml pressure lock gas syringe (Series A, Hamilton) was injected into prepacked carbosieve S columns (Supelco, Inc.). The thermal conductivity detector (TCD) temperature was at 215°C, and the injection port temperature started at 210–215°C and was increased at a rate of 25°C/min.

Peak area was calculated as 1/2 the base times height (CO and H_2O) or as the width at 1/2 height times height (O_2, N_2 and CO_2). The ratio of the areas of each peak to the sum of all peaks gave an estimate of the percentage composition of each gas.

Other methods of analysis

Various analyses were performed on a one-time basis.

A protein determination of the drip from red hake packaged in a 21% O_2-1% CO-78% N_2, and in a 60% CO_2-21% O_2-1% CO-18% N_2 modified atmosphere was carried out by the method of Lowry et al. (1951).

The proximate composition of red hake was determined from ground fillet meat previously frozen at −15°C for 8 days. Six 1g samples were used in the determination of protein by the Kjeldahl method. A conversion factor of 6.25g protein/g nitrogen was used.

The fat content of ground red hake meat was determined in duplicate using 5g samples by the Goldfisch method (AOAC, 1965). The samples were then analyzed for ash content in a muffle furnace (525°C for 8 hr). A more complete set of experimental details are given by Fey (1980).

Table 1—Effect of potassium sorbate dip on the microbiological count and TMA values in red hake

Attribute	Time in storage (hr)	Control[a]	Treatment: 1% w/v Potassium sorbate dip	Treatment: 3% w/v Potassium sorbate dip	Treatment: 5% w/v Potassium sorbate dip
Psychrotroph count[b]	4	4.5	5.2	4.9	5.0
	162	6.6	6.5	6.5	5.6
	331	>8.5	>8.5	>8.5	>8.5
TMA value[c,d]	20	187	–	–	130
	188	580	310	1120	290
	356	3720	2460	1830	1720

[a] The control for all tables is fish stored in ice.
[b] Log of the average count of duplicate plates.
[c] Average of two fish per treatment with a 1 min dip in 0 (control), 1, 3, and 5% w/v potassium sorbate.
[d] TMA value: μmoles TMA/100g wet fish weight.

Table 2—Effect of potassium sorbate ice on some taste panel values of red hake

Attribute[a]	Time in storage (hr)	Control	Treatment: 0.1% w/v Potassium sorbate ice	Treatment: 1% w/v Potassium sorbate ice	Treatment: 2% w/v Potassium sorbate ice
Odor	285	5.1	5.8	5.0	5.4
	453	3.3[c]	4.6[d]	5.9[e]	5.1[d]
Flavor	285	4.6[c]	6.4[d]	5.3[c]	4.9[c]
	453	3.2[c]	4.8[d]	5.4[d]	4.9[d]
Overall acceptability	285	4.4[c]	6.2[d]	5.1[c]	5.3[d]
	453	3.4[c]	4.9[d]	5.6[d]	5.1[d]

[a] Average of two fish per treatment; 8 panel members for all taste panels. A 9-point scale was used; a score of 4.0 or below indicates poor quality.
[c,d,e] Rows of values with the same superscript, or rows of values containing no superscripts are not significantly different at $P<0.05$.

RESULTS

THE TABLES are limited to those results which showed differences between treatments. Only the initial and final time values are shown unless intermediate time values aid in the understanding of the results. The complete results are given by Fey (1980).

The effects of 1, 3 and 5% potassium sorbate dips on the shelf-life of gutted red hake did not improve the final sensory properties at 356 hr. The microbiological counts (Table 1) were high after 331 hr in all samples, yet the fish were not considered spoiled by the panel. The TMA data suggests a possible selective benefit of the dip in inhibiting specific TMA-producing spoilage organisms.

The TBA values in red hake did not show any measurable increases.

The sensory attributes of cooked red hake, after exposure to 0.1, 1 and 2% potassium sorbate ices, for which differences ($P < 0.05$) were found are shown in Table 2. Note that at 453 hr the flavor and odor of the 1% ice-treated fish was better than that of the 2% ice-treated fish. At 285 hr the 0.1% ice gave better results, than the higher levels.

Improvements in the bacterial counts were observed earlier during storage (for 1% and 2% treatments) but were lost by 432 hr (e.g., 265 hr: log control = 7.1, 1% = 5.3, 2% = 4.8). The major benefit during prolonged storage in sorbate ice, like the dip, seemed to be its effect on inhibiting TMA formation (e.g., 453 hr: TMA (μmoles/100g fish): control = 2400, 0.1% = 1800, 1% = 400, 2% = 900).

The effect of various gases and gas mixtures on the shelf-life of red hake was studied in four separate experiments. The first series of gases did not contain CO_2 or CO. Dry air was included to test the effect of differences in humidity, build up of metabolic gases, consumption of naturally present gases, etc.). The 100% nitrogen pack had a lower aerobic bacterial count (Table 3) throughout the storage period but this did not improve the sensory response.

The actual composition of the gases used experimentally and their change over time both with and without the presence of fish are shown in Table 4. The oxygen-containing samples lost oxygen during storage.

Samples of red hake containing 20% CO_2 (21% O_2) or 60% CO_2 (5 and 21% O_2) generally showed an improve-

ment in sensory values as a result of treatment at 488 and 656 hr (Table 5). The control was spoiled at 656 hr while the treated fish were still acceptable. Both the microbiology and the Torrymeter numbers as shown in Table 6 also suggest that higher CO_2 levels did have a beneficial effect. In commercial transportation systems it is necessary to expect a higher leakage rate than in gas "impermeable" bags and thus higher initial amounts of CO_2 might be beneficial. 60% CO_2 seemed to decrease the initial pH slightly, from pH 6.8 to pH 6.5, and even after 656 hr the pH had only risen about 0.4 units as compared to almost 0.9 units for the control. But, unlike sorbate, CO_2 did not decrease the TMA values, although initially a beneficial effect was seen. Thus, the benefits of CO_2 and sorbates may be different.

CO, in the presence of CO_2, was also tested and had, if anything, a negative effect, e.g., overall sensory acceptability with CO in 60% CO_2:21% O_2 decreased to 4.4 from the 5.2 of the control at 600 hr; color was also significantly lower (6.2 vs 7.4). Work with this compound was discontinued.

The final red hake experiments combined the use of modified gas atmosphere (60% CO_2) and potassium sorbate ice (1%). Since very little difference had been observed, the 5 and 21% oxygen samples were each used in different experiments. The bulk storage samples were only tested initially and at the end of a particular experiment.

The gas pack had a beneficial effect and the gas-sorbate ice samples had the best raw appearance (Table 7). Fran-Kem ice alone was not as effective as the potassium sorbate ice. The consistency of the flesh was best in the control and in the gas-sorbate ice sample. The full treatment (gas with sorbate ice) was not as effective for gill odor as ice.

The taste panel results (Table 8) suggest that the full treatment significantly improved flavor and overall acceptability. These two scores often closely approximated each other, reminding us of the importance of flavor in the overall perception of fish. Other tests are summarized in Table 9. The lower values for uniced fish may be due to desiccation of the skin surface during storage. The Torrymeter numbers parallel the overall acceptability rating. The higher drip loss of most of the treated samples would have to be taken into account in any economic evaluation of potential commercial shipping and storage methods.

Red hake which had been stored for 2 months in the gas pack system was still edible; the flavor was acceptable, but the texture was similar to that found with frozen red hake, i.e., the tough "cottony-spongy" texture that is associated with trimethylamine oxide breakdown to dimethylamine and formaldehyde.

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Table 3—Effect of Non-CO_2 atmospheres on microbiological counts of red hake

Analysis[a]	Time in storage (hr)	Control	Barrier Bags® containing: Dry air	100% N_2	5%O_2-95%N_2
Log standard plate count	61	4.1	4.1	3.7	3.7
	228	7.0	5.9	3.9	6.3
	396	8.9	8.2	5.8	7.9
Log psychrotroph count	61	4.0	4.1	3.4	2.4
	228	7.0	5.9	4.1	6.3
	396	8.5	8.2	5.8	7.9

[a] Log of the average (duplicate plates) of two fish per treatment.

Table 4—Changes in the gas composition of the Non-CO_2 atmospheres during storage of red hake

Atmosphere	Time in storage (hr)	% Gas[a] N_2	O_2	CO_2	H_2O
Dry air	12*	82.6	17.3	0.0	0.1
	43	84.2	14.5	0.2	0.5
	499	83.0	9.7	6.3	1.0
	499*	79.9	19.1	0.0	1.0
100%N_2	12*	99.9	0.0	0.0	0.2
	43	98.9	0.0	0.1	1.0
	499	95.9	0.0	2.6	1.5
	499*	93.3	5.8	0.0	0.9
5%O_2-95%N_2	12*	97.7	2.1	0.0	0.2
	43	97.5	1.7	0.1	1.2
	499	93.7	1.2	3.9	1.2
	499*	91.2	8.2	0.0	0.7

[a] Results are the average of two bags per modified atmosphere treatment, where the value given is the % of the area under the peak/total area under all peaks.
* Control bags containing no fish.

Table 5—Effect of CO_2-O_2 atmospheres on some sensory values of red hake

Attribute[a]	Time in storage (hr)	Control	Barrier Bags® Containing: 20%CO_2: 21%O_2: 59%N_2	60%CO_2: 21%O_2: 19%N_2	60%CO_2: 5%O_2: 35%N_2
Color	152	7.4	7.4	7.6	7.8
	656	4.9[c]	6.7[d]	6.8[d]	6.8[d]
Odor	152	6.6	6.3	6.6	7.1
	656	3.3[c]	5.3[d]	4.8[d]	5.0[d]
Juiciness	152	6.4	6.8	6.4	6.3
	656	3.8[c]	5.4[d]	5.6[d]	5.0[d]
Tenderness	152	7.0	6.9	6.6	6.6
	656	4.0[c]	6.7[d]	6.1[d]	5.5[d]
Flavor	152	6.6[c,d]	6.6[c,d]	6.4[c]	7.2[d]
	320	5.9[c]	6.1[c,d]	6.2[c,d]	6.6[d]
	488	5.3[c]	5.9[c,d]	6.2[d]	6.1[c,d]
	656	2.8[c]	4.9[d]	4.4[d]	4.9[d]
Overall Acceptability	152	6.6	6.7	6.4	6.8
	656	2.7[c]	5.0[d]	4.6[d]	4.8[d]

[a] Average of two fish per treatment; 8 panel members for all taste panels. A 9-point scale was used; a score of 4.0 or below indicates poor quality.
[c,d,e] Rows of values with the same superscript, or rows of values containing no superscripts are not significantly different at $P<0.05$.

Table 6—Effect of CO_2-O_2 atmospheres on microbiological counts and chemical parameters of red hake

Analysis[a]	Time in storage (hr)	Control	Barrier Bags® containing: 20%CO_2: 21%O_2 59%N_2	60%CO_2: 21%O_2: 19%N_2	60%CO_2: 5%O_2: 35%N_2
Standard plate count	128	3.7	2.1	3.3	2.5
	632	9.2	5.8	2.4	2.4
Psychrotroph count	128	3.7	2.1	3.1	2.0
	632	9.2	6.1	4.9	4.1
Torrymeter number[b]	0	15.4	14.9	14.2	14.6
	632	3.0	6.3	9.0	8.0
TMA Value[c]	155	20	40	30	30
	659	1560	1530	1580	1260

[a] Average of two fish per treatment.
[b] Time 0 represents the average of two readings for 12 fish per treatment; all other values represent the average of two readings for two fish per treatment.
[c] TMA value: μmoles TMA/100g wet fish weight.

The two experiments with the more commercially important salmon gave results that were consistant with those for red hake, suggesting that the combined gas-sorbate system may be applicable to various species and types of fish.

Table 7—Raw-panel scores for red hake held in ice, potassium sorbate ice, and modified atmosphere-ice combinations

Attribute[a]	Control	Fran-Kem ice	Barrier Bags® Containing Gas[b]: 1% w/v Potassium sorbate ice	No Ice	Ice	1% w/v Potassium sorbate ice
General appearance	2.5[c]	2.5[c]	2.1[c,d]	2.0[c,d,e]	1.5[e]	1.8[d,e]
Eyes	2.6[c,d]	2.8[c]	2.0[e]	2.1[d,e]	1.8[e,f]	1.4[f]
Gills:						
Color:	2.9[c]	2.8[c,d]	2.0[e]	2.3[d,e]	2.1[e]	2.1[e]
Odor:	2.8[c]	2.5[c,d]	2.3[c,d]	2.1[d]	1.4[e]	2.0[d]
Slime	2.3[c]	2.5[c]	1.6[d,e]	2.0[c,d]	1.3[e]	1.5[d,e]
Odor	2.5[c]	2.4[c,d]	2.4[c,d]	1.9[d,e]	1.1[f]	1.5[e,f]
Consistency of Flesh	1.5[e]	2.0[c,d]	2.0[c,d]	2.4[c]	1.9[c,d,e]	1.4[d,e]
Belly flaps	2.5[c]	2.4[c,d]	1.9[d,e]	1.9[d,e]	1.4[e]	1.5[e]
Overall score	2.6[c]	2.4[c,d]	2.0[d,e]	1.9[e]	1.6[e,f]	1.4[f]

[a] Average scores at 699 hr of two fish per treatment; 8 panel members. Selected criteria of the Fish Inspection Service of Canada (Anderson, 1965) for groundfish. Grading scale 1—3, where lower scores indicate fish of better quality.
[b] Atmosphere was 60%CO_2: 21%O_2: 19%N_2, however, results from gas chromatography revealed that the bags were poorly sealed, so bags were recharged approximately every 2 days.
[c,d,e] Rows of values with the same superscript, or rows of values containing no superscripts are not significantly different at $P<0.05$.

Table 10 shows the attributes scored by the grading panel for chinook salmon where the three gas treated samples at 0°C showed differences. There was the better overall acceptability for the gas-without-ice sample (though not as good as gas plus sorbate ice) as compared to the sample of gas-with-ice. If no sorbate is used, or sorbate were applied with a dip prior to packaging, ice might be eliminated altogether at a cost savings. Humidity control would, however, be more critical. A fully treated sample subject to an abuse temperature of 6°C was also tested, and like the control, was completely spoiled after 586 hr of storage. An antioxidant dip to prevent both rancidity and off-color problems with the pigmented salmon did not yield the desired benefits. The taste panel data (Table 11) show some quality decreases with the sorbate-treated gas pack. These results were not confirmed with sockeye salmon (Table 13) except possibly for the color problems. The reason for this difference is not known. Table 10 also summarizes some of the other results with chinook salmon. Bacterial counts were lowest for the sample without ice, while TBA values were increased, especially for the sorbate treatment which may explain the decreased flavor score.

The Torrymeter did not work for salmon; the initial readings were low and remained low throughout the study. Because the samples were shipped to us with a frozen gel, it is possible that surface freezing occurred and that this was sufficient to invalidate the Torrymeter. Kramer et al.

Table 8—Taste panel scores at 688 hr for red hake in ice, Fran-Kem ice, potassium sorbate ice, and modified atmosphere-ice combinations

Attribute[a]	Initial[c]	Control	Fran-Kem ice	Barrier Bags® Containing Gas[b]: 1% w/v Potassium sorbate ice	No ice	Ice	1% w/v Potassium sorbate ice
Color	7.6	6.4	6.2	5.6	6.4	6.5	6.6
Odor	6.3	3.2[d]	3.2[d]	3.0[d]	5.5[e]	5.2[e]	5.6[e]
Texture	6.5	3.7[d]	2.8[d]	2.6[d]	5.3[e]	5.6[e]	5.2[e]
Juiciness	6.4	3.8[d]	3.2[d]	3.4[d]	5.6[e]	5.8[e]	6.1[e]
Tenderness	6.9	3.5[d,e]	2.4[d]	3.0[d,e]	5.4[f]	5.0[e,f]	5.8[f]
Flavor	5.9	2.8[d]	2.0[d]	2.4[d]	4.1[e]	4.3[e]	5.8[f]
Overall acceptability	5.9	2.9[d,e]	2.4[d]	2.4[e]	4.1[e,f]	4.5[f,g]	5.7[g]

[a] Average of two fish per treatment for the fish packaged in modified atmospheres and one fish for the remaining treatments; 5 panel members. A 9-point scale was used; where a score of 4.0 or below indicates poor quality.
[b] Atmosphere was 60%CO_2:21%O_2:19%N_2, however, results from gas chromatography revealed that the bags were poorly sealed, so bags were recharged approximately every 2 days.
[c] From an earlier experiment at 14 hr of storage. Also see Table 5 for data at 152 hr with a different batch of red hake.
[d,e,f,g] Rows of values with the same superscript, or rows of values containing no superscripts are not significantly different at $P<0.05$.

Table 9—Selected physical and chemical results for red hake in ice, Fran-Kem ice, potassium sorbate ice, and modified atmosphere-ice combinations

Analysis (time)[a]	Control	Fran-Kem Ice	Barrier Bags® Containing Gas[b]: 1% w/v Potassium sorbate ice	No ice	Ice	1% w/v Potassium sorbate ice
TMA value[c] (670 hr)	2430	2310	1170	2040	3490	1400
pH (667 hr)	7.40	7.74	7.94	6.71	6.73	6.85
Torrymeter No.						
Initial (0 hr)	15.7	16.0	15.3	15.7	15.7	16.0
Final (644 hr)	3.3	3.0	5.5	3.3	8.0	11.3
% Drip Loss[d] (664 hr)	3	8	6	7	8	10

[a] Average of two fish per treatment for the TMA value and pH. Initial Torrymeter numbers are the average of five fish per treatment, while final values represent an average of two readings for two fish per treatment.
[b] Atmosphere was 60%CO_2:21%O_2:19%N_2; however, results from gas chromatography revealed that the bags were poorly sealed, so bags were recharged approximately every 2 days.
[c] TMA value: μmoles TMA/100g wet fish weight.
[d] Drip loss values represent the average wet weight change in grams of five fish per treatment.

(1978) have reported on the successful use of the Torrymeter with salmon.

The final series of experiments with sockeye salmon generally confirmed the previous results, but did show some benefit for the antioxidant dip (Table 12 and 13). The TBA data suggested that sorbate might be increasing the TBA value of the darker (red) muscle. The drip losses were lower than those observed with chinook salmon.

DISCUSSION

THE EXPERIMENTS reported in this paper suggested a potential for the use of MA and potassium sorbate to extend the shelf-life of fresh fish. However, the evaluation of these results must include certain considerations before the method is ready for commercial adoption:

First, the experiments were done under laboratory conditions where many variables are more easily controlled.

Second, the samples in the bulk storage experiments had some fish-to-fish contact but were not as tightly packed as might be the case in a real fish box. Tighter packing would of course decrease the flow of gases and ice to the surface of the fish. Flatfish might be of particular concern.

Third, we are currently operating with conditions that are in excess of what might be used commercially: there is probably more ice at a higher sorbate concentration than necessary, and an excess of gas relative to the amount of fish.

Fourth, we worked with fish that were a few days old by the time they arrived in Ithaca; a "Delay-Pack" hypothesis is discussed by Regenstein and Regenstein (1981b).

Fifth, because of limitations of time and fish availability, specific experiments were not generally duplicated. Thus, results may depend on the many variables that affect fish

Table 10—Selected results for Chinook salmon held in ice, in dips and ices, and in modified atmosphere-ice combinations under abuse and normal handling

		Barrier Bags® Containing Gas[b]				
Criteria	Control	Antioxidant dip[c] + ice	Ice (6°C)	No ice	Ice	1% w/v Potassium sorbate ice
General appearance[a]	3.0[e]	3.0[e]	2.9[e]	1.0[g]	1.7[f]	1.0[g]
Slime[a]	3.0[e]	2.9[e]	3.0[e]	1.0[g]	1.6[f]	1.1[g]
Odor[a]	2.7[e]	2.4[e]	2.9[e]	1.1[g]	1.9[f]	1.3[g]
Overall score[a]	3.0[e]	3.0[e]	3.0[e]	1.4[g]	1.9[f]	1.2[g]
Log psychrotroph count (562 hr)	10.2	–	9.5	7.8	8.3	8.2
TMA value[h] (588 hr)	320	340	800	760	310	80
TBA Value[i] (588 hr)	0.3	0.6	1.9	3.2	3.4	5.8
pH (585 hr)	7.0	7.1	6.3	6.2	6.3	6.4
% Drip Loss[j] (562 hr)	−3		4	4	2	7

[a] Average grading scores at 564 hr of two fish per treatment, except one fish for the antioxidant dip; 8 panel members. Selected criteria of the Fish Inspection Service of Canada (Anderson, 1965) for groundfish. Grading scale 1—3, where lower scores indicate fish of better quality.
[b] Atmosphere was 60%CO_2:5%O_2:35%N_2.
[c] Dipped for 5 min in 0.2% w/v sodium erythorbate, 0.2% w/v citric acid, and 0.5% w/v sodium chloride solution.
[e,f,g] Rows of values with the same superscript, or rows of values containing no superscripts are not significantly different at $P<0.05$.
[h] TMA value: μmoles TMA/100g wet fish weight.
[i] TBA value: μmoles malonaldehyde/100g wet fish weight.
[j] Drip loss values represent the average wet weight in grams of two fish per treatment.

Table 11—Taste panel scores at 586 hr for Chinook salmon held in ice, in dips and in modified atmosphere-ice combination under abuse and normal handling

		Barrier Bags® containing gas[b]		
Attribute[a]	Control	No ice	ice	1% w/v Potassium sorbate ice
Color	5.1[e]	6.6[f]	6.9[f]	5.6[e]
Odor	1.5[e]	6.6[f]	5.7[f]	5.4[f]
Texture	3.0[e]	6.4[f]	6.5[f]	6.6[f]
Juiciness	3.5[e]	6.1[f]	6.8[f]	5.9[f]
Tenderness	2.1[e]	6.6[f]	6.4[f]	6.3[f]
Flavor	2.1[e]	6.4[g]	5.8[f,g]	5.3[f]
Overall Acceptability	1.9[e]	6.4[g]	5.8[f,g]	5.3[f]

[a] Average of two fish per treatment, except for the control which was only one fish; 8 panel members. Hedonic scale 1—9, where a score of 4.0 or below indicates poor quality. The samples dipped for 5 min in a 0.2% w/v sodium erythorbate, 0.2% w/v citric acid, and 0.5% w/v sodium chloride solution and the iced fish held at 6° C were unfit for human consumption.
[b] Atmosphere was 60%CO_2:5%O_2:35%N_2.
[e,f,g] Rows of values with the same superscript, or rows of values containing no superscripts are not significantly different at $P<0.05$.

Table 12—Selected results for Sockeye salmon in modified atmosphere-ice combinations

	Barrier Bags® Containing:							
Criteria[a,j]	60%CO_2: 5%O_2:35%N_2 + ice (bag control)	60%CO_2: 5%O_2:35%N_2 No ice	60%CO_2: 5%O_2:35%N_2 + Antioxidant dip[b] + ice[g]	60%CO_2: 5%O_2:35%N_2 + Antioxidant dip[b] + ice[g]	60%CO_2:5%O_2 1%CO:34%N_2 + Antioxidant dip[b]	40%CO_2:60%N_2 + Antioxidant dip[b] + ice	60%CO_2:5%O_2 1%CO:34%N_2 + Antioxidant dip[b] + 1%w/v potassium sorbate ice	60%CO_2:5%O_2: 35%N_2 + Antioxidant dip[b] + 1%w/v potassium sorbate ice
General appearance	1.4[e,f]	1.9[c,d]	1.6[d,e]	1.7[c,d]	2.1[c]	1.4[e,f]	1.2[f]	1.1[f]
Slime	1.1[d,e]	1.7[c]	1.3[d,e]	1.3[d,e]	1.4[c,d,e]	1.5[c,d]	1.1[d,e]	1.0[e]
Odor	1.7[c,d,e]	2.0[c,d,e]	2.1[c,d]	2.2[c]	2.0[c,d,e]	1.8[c,d,e]	1.6[d,e]	1.6[e]
Consistency of flesh	1.5[c,d]	1.8[c]	1.7[c,d]	1.8[c]	1.6[c,d]	1.5[c,d]	1.5[c,d]	1.4[d]
Belly flaps	1.6[c,d]	2.5[c]	1.9[c,d]	1.7[c,d]	1.4[d]	1.5[c,d]	1.4[d]	1.7[c,d]
Internal browning	1.6[d,e]	2.2[c]	1.9[c,d]	1.9[c,d]	1.6[d,e]	1.5[e]	1.4[e]	1.6[d,e]
Overall score	1.8[c,d]	2.1[c]	1.8[c,d]	1.8[c,d]	1.6[d,e]	1.6[d,e]	1.3[e]	1.5[d,e]
Log psychrotroph count (402 hr)	6.4	6.9	–	6.3	7.7	–	5.1	6.0
TBA value[h] (433 hr)	1.9	2.4	1.7(7.2)	1.1(6.3)	–	(6.5)	(5.7)	(10.1)
Drip loss[i]	0.3	1.4	1.2	1.3	1.2	1.7	0.4	0.1

[a] Average scores at 405 hr of two fish per treatment; 11 panel members. Selected criteria of the Fish Inspection Service of Canada (Anderson, 1965) for groundfish. Grading scale 1—3, where lower scores indicate fish of better quality.
[b] Dipped for 5 min in a 0.2% w/v sodium erythorbate, 0.2% w/v citric acid, and 0.5% w/v sodium chloride solution.
[c,d,e,f] Rows of values with the same superscript are not significantly different at $P<0.05$.
[g] Identical treatments
[h] TBA value: μmoles malonaldehyde/100g wet fish weight. Values in parentheses represent TBA values for browned flesh samples.
[i] Drip loss values represent the average wet weight in grams of two fish per treatment.
[j] Average of two fish per treatment for psychrotroph count and TBA.

Table 13—Taste panel scores at 429 hr for Sockeye salmon in modified atmosphere-ice combinations

Attribute[a]	$60\%CO_2$:$5\%O_2$: $35\%N_2$ + ice (control bag)	$60\%CO_2$:$5\%O_2$: $35\%N_2$ No ice	Barrier Bags® Containing $40\%CO_2$:$60\%N_2$ w/Antioxidant dip[b] + ice	$60\%CO_2$:$5\%O_2$: $34\%N_2$:1%CO w/Antioxidant dip[b] + 1% w/v potassium sorbate ice	$60\%CO_2$:$5\%O_2$: $35\%N_2$ w/Antioxidant dip[b] + 1% w/v Potassium sorbate ice
Color	7.2[c,d]	7.3[d]	7.6[d]	7.2[c,d]	6.4[c]
Odor	6.7	6.8	6.8	6.3	7.0
Texture	7.3	7.1	6.9	6.8	7.1
Juiciness	6.6[c,d]	6.4[c,d]	5.9[c,d]	5.6[c]	6.5[c,d]
Tenderness	6.9	7.1	6.8	7.0	6.9
Flavor	6.4	6.4	6.1	6.3	6.5
Overall Acceptability	6.6	6.7	6.1	6.4	6.8

[a] Average of two fish per treatment except for treatment 8 which was only one fish; 8 panel members. Hedonic scale 1—9, where a score of 4.0 or below indicates spoilage.
[b] Dipped for 5 min in 0.2% w/v sodium erythorbate, 0.2% w/v citric acid, and 0.5% w/v sodium chloride solution.
[c,d] Rows of values with the same superscript or rows of values containing no superscript, are not significantly different at $P<0.05$.

such as age, time of year, method of harvesting, region of catch, etc. However, the overall results of these experiments were fairly consistent and do suggest the positive benefits of these treatments.

Sixth, because of our being inland, the sensory panel was well aware of the age of the fish at the time they tested the sample, and we believe there was a negative bias against older fish. The fact that the panel still gave such high ratings after as long as 4 wk suggested that these treatments did have an effect. On the other hand, the panel was not that experienced with fish and would not be as critical as a coastal panel comprised of seafood experts.

Seventh, in some cases, the cooked quality of the fish was fine but the raw appearance of the whole fish was not as good. Thus, the potential end-use of the product would be of some importance: these fish might be more appropriate for filleting.

Some of the bags developed an odor that led to an overnegative score if the fish weren't left out of the bag for a little while before grading. This "bag effect" would limit the shelf-life of retail packages.

It is important to emphasize the need for good temperature control. Clearly the abuse temperature (6°C) sample did not do nearly as well as the colder samples.

Though potassium sorbate dips alone were not particularly effective, they might be more effective in conjunction with gas or gas plus potassium sorbate treatments (e.g., Regenstein and Regenstein, 1981a, b). It is important, however, that the dip treatment not become a source of contamination.

The more drastic decrease in TMA values at times when compared to the total counts suggested that the flora was either being changed or that the TMA formation reaction was being blocked.

The TBA results suggested that there was no need for concern about rancidity with red hake. Even the slightly higher TBA numbers with salmon did not lead to a taste panel perception of off-flavor.

The weight loss due to drip is clearly of some concern. Fish in general seem to have a high drip level, running as high as 3–8% (Connell, 1975). Presumably, the longer storage periods might have a more marked effect.

The results of this study suggest real potential for the commercial use of this type of system, but the consumer remains the ultimate judge. As with any new technology, caution is advised at this time. It is our hope that industry will work closely with researchers in an effort to improve and develop these systems for commercial use.

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Teflon and non-Teflon Lined Dies: Effect on Spaghetti Quality

B. J. DONNELLY

ABSTRACT

Six samples of semolina, milled from durum wheats of varying quality, were extruded through Teflon and non-Teflon lined dies. Extruded spaghetti was evaluated for color and cooking quality. The effect of cooking time on cooking quality was also evaluated. Teflon extruded spaghetti had better appearance and cooking quality than its non-Teflon extruded counterpart. Strong gluten, whether in the Teflon or non-Teflon extruded products, improved cooking quality and tolerance to over-cooking. Scanning electron microscopy of the outer surface of dry and cooked spaghetti elucidated, in part, the differences in appearance and cooking quality of the products extruded through both die forms.

INTRODUCTION

DURUM WHEAT grown in the Upper Great Plains of the United States, primarily North Dakota, is generally accepted as the raw material of choice for the commercial production of high quality pasta products. Pasta is used here in the generic sense to mean such products as macaroni, spaghetti, and noodles, each of which can be marketed in a wide variety of shapes and sizes. Pasta consumption in the U.S. is approximately 10 pounds (4.6 Kg) per capita per year (Donnelly, 1979).

With the exception of lasagna, most of the pasta currently being processed in the U.S. is extruded through Teflon-lined dies. Teflon is used for three basic reasons, namely, (a) it extends the life-time of the dies by reducing wear on the brass and/or steel; (b) it provides a smoother surface to the pasta; and (c) it improves the general appearance of the dried product.

No published information is available detailing the relative quality merits of pasta processed through both die forms. The purpose of this paper is to review some research that was done in this laboratory on the effect on spaghetti color and cooking quality of processing durum wheat semolina through Teflon and non-Teflon lined dies. In addition scanning electron microscopy (SEM) of the dry and cooked spaghetti was utilized to determine what gross structural differences were present and if these could contribute to quality differences.

MATERIALS & METHODS

Durum wheat

Five durum wheat varieties (Crosby, Edmore, Macoun, Wakooma, and Wells) and a commercial blend were used in this study. These were selected on the basis of potential differences in wheat quality, milling, and processing characteristics.

Test weights

Test weight (lb/bu) was determined using the standard AACC method 84-10 (1961).

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Vitreous kernel content

The vitreous kernels from 50 grams of wheat were hand picked, weighed, and expressed as percent.

Falling Number

Falling Number values of the ground wheat were determined using the standard AACC method 56-81B (1972).

Wheat and semolina protein

Protein conent was determined by the Kjeldahl procedure using the standard AACC method 46-10 (1961). Results are expressed on a 14.0% moisture basis.

Wheat and semolina ash

Ash content was determined by the standard AACC method 08-01 (1961). Results are expressed on a 14.0% moisture basis.

Semolina extraction

Semolina extraction was determined using the standard AACC method 26-30 (1961) as modified for the Buhler mill.

Bran speck count

The average of three replications of the number of visible specks per 10 square inches (25.4 sq cm) of semolina surface area.

Gluten strength

Farinograms were obtained using the standard AACC method 54-21 (1961). Absorptions were adjusted so that the resulting farinograph peaks touched the 500 B.U. line. The curves were compared with standard curves developed in this department for numerical score assignment (Fig. 1). Low numbers indicate weak gluten and higher numbers indicate progressively stronger gluten or mixing tolerance.

Spaghetti processing

The method of Walsh et al. (1971) was used to prepare spaghetti from the semolina. Average dry spaghetti diameter after processing through spaghettini Teflon and non-Teflon lined 85 hole brass dies was 1.52 mm.

Spaghetti color

Spaghetti color was determined using the reflectance technique described by Walsh et al. (1969).

Spaghetti cooking quality

Spaghetti cooked weight (g), cooking loss (%), and cooked firmness (g cm) values were determined using the procedure of Walsh et al. (1971) with 15 min as the standard cooking time. The effect of cooking time on spaghetti cooking quality was also determined by varying cooking time from 5 to 10, 15, and 20 min.

Grzybowski and Donnelly (1979) reported spaghetti of 1.52 mm average diameter cooked for 15 min had S.D. = 0.37 for cooked weight, S.D. = 0.24 for cooking loss, and S.D. = 0.2 for cooked firmness. Standard deviations for each of these cooking quality traits at 5, 10, and 20 min cook times were also reported. Cooking quality data presented in this study were not replicated.

Scanning electron microscopy (SEM)

Samples of dry and cooked spaghetti (15 min) were scanned longitudinally on a Jeol/JSM-35 scanning electron microscope. All samples were viewed at 15 mm working distance using 20 kV and 1200X magnification.

–Continued on next page

Samples of cooked spaghetti were prepared for SEM by cutting into 2.54 cm lengths and fixed in a 5% solution of glutaraldehyde in Millonig's phosphate buffer (pH 7.4; D-glucose deleted) for 2.25 hr (Millonig, 1961). Samples were subsequently dehydrated in ethanol series: 30, 50, 70, and 100 (2X) for 30 min each, and then critical point dried in a Tousimis critical point drier for 20 min. Specimens were placed on an aluminum disk specimen stage and gold coated (10–15 nm). Pictures were taken using Polaroid type 55 P/N film.

Uncooked spaghetti was gold coated without pre-treatment.

RESULTS & DISCUSSION

Wheat quality

Wheat quality characteristics of the six samples used in this study are presented in Table 1. Since these samples were chosen for their potential quality differences, the actual differences are apparent in the data. Test weight ranged from a low of 54.4 pounds per bushel for Wakooma to a high of 62.0 pounds per bushel for the wheat blend. In this particular selection Wakooma also had the lowest percent vitreousness of 54 with Edmore having the highest level of 92%. Although there was no visible evidence of sprouting, two samples (Wakooma and Macoun) with Falling Number values of 213 and 231 sec respectively, may have had some incipient sprouting. With the exception of Wells and Edmore, protein levels were generally in the range expected for commercially marketed North Dakota durum wheat. Wheat ash levels were in the acceptable range, although Wakooma and Macoun were relatively high.

Semolina quality

Semolina extraction levels obtained from the six wheat samples are presented in Table 2. Differences in extraction can be explained by the differences in test weight, vitreousness and kernel distribution (data not shown). The relatively low yield from Crosby is due parimarily to the high level of small kernels (8%) and low level of large kernels (9%) for this particular sample. Macoun had a higher extraction level due to the significant difference in large kernel content, 75 vs 58%, respectively, when compared with Edmore. With the exception of Wakooma all samples had acceptable bran speck counts. The high wheat ash and bran level in the semolina contributed to the higher ash level of Wakooma, whereas the remaining samples were in the normal range for ash content. Variability in protein quantity and quality are evident from the data in Table 2. Protein content ranged from a low of 10.7% for Wells to a high of 14.2% for Edmore. With the exception of Wells, semolina protein are close to or higher than the 12.5% average level used in commercial pasta production.

Protein quality or gluten strength, as represented by the values presented in Table 2, show Crosby, Wells, and the wheat blend to have the characteristic weak gluten mixing curves associated with varieties currently in major production in North Dakota. Both Wakooma and Macoun have stronger gluten, particularly Macoun but neither exhibited as strong a mixing curve as Edmore.

Spaghetti quality

Color. After extrusion and drying, the semolina dough extruded through Teflon and non-Teflon lined dies produced spaghetti color scores presented in Table 3. Scores of 8.5 or higher reflect a desireable bright, amber colored product. It is apparent that with the exception of Wakooma, processing through a non-Teflon die did not adversely affect the amber appearance of the products. The scores of 7.0 and 7.5 for Wakooma reflect a dull, amber product, and may in part be the result of the high semolina speck count, and/or ash content.

These objective color scores can be misleading. Visual inspection clearly indicated that the samples processed through the Teflon die had a smoother, glassier appearance than the non-Teflon processed spaghetti. This discrepancy

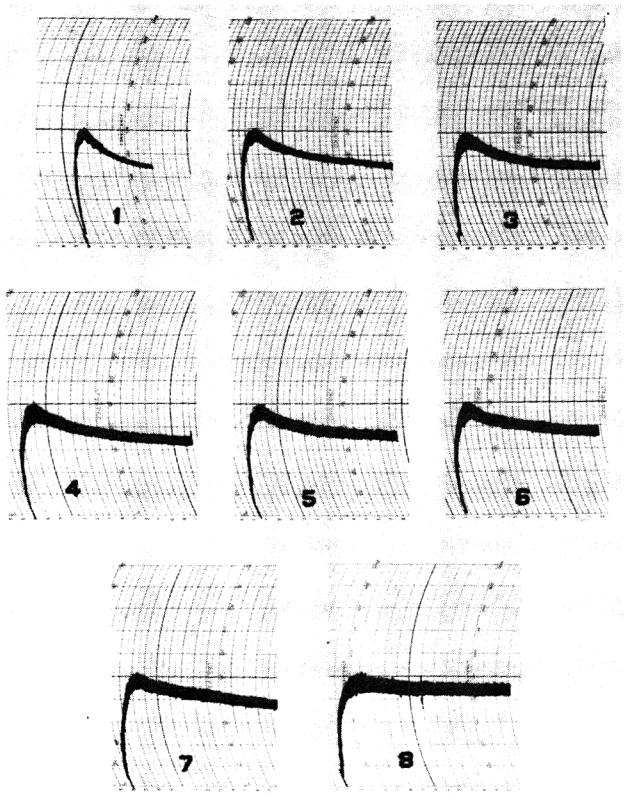

Fig. 1—Standard farinograph curves of durum wheat semolina.

Table 1—Wheat quality data for durum wheat

Variety	Vit. (%)	Test wt. (lb/bu)	Falling No. (sec)	Wheat protein[a] (%)	Wheat ash[b] (%)
Wells	60	61.9	565	11.2	1.44
Blend	88	62.0	480	14.7	1.54
Crosby	80	59.1	559	14.7	1.53
Wakooma	54	54.4	231	15.1	1.70
Macoun	78	60.4	213	13.3	1.62
Edmore	92	57.7	574	15.6	1.54

[a] Expressed on a 14.0% moisture basis; N x 5.7.
[b] Expressed on a 14.0% moisture basis.

Table 2—Milling performance and semolina quality

Variety	Semolina extraction (%)	Semolina protein[a] (%)	Semolina ash[b] (%)	Bran speck count/ 25.4 cm^2	Gluten strength[c]
Wells	52.1	10.7	0.59	30	2
Blend	56.3	13.6	0.55	30	3
Crosby	51.6	13.7	0.60	27	4
Wakooma	52.3	14.0	0.66	100	5
Macoun	58.0	12.2	0.59	33	7
Edmore	53.7	14.2	0.59	30	8

[a] Expressed on a 14.0% moisture basis; N x 5.7
[b] Expressed on a 14.0% moisture basis.
[c] Higher numbers indicate stronger gluten; derived from comparison with standard curves.

between objective and subjective evaluation can be explained by the fact that the instrumental reflectance procedure for measuring product lightness or brightness (L value; Hunter Color Difference meter) is based upon light reflection from the product surface at 660 nm. Such measurement at a specific wavelength is unable to differentiate beteen specular reflection which contributes to glossy appearance, and specular diffuse reflection which gives a product its duller appearance (Hunter, 1975). Thus the instrument "sees" both Teflon and non-Teflon extruded products as being bright in appearance.

Cooking quality. Spaghetti cooked for the standard cooking time of 15 min exhibited differences in cooked weight (CW), cooking loss (CL), and cooked firmness (CF) within samples processed though the same die and between samples processed through both die types (Table 3); In all cases, with the exception of Macoun, samples processed through Teflon had lower CW, CL, and higher CF values than the non-Teflon processed spaghetti. In general the spaghetti extruded through Teflon exhibited superior cooking qualities when compared with the non-Teflon processed samples. The effect of cooking time on CW, CL, and CF, as shown in Table 3, shows the same trends indicated for the standard cooking time. An important feature of the results is the fact that spaghetti processed through Teflon maintained superior CF values between 10 and 20 min cooking while maintaining lower levels of CL. Another important feature is the ability of the stronger gluten varieties Wakooma and Edmore to maintain better CF or "al dente" characteristics for the extended cooking period of 20 min. This ability of stronger gluten to provide spaghetti with superior cooking qualities has previously been demonstrated by Matsuo and Irvine (1970), and Grzybowsky and Donnely (1979).

Scanning electron microscopy (SEM)

Differences in cooking quality between Teflon and non-Teflon processed spaghetti can also be explained in part by examination of selected SEM's of both dried and cooked products. Since all products processed through Teflon showed similar SEM's as did the non-Teflon processed samples, only the SEM's for Edmore are presented here. As was more generally apparent with visual observation, the SEM's clearly define surface differences at a highly magnified level. A significant feature of the spaghetti processed through the non-Teflon lined die (Fig. 2A) is its generally open, porous structure, which obviously would allow for rapid water penetration during the cooking process. Leaching of water-soluble material during cooking would also be enhanced with this type of structure. Starch granules of varying size, covered with a layer of protein, are distinct and more clearly defined than those visible in the spaghetti processed through the Teflon die (Fig. 2B). In addition the Teflon processed spaghetti has a continuous layer of protein essentially covering the starch granules and outer surface of the product. The existence of this protein film was also reported by Evans et al. (1975). Some small holes and cracks are present on the surface, a feature noted by Dexter et al. (1978), which would facilitate water penetration during cooking.

Fig. 3A and 3B show the effect cooking the spaghetti for 15 min had on the surface of the non-Teflon and Teflon processed Edmore respectively. A distinctive feature of these SEMs is the loss of starch integrity due to gelatinization and its envelopment and fusion with the surrounding protein network. There was a tendency for the protein in the non-Teflon processed spaghetti (Fig. 3A) to form a fibrous, more open, pitted structure than the teflon processed past (Fig. 3B) which retained its film structure somewhat better. This feature was noted generally with all the paste processed through Teflon. Such structural differences between the non-Teflon and Teflon extruded cooked products could be explained by the fact that during cooking the spaghetti strand expands in volume, imposing a great deal of stress on surface protein. In the case of the non-Teflon processed spaghetti this protein coagulates into a filamentous network that shows a more ruptured framework than the corresponding teflon processed material. Dexter et al. (1978) reporting on Teflon processed spaghetti noted a similar structural change with cooking.

CONCLUSIONS

PASTA COOKING QUALITY is related to both protein content (Matsuo et al., 1972; Dexter and Matsuo, 1977) and gluten quality (Matsuo and Irvine, 1970; Grzybowski and Donnelly, 1979). This study provides additional possible explanations on the importance of die type to provide a quality pasta product. Extrusion of semolina dough through dies without a Teflon liner produces products that are less glossy in appearance than Teflon processed products. Cooking quality of non-Teflon extruded spaghetti shows higher CW, CL, and lower CF values than the Teflon extruded spaghetti. Protein quality (gluten strength) had a beneficial effect on the cooking quality of both non-Teflon and Teflon extruded spaghetti. Differences in cooking quality between non-Teflon and Teflon processed spaghetti

Table 3—Effect of cooking time on the cooking properties of spaghetti processed through Teflon and non-Teflon lined dies

				Cooked weight, g				Cooking loss, %				Cooked firmness, g cm			
				Cooking time, min				Cooking time, min				Cooking time, min			
Variety	Die	Color	Gluten strength	5	10	15	20	5	10	15	20	5	10	15	20
Wells	Teflon	9.0	2	23.4	23.8	34.1	39.8	4.1	5.6	7.0	7.4	5.9	6.5	4.1	3.7
	(–) Teflon	9.0	2	24.4	30.0	36.5	40.5	4.9	6.3	7.5	8.3	7.1	4.4	3.2	3.1
Blend	Teflon	9.0	3	22.5	23.4	34.2	37.3	4.1	5.7	6.5	7.4	8.1	5.4	4.5	4.2
	(–) Teflon	9.0	3	25.0	33.6	35.9	43.3	6.4	6.7	8.3	10.0	6.3	3.4	3.1	2.2
Crosby	Teflon	9.0	4	23.0	29.2	33.2	41.8	4.2	6.0	6.8	8.0	8.2	5.4	5.0	3.0
	(–) Teflon	9.5	4	24.2	30.3	34.9	41.3	4.9	6.6	7.2	8.1	7.4	4.3	4.3	2.8
Wakooma	Teflon	7.0	5	22.9	29.1	32.8	35.9	5.2	6.1	8.3	9.0	11.0	5.8	5.3	5.1
	(–) Teflon	7.5	5	23.8	29.4	34.5	39.1	5.7	6.4	9.6	9.8	7.9	5.2	4.2	3.8
Macoun	Teflon	9.0	7	22.1	28.4	33.6	37.5	6.3	7.0	8.0	8.4	8.7	5.8	5.3	4.5
	(–) Teflon	8.5	7	23.4	31.2	35.6	39.1	6.1	7.1	7.2	8.7	6.6	4.0	3.8	3.5
Edmore	Teflon	9.0	8	22.7	27.2	33.1	37.0	4.3	4.4	5.9	6.9	8.1	7.7	6.0	5.4
	(–) Teflon	9.5	8	23.7	29.9	34.5	39.4	4.8	6.0	7.6	8.6	10.0	5.4	5.1	4.0

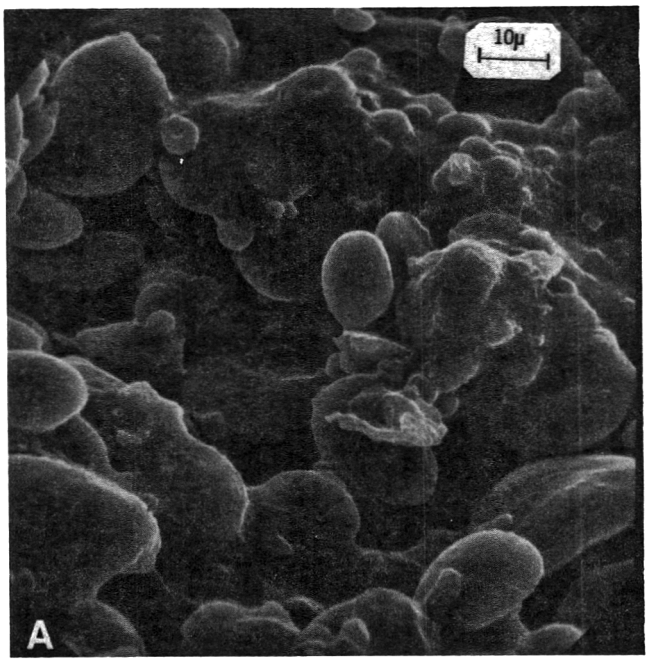

Fig. 2A—SEM of outer surface of spaghetti processed from Edmore semolina using non-Teflon lined die.

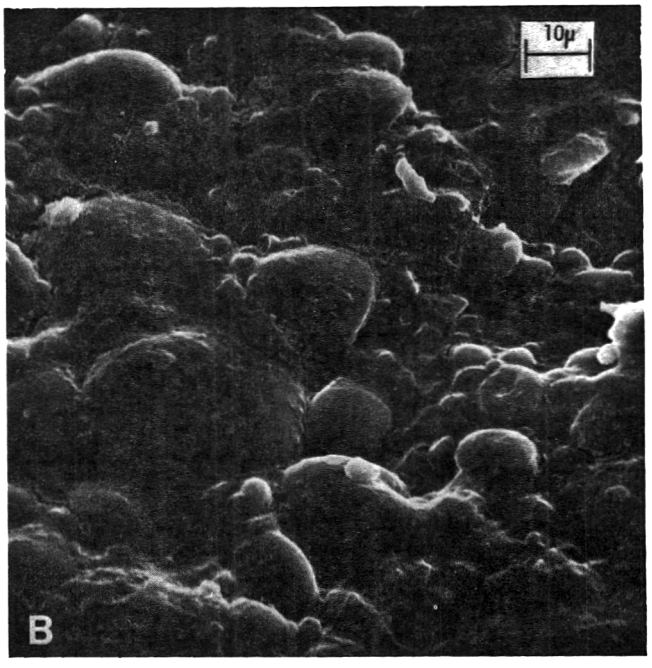

Fig. 2B—SEM of outer surface of spathetti processed from Edmore semolina using Teflon lined die.

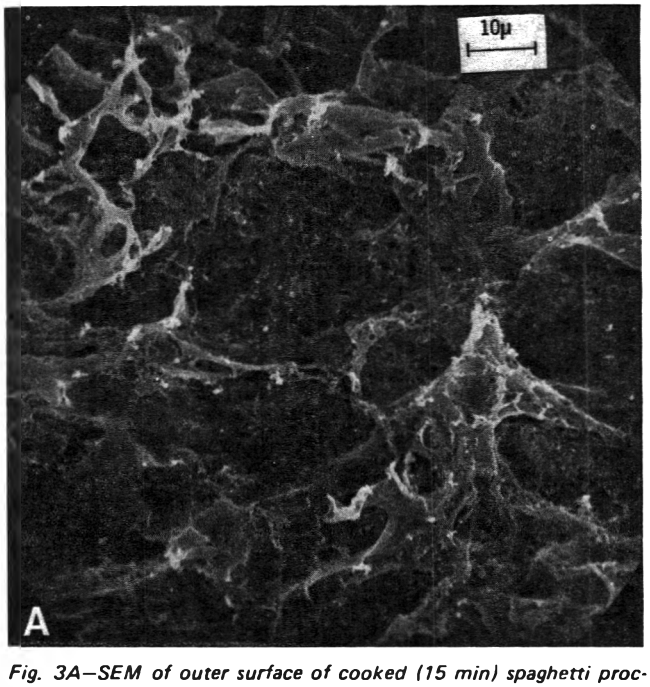

Fig. 3A—SEM of outer surface of cooked (15 min) spaghetti processed from Edmore semolina using non-Teflon lined die.

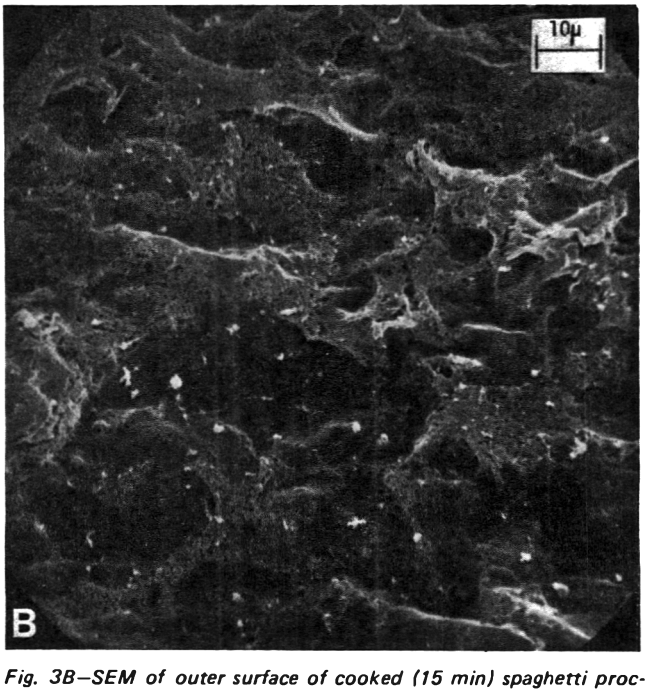

Fig. 3B—SEM of outer surface of cooked (15 min) spaghetti processed from Edmore semolina using Teflon lined die.

could be explained in part by examination of the SEMs of the outer surface of the uncooked and cooked products. However, SEM in this study did not differentiate between sample product cooking quality on the basis of gluten quality. A separate study using SEM to examine the interior areas of cooked spaghetti with the range of gluten quality similar to those used in this study could possibly delineate some morphological characteristics associated with gluten quality as it relates to cooking quality.

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Centralized Packaging of Beef Loin Steaks with Different Oxygen-Barrier Films: Physical and Sensory Characteristics

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ABSTRACT

Vacuum packaged beef strip loins (n = 72) were stored (2 ± 1°C) for either 0, 12 or 24 days before fabrication; steaks were packaged and displayed (2°C or 7°C) up to 6 days in oxygen-permeable film or up to 30 days in vacuum packages (medium or high oxygen-barrier film). Steaks displayed at 2°C, rather than 7°C, tended to have higher overall appearance scores especially when steaks were from 12 or 24 day subprimals. Overall palatability of vacuum packaged steaks was unacceptable after 10–15 days of display. Vacuum packaged steaks can be displayed for 10 days if: (1) steaks are from relatively fresh subprimals, (2) steaks are vacuum packaged with high oxygen-barrier film, and (3) steaks are displayed at 2°C. Although visual scores for vacuum packaged steaks were acceptable for 20–30 days, off-odors and off-flavors were limiting factors in determining shelf-life.

INTRODUCTION

AS ECONOMIC PRESSURES on the meat industry continue to mount, the advantages of centrally cutting and packaging steaks and sending them to the retailer as a case-ready product becomes more evident. Volz and Marsden (1963) identified numerous advantages for centralized prepackaging of meat in a 40-store operation utilizing cuts wrapped in oxygen-permeable polyvinyl chloride (PVC) film. A limiting factor of their system was the short period of product acceptability (3–4 days) after cutting and packaging. Research has been limited on the use of vacuum packaged retail cuts in a centralized distribution system. Because the appearance – especially the lean color – of meat in the retail case is a major factor in consumer acceptance (Kropf, 1980; Walker, 1980), the purple-red color of the reduced myoglobin pigment found on vacuum packaged cuts (Ernst, 1980; Kropf, 1980; Lawrie, 1979) may be a limiting factor in consumer acceptance of vacuum packaged retail cuts. Short time intervals between cutting and packaging along with extremely low partial pressures of oxygen in the package must also be achieved to ensure against formation of metmyoglobin – the pigment associated with brown lean color and most often equated with spoilage in fresh meat by consumers (Ernst, 1980). Jeremiah et al. (1972) indicated that consumers prefer steaks that are neither extremely dark nor extremely pale in muscle color. Griffin et al. (1982) found that individual muscles from beef round steaks have unique lean color characteristics when vacuum packaged. Ernst (1980) reported that although consumers commented on the dark lean color of vacuum packaged retail cuts as compared with the cherry-red color of PVC wrapped cuts, when this color phenomenon was understood by consumers, the dark lean color was not reported as a deterrent to sales.

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Because limited research has been conducted to evaluate efficacy of vacuum packaging of fresh retail cuts, questions regarding maximum postmortem age of subprimals prior to cutting of steaks, maximum display times and optimum display temperatures for such steaks have arisen. Methods to maximize retail appearance and case-life while maintaining desirable palatability attributes must be developed before the retail industry regards vacuum packaged retail cuts as a viable means of marketing fresh beef. The present study was conducted to compare physical and sensory characteristics of beef loin steaks packaged in oxygen-permeable polyvinyl chloride (PVC) film with those of steaks which were vacuum packaged in either medium or high oxygen-barrier films, using different retail display conditions to determine the physical and sensory characteristics of centrally packaged beef steaks.

MATERIALS & METHODS

Selection of carcasses/cuts

Fifty pairs of beef strip loins were selected at a commercial packing plant. For purposes of standardizing the size and quality of retail cuts, U.S. Choice carcasses were selected with the following characteristics: USDA lean maturity of A^{30}–A^{60}; ribeye area of 70.0–84.0 sq cm; subcutaneous fat thickness of 8.9–12.7 mm; marbling of "Small" to "Modest" amounts; and warm carcass weights of 318–363 kg. After selection, carcasses were fabricated at 48 hr postmortem and beef strip loins, short cut (I.M.P.S. #179) were vacuum packaged, boxed and shipped to the Texas A&M University Meat Laboratory.

Fabrication and packaging

Upon arrival at the TAMU Meat Laboratory (7 days postmortem), 36 pairs of strip loins that did not have package failure and that had the highest comparative degrees of package vacuum were selected for further study. Paired strip loins were randomly assigned to a storage period of either 0, 12 or 24 days (n = 12 paired loins per storage period). At the completion of each storage period, 12 paired strip loins were removed from vacuum and cut – beginning at the anterior end of the loin – into nine, 2.54 cm thick, steaks.

One-third of the paired strip loin steaks were placed in plastic foam trays and overwrapped in an oxygen-permeable polyvinyl chloride (PVC) film (Oxygen Transmission Rate or OTR = 6,500 $cc/m^2/24$ hr at 0% RH). One-third of the steaks were placed in transparent medium oxygen-barrier bags (OTR = 30 $cc/m^2/24$ hr at 22.8°C and at 0% RH) and were packaged utilizing a Multivac A300 vacuum packaging machine. The remaining third of the paired steaks were packaged in a high oxygen-barrier film (OTR = 10 $cc/m^2/24$ hr at 22.8°C and at 0% RH) using a Bivac® packaging machine. (Although the primary emphasis of this research was evaluating various films, the packaging methods used undoubtedly made small contributions to the outcome of this study.) All strip loin steaks were weighed prior to packaging and following retail display to determine total weight loss.

Retail display

Steaks were displayed in Tyler (model number DM8) retail cases; steaks from left side strip loins were displayed at an air temperature of 2°C, while steaks from right side strip loins were displayed at an air temperature of 7°C. All steaks were displayed under 1614 lux of GE "Natural" (model number F40N) fluorescent light following commercial time-patterns of lighting (14 hr on, 10 hr off).

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Evaluation

All retail cuts were evaluated 12 hr after packaging and daily (every 24 hr) thereafter by a trained six-member panel for the following traits: oxygenated lean color (15 = bright cherry-red, 1 = extremely dark brown) or reduced myoglobin lean color (15 = bright purple-red, 1 = extremely dark brown); fat color (15 = white, 1 = extremely dark brown or green); surface discoloration (15 = 0% discoloration, 1 = 100% discoloration) and overall appearance (15 = extremely desirable, 1 = extremely undesirable). Steaks wrapped in oxygen-permeable film were sampled at 0, 1, 2, 3, 4, 5 or 6 days of display; steaks packaged in medium or high oxygen-barrier film were sampled at 0, 5, 10, 15, 20, 25 or 30 days of display. At these time intervals, five pairs of steaks were removed from each packaging treatment and the packages were opened and each steak was immediately evaluated for off-odor (10 = no off-odor, 1 = abundant off-odor). After 15 min of exposure to air, two of the five pairs of steaks that had been vacuum packaged were evaluated for lean color (15 = bright cherry-red, 1 = extremely dark brown) and for off-odor (10 = no off-odor, 1 = abundant off-odor).

Microbiological sampling

Selected samples for microbiological assay were obtained from the remaining three of five pairs of steaks for each packaging method as they were removed from display. Numbers and types of microorganisms are reported in the companion report by Vanderzant et al. (1982).

After microbiological sampling, all steaks were repackaged with high oxygen-barrier film using the Bivac® packaging machine, blast-frozen at −30°C and stored until subsequent palatability evaluations were performed.

Palatability evaluations

Representative numbers of strip loin steaks from each treatment were removed from the freezer, thawed at 2°C and broiled on Farberware Open-Hearth broilers to an internal temperature of 70°C. The lateral half of each steak was served, while warm, to a trained, eight-member sensory panel and each member independently evaluated each sample for juiciness (8 = extremely juicy, 1 = extremely dry), tenderness (8 = extremely tender, 1 = extremely tough), flavor desirability (8 = extremely desirable, 1 = extremely undesirable) and overall palatability (8 = extremely desirable, 1 = extremely undesirable). The medial half of each steak was cooled to room temperature (23°C) and cored (1.27 cm cores) parallel to the muscle fiber orientation for shear force determinations, using a Warner-Bratzler shear machine. Each steak was weighed before and after cooking to determine cooking loss.

Statistical analysis

Data were analyzed by analysis of variance and mean separation utilizing the Statistical Analysis System package of Barr et al. (1979).

RESULTS & DISCUSSION

LEAN COLOR CHARACTERISTICS of PVC overwrapped and vacuum packaged strip loin steaks displayed at different temperatures are reported in Table 1. Steaks from subprimals that were stored for 0 days and then packaged with oxygen-permeable (PVC) film were not different ($P > 0.05$) in lean color as a function of display temperature (2°C vs 7°C); however, steaks from subprimals that were stored for

Table 1 — Mean lean color[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
	0	12.98^{c}	12.91^{c}	0	11.64^{f}	11.02^{f}	12.12^{e}	11.46^{e}
	1	11.28^{d}	11.42^{d}	5	13.16^{de}	13.42^{de}	13.60^{d}	13.52^{d}
	2	10.94^{de}	10.55^{e}	10	13.17^{de}	13.27^{de}	13.52^{d}	13.48^{d}
0	3	9.96^{ef}	9.11^{f}	15	13.65^{d}	13.61^{d}	13.76^{d}	13.58^{d}
	4	9.56^{f}	8.40^{f}	20	13.09^{de}	13.38^{de}	13.47^{d}	13.51^{d}
	5	7.83^{g}	6.32^{g}	25	12.45^{e}	13.03^{de}	13.24^{d}	13.36^{d}
	6	4.83^{h}	3.57^{h}	30	12.93^{de}	12.57^{e}	13.37^{d}	13.01^{d}
	0	14.00^{c}	13.89^{c}	0	9.71^{e}	8.88^{f}	10.76^{e}	10.41^{f}
	1	12.88^{d}	11.53^{d}	5	12.77^{d}	13.10^{d}	13.12^{d}	13.15^{d}
	2	11.89^{d}	9.71^{e}	10	12.37^{d}	12.55^{e}	12.74^{d}	12.70^{e}
12	3	9.72^{e}	6.37^{f}	15	12.61^{d}	12.83^{de}	13.05^{d}	13.06^{de}
	4	8.20^{f}	4.77^{f}	20	12.65^{d}	13.10^{de}	12.78^{d}	13.32^{d}
	5	8.28^{ef}	5.77^{f}	25	12.93^{d}	12.77^{de}	13.20^{d}	12.85^{de}
	6	5.20^{g}	4.90^{f}	30	12.73^{d}	12.65^{de}	13.14^{d}	12.90^{de}
	0	13.59^{c}	13.60^{c}	0	11.76^{f}	11.50^{h}	11.88^{h}	11.56^{h}
	1	12.35^{d}	10.84^{d}	5	12.78^{d}	12.81^{e}	12.88^{ef}	12.79^{f}
	2	10.13^{e}	8.50^{e}	10	12.95^{d}	13.25^{d}	13.36^{d}	13.26^{d}
24	3	8.95^{f}	5.21^{f}	15	11.96^{f}	11.96^{g}	12.23^{g}	12.27^{g}
	4	7.86^{f}	4.96^{f}	20	12.79^{d}	12.53^{ef}	13.08^{e}	13.00^{e}
	5	5.80^{g}	5.16^{f}	25	12.45^{e}	12.09^{g}	13.03^{ef}	12.38^{g}
	6	4.70^{g}	4.23^{f}	30	12.37^{e}	12.30^{fg}	12.67^{f}	12.44^{g}

[a] Means based on 15-point scales (oxygen-permeable film: 15 = bright cherry red, 1 = extremely dark brown; medium and high oxygen-barrier films: 15 = bright purple-red, 1 = extremely dark brown).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-h] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

12 or 24 days after arrival had higher ($P < 0.05$) lean color scores when steaks were displayed at 2°C, rather than 7°C, on days 1 through 4 of retail display. PVC-overwrapped steaks displayed acceptable (7.5 or greater) lean color scores for 5 days when subprimals had been stored for 0 or 12 days, cut and displayed at 2°C, while steaks from subprimals that had been stored for 24 days exhibited acceptable lean color characteristics for 4 days when steaks were displayed at 2°C. Lean color scores of steaks overwrapped in PVC and displayed at 7°C were desirable for 4, 2 or 2 days, respectively, for cuts from subprimals that had been stored for 0, 12 or 24 days. No consistently significant differences were observed between steaks displayed at 2°C and steaks displayed at 7°C after vacuum packaging in either medium oxygen-barrier bags or high oxygen-barrier film. All lean color scores for steaks displayed at either temperature (2°C or 7°C) in medium oxygen-barrier bags or high oxygen-barrier film were acceptable (7.5 or greater) at all display periods; however, lean color scores at day 0 were invariably lower ($P < 0.05$) than were those at 5 to 30 days of display for steaks cut from all subprimals. When significant differences were found between steaks packaged in medium oxygen-barrier bags and those packaged in high oxygen-barrier film, those packaged in the latter film had higher ($P < 0.05$) lean color scores than those of the former type, regardless of display temperature (data not presented).

Results of lean color evaluations allowing 15 min for reoxygenation after opening the packages (data not presented) revealed that almost complete reoxygenation and development of the characteristic cherry-red lean color of steaks occurred. No significant differences in 15-min color scores were found between type of package, display temperature or increasing display time in the retail case. Steaks wrapped in oxygen-permeable PVC film will maintain the characteristic cherry-red color of oxygenated lean longer if steaks are fabricated from fresher subprimals and if they are displayed at 2°C rather than 7°C. Furthermore, steaks vacuum packaged in medium oxygen-barrier bags or high oxygen-barrier film will maintain acceptable lean color characteristics for 30 days, although evaluations at 12 hr after packaging (day 0 evaluations) indicate that the reaction converting oxymyoglobin to reduced myoglobin had not been completed; this resulted in low initial lean color scores at day 0.

Differences were not consistent in fat color scores (Table 2) between steaks overwrapped in oxygen-permeable PVC film and displayed at 2°C and those displayed at 7°C for steaks cut after subprimals had been stored for 0 or 12 days; however, all steaks cut after 24 days of subprimal storage and displayed at 2°C had higher ($P < 0.05$) fat color scores (whiter fat) than those displayed at 7°C, irrespective of display period. Steaks that were from subprimals stored for 0 or 12 days that were vacuum packaged in medium oxygen-barrier bags were not consistently different in fat color when steaks were compared at 2°C and 7°C; however, steaks displayed at 2°C that were from subprimals stored for 24 days had higher ($P < 0.05$) fat color scores than those displayed at 7°C in all but one comparison (day 0 for medium oxygen-barrier bags). Steaks packaged in high oxygen-barrier film revealed no differences ($P > 0.05$) in fat color scores between steaks displayed at 2°C and those displayed at 7°C when steaks were fabricated after no (0

Table 2 – Mean fat color[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
0	0	13.34^{c}	13.01^{c}	0	12.12^{c}	11.97^{c}	13.10^{c}	13.10^{c}
	1	12.90^{c}	12.55^{c}	5	10.77^{d}	9.87^{e}	11.43^{d}	11.31^{d}
	2	12.21^{d}	11.52^{d}	10	10.67^{d}	10.55^{d}	11.32^{d}	11.33^{d}
	3	11.26^{e}	10.72^{e}	15	9.97^{e}	10.08^{e}	10.12^{e}	10.91^{de}
	4	11.27^{e}	10.19^{ef}	20	9.63^{e}	9.33^{f}	10.33^{e}	10.73^{ef}
	5	9.90^{f}	9.47^{fg}	25	9.42^{e}	9.25^{f}	10.21^{e}	10.08^{f}
	6	9.20^{f}	8.53^{g}	30	9.70^{e}	7.37^{g}	10.27^{e}	8.61^{g}
12	0	13.69^{c}	13.76^{c}	0	11.88^{c}	11.85^{c}	11.85^{c}	12.19^{c}
	1	11.97^{d}	11.34^{d}	5	10.81^{d}	10.49^{d}	11.12^{d}	10.93^{d}
	2	11.75^{d}	11.01^{d}	10	10.05^{e}	9.77^{e}	10.78^{de}	10.70^{d}
	3	10.66^{e}	9.68^{e}	15	10.07^{e}	9.30^{e}	10.67^{def}	9.84^{e}
	4	10.34^{e}	9.74^{e}	20	8.59^{f}	8.15^{f}	10.07^{f}	8.55^{f}
	5	9.82^{e}	9.20^{ef}	25	9.25^{f}	7.25^{f}	10.27^{ef}	7.78^{g}
	6	10.20^{e}	8.07^{f}	30	8.80^{f}	7.20^{f}	10.36^{def}	7.37^{g}
24	0	13.96^{c}	13.69^{c}	0	12.37^{c}	12.36^{c}	13.00^{c}	12.60^{c}
	1	12.59^{d}	11.35^{d}	5	10.12^{d}	9.46^{d}	12.50^{c}	11.47^{d}
	2	11.39^{e}	9.40^{e}	10	9.71^{de}	8.82^{e}	11.71^{d}	10.54^{e}
	3	10.97^{e}	9.00^{e}	15	9.50^{e}	8.08^{f}	11.18^{de}	9.64^{f}
	4	9.91^{f}	6.98^{f}	20	9.52^{e}	6.91^{g}	10.90^{ef}	8.28^{g}
	5	8.32^{g}	6.28^{fg}	25	8.47^{f}	6.64^{g}	10.40^{ef}	7.68^{gh}
	6	9.00^{fg}	5.80^{g}	30	7.14^{g}	5.33^{h}	9.97^{f}	7.02^{h}

[a] Means based on a 15-point scale (15 = white, 1 = extremely dark brown or green).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-h] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

days) additional storage of subprimals, while for steaks fabricated from subprimals that had been stored for 12 days prior to cutting, steaks displayed at 2°C were given higher ($P < 0.05$) fat color scores than those displayed at 7°C at 15 days of display or longer. Furthermore, steaks cut from subprimals after 24 days of storage and displayed at 2°C were given higher ($P < 0.05$) fat color scores than those displayed at 7°C at all display periods. When comparisons were made between steaks packaged in medium vs high oxygen-barrier films (data not presented), fat color scores were higher ($P < 0.05$) for steaks in high oxygen-barrier packages in 14 of 21 comparisons when steaks were displayed at 2°C, and in 12 of 21 comparisons when steaks were displayed at 7°C. All fat color scores at day 0 were higher ($P < 0.05$) than those at the longer periods of retail display, regardless of type of film, days of additional storage as subprimals, or temperature of display. Evaluations of fat color in this study indicate that steaks can be displayed at 2°C or 7°C as long as relatively fresh subprimals are used and regardless of the type of oxygen-barrier film used. As subprimals reach advanced postmortem ages, fat color scores of steaks displayed at 2°C are superior to those displayed at 7°C.

Mean surface discoloration scores for steaks packaged in each of three films and displayed at each of two temperatures are reported in Table 3. Steaks cut from subprimals with no additional storage after arrival and overwrapped in oxygen-permeable film did not differ ($P > 0.05$) in surface discoloration when displayed at 2°C vs 7°C. Steaks from subprimals that had been stored for 12 days showed less ($P < 0.05$) surface discoloration on days 1 through 5 when steaks were displayed at 2°C. Less surface discoloration ($P < 0.05$) was observed during the first 4 days of retail display if steaks from subprimals stored 24 days were displayed at 2°C rather than 7°C. Steaks from subprimals stored for 0 days that were vacuum packaged in medium oxygen-barrier bags and displayed for 30 days did not differ significantly in surface discoloration in response to display temperature (2°C vs 7°C); steaks cut after storing subprimals for 12 days had less surface discoloration if stored at 2°C rather then at 7°C on the 15th through 25th days of retail display, and steaks cut from subprimals stored for 24 days had less ($P < 0.05$) surface discoloration if stored at 2°C rather than at 7°C on the 10th through 30th days of retail display. Regardless of display temperature or storage period as subprimals, surface discoloration of steaks at day 0 of retail display was significantly lower than for steaks displayed for longer periods of time. Steaks from strip loins stored for 0 days and vacuum packaged in high oxygen-barrier film revealed no differences ($P > 0.05$) in surface discoloration scores between paired steaks displayed at 2°C vs 7°C until day 30 of retail display, while steaks cut from subprimals stored for 12 days had less surface discoloration if displayed at 2°C rather than 7°C after 4 of 7 display periods (both early and late in the display periods). Steaks cut from subprimals that had been stored for 24 days and displayed at 2°C had less ($P < 0.05$) surface discoloration after 10, 15, 20, 25, and 30 days of display than did those displayed at 7°C.

In data not presented, surface discoloration at day 0 was significantly less evident than it was after 5 through 30 days of retail display in all comparisons when steaks were vac-

Table 3 — Mean surface discoloration[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
	0	14.85^{c}	14.87^{c}	0	11.71^{d}	11.09^{e}	12.22^{d}	11.13^{d}
	1	11.64^{d}	12.08^{d}	5	14.40^{c}	14.52^{c}	14.61^{c}	14.64^{c}
	2	10.63^{de}	10.28^{e}	10	14.24^{c}	14.05^{cd}	14.51^{c}	14.45^{c}
0	3	9.92^{e}	8.79^{f}	15	13.85^{c}	14.27^{cd}	14.36^{c}	14.13^{c}
	4	7.78^{f}	6.24^{g}	20	13.08^{c}	13.92^{cd}	13.86^{c}	14.14^{c}
	5	5.75^{g}	4.03^{h}	25	12.80^{cd}	13.45^{cd}	13.57^{c}	14.23^{c}
	6	4.17^{g}	2.50^{h}	30	14.40^{c}	12.63^{d}	14.43^{c}	13.03^{c}
	0	14.82^{c}	14.47^{c}	0	6.18^{d}	4.27^{e}	8.77^{d}	7.30^{f}
	1	13.74^{c}	12.14^{d}	5	14.24^{c}	14.24^{c}	14.59^{c}	14.21^{c}
	2	11.43^{d}	7.51^{e}	10	14.08^{c}	13.95^{c}	14.17^{c}	13.98^{c}
12	3	9.67^{e}	5.77^{e}	15	14.13^{c}	13.75^{c}	14.13^{c}	13.54^{cd}
	4	7.20^{f}	3.70^{f}	20	14.33^{c}	13.71^{c}	14.18^{c}	13.40^{cd}
	5	6.60^{f}	3.49^{f}	25	13.89^{c}	10.83^{d}	14.16^{c}	10.57^{e}
	6	3.90^{g}	3.24^{f}	30	13.13^{c}	11.87^{d}	13.69^{c}	11.93^{de}
	0	14.83^{c}	14.95^{c}	0	5.80^{f}	5.95^{g}	6.73^{e}	6.34^{f}
	1	12.48^{d}	10.47^{d}	5	14.47^{c}	14.51^{c}	14.44^{c}	14.49^{c}
	2	9.92^{e}	6.59^{e}	10	14.49^{c}	14.09^{c}	14.53^{c}	14.10^{c}
24	3	7.41^{f}	3.85^{f}	15	14.15^{c}	12.41^{d}	14.03^{c}	12.54^{d}
	4	6.90^{fg}	3.49^{f}	20	13.98^{c}	11.78^{e}	14.12^{c}	11.86^{de}
	5	5.22^{gh}	3.70^{f}	25	13.35^{d}	11.05^{f}	13.75^{cd}	11.53^{de}
	6	3.53^{h}	3.80^{f}	30	12.48^{e}	10.43^{f}	12.61^{d}	11.16^{e}

[a] Means based on a 15-point scale (15 = 0% discoloration, 1 = 100% discoloration).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-h] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

uum packaged in high oxygen-barrier film. Comparisons of medium vs high oxygen-barrier packages revealed no consistent differences in surface discoloration scores when steaks were displayed at either temperature (2°C or 7°C). Steaks overwrapped in PVC tended to have the least surface discoloration immediately upon placement in the display case, while steaks vacuum packaged in medium and high oxygen-barrier films tended to have unacceptable levels of surface discoloration on day 0, suggesting that the reaction involved in conversion of oxymyoglobin to metmyoglobin and subsequently to reduced myoglobin may not have been complete at 12 hr after packaging. In addition, surface discoloration scores of steaks from relatively fresh subprimals are not greatly affected by display temperatures, while steaks cut from subprimals that had been subjected to additional periods of storage tend to have more extensive surface discoloration when displayed at 7°C, rather than at 2°C, and thereby have reduced shelf-life.

Mean overall appearance scores of strip loin steaks packaged in each of three films and displayed at each of two temperatures are reported in Table 4. There were no differences ($P > 0.05$) between display temperatures for steaks overwrapped in oxygen-permeable film regardless of the number of days of display when steaks were from subprimals stored for 0 days. With longer periods of storage (12 or 24 days), retail display of PVC overwrapped steaks at 2°C resulted in higher ($P < 0.05$) overall appearance scores in 9 of 14 comparisons with steaks displayed at 7°C (although steaks from both display temperatures received low and nonsignificant scores at days 5 and 6). When PVC-overwrapped loin steaks were displayed at 2°C, acceptable overall appearance scores (greater than 8.0) were maintained for 3, 3 or 2 days, for steaks from subprimals stored for 0, 12, or 24 days, respectively; steaks displayed at 7°C maintained acceptable overall appearance scores for 2, 1, or 1 days from subprimals stored 0, 12, or 24 days, respectively. Steaks packaged in medium oxygen-barrier bags displayed no consistent differences in overall appearance between steaks displayed at 2°C and those displayed at 7°C when steaks were from subprimals with no additional storage (0 days). Steaks fabricated from subprimals after 12 or 24 days of storage and packaged in medium oxygen-barrier bags had higher ($P < 0.05$) overall appearance scores when displayed at 2°C in 4 of 7 comparisons with steaks displayed at 7°C within their respective storage periods. Steaks packaged in medium oxygen-barrier bags and displayed at 2°C or 7°C exhibited desirable overall appearance scores in all day 0 storage display periods. Overall appearance scores of steaks from loins stored for 12 days were undesirable on day 0 if displayed at 2°C and on days 0 and 25 if displayed at 7°C; steaks packaged in medium oxygen-barrier bags from loins stored for 24 days were desirable on days 5 through 25 when displayed at 2°C and were desirable on days 5 through 20 when displayed at 7°C. Steaks packaged in high oxygen-barrier film and from subprimals stored for 0 days revealed no differences ($P > 0.05$) between display temperatures (2°C vs 7°C) in overall appearance until day 30 evaluations. With longer storage periods (12 and 24 days), steaks displayed at 2°C received higher overall appearance scores in all but one (12 days storage; 5 days display) comparison with steaks stored at 7°C. Steaks packaged in high oxygen-barrier film and displayed at 2°C received accepta-

Table 4 — Mean overall appearance[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film		Display period (days)	Medium oxygen-barrier		High oxygen-barrier	
		2°C	7°C		2°C	7°C	2°C	7°C
0	0	13.54^{c}	13.52^{c}	0	11.84^{e}	11.42^{f}	12.38^{d}	11.64^{f}
	1	10.54^{d}	11.02^{d}	5	13.24^{cd}	13.67^{c}	13.91^{c}	13.84^{c}
	2	9.30^{e}	9.11^{e}	10	12.97^{cd}	13.07^{cd}	13.57^{c}	13.51^{cd}
	3	8.34^{e}	7.48^{f}	15	13.47^{c}	13.47^{cd}	13.76^{c}	13.60^{cd}
	4	6.50^{f}	5.05^{g}	20	12.18^{de}	12.69^{de}	12.76^{d}	13.08^{de}
	5	5.13^{fg}	3.52^{gh}	25	11.15^{e}	11.76^{ef}	12.48^{d}	12.68^{e}
	6	3.63^{g}	2.20^{h}	30	12.70^{cde}	10.13^{g}	12.97^{cd}	11.11^{f}
12	0	14.61^{c}	14.65^{c}	0	6.05^{f}	4.71^{h}	8.23^{d}	6.95^{f}
	1	12.98^{d}	11.04^{d}	5	12.83^{c}	12.93^{c}	13.30^{c}	13.02^{c}
	2	10.73^{e}	6.97^{e}	10	12.47^{cd}	12.31^{d}	12.90^{c}	12.61^{cd}
	3	8.47^{f}	4.72^{f}	15	12.46^{cd}	12.24^{d}	12.83^{c}	11.83^{cd}
	4	7.11^{fg}	3.70^{f}	20	11.92^{cd}	10.67^{e}	12.54^{c}	10.83^{e}
	5	5.35^{gh}	3.20^{f}	25	11.40^{de}	6.40^{g}	12.50^{c}	6.60^{f}
	6	3.37^{h}	2.30^{f}	30	10.37^{e}	8.28^{f}	12.25^{c}	8.03^{f}
24	0	14.73^{c}	14.55^{c}	0	7.09^{f}	7.00^{g}	8.07^{f}	7.27^{f}
	1	12.21^{d}	10.41^{d}	5	12.75^{c}	12.76^{c}	13.81^{c}	13.47^{c}
	2	9.37^{e}	6.07^{e}	10	13.07^{c}	11.75^{d}	13.88^{c}	13.07^{c}
	3	7.39^{f}	3.31^{f}	15	11.87^{d}	9.49^{e}	12.42^{d}	10.32^{d}
	4	5.39^{g}	2.13^{g}	20	11.91^{d}	8.06^{f}	12.47^{d}	8.30^{e}
	5	3.45^{h}	2.31^{fg}	25	10.57^{e}	7.10^{g}	12.48^{d}	7.36^{f}
	6	2.60^{h}	2.33^{fg}	30	5.83^{g}	3.83^{h}	10.69^{e}	4.93^{g}

[a] Means based on a 15-point scale (15 = extremely desirable, 1 = extremely undesirable).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-h] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

ble overall appearance scores after all display periods, regardless of storage time, while steaks from subprimals fabricated after 0, 12 or 24 days of storage received acceptable scores for 30, 20 and 20 days of display, respectively, at 7°C.

In data not presented, analysis of day 0 through day 5 overall appearance scores revealed that although day 0 scores for all treatments were significantly lower, days 1 through 5 were not different ($P > 0.05$), suggesting that the conversion of oxymyoglobin to reduced myoglobin was completed by day 1 (36 hr after packaging). When comparisons were made between steaks packaged in medium vs high oxygen-barrier films (data not presented), steaks packaged in high oxygen-barrier films were superior in overall appearance to those packaged in medium oxygen-barrier films in 13 of 21 comparisons when steaks were displayed at 2°C, and in 6 of 21 comparisons when steaks were displayed at 7°C. Results of overall appearance evaluations suggest that medium and high oxygen-barrier films, used in their respective packaging systems, are superior to oxygen-permeable PVC film in maintaining acceptable visual properties when strip loin steaks are to be held longer than 3 days. In addition, unless fresh subprimals are used exclusively, beef strip loin subprimals and steaks must be held at or below 2°C to ensure maximum product appearance. Steaks from subprimals stored 12 or 24 days and packaged in medium or high oxygen-barrier films had low overall appearance scores after extended display (20–30 days) at 7°C as a result of apparent gas formation which caused many packages to "balloon" and resulted in metmyoglobin formation on the surface of the steak. The accumulation of gas in many medium oxygen-barrier and high oxygen-barrier vacuum packages of steaks that had been displayed for extended periods at 7°C and that had been fabricated from subprimals that had been stored for 12 or 24 days probably resulted from the activity of heterofermentative *Lactobacillus* spp. Data in a companion paper indicate that these species were a major or dominant part of the microflora of these steaks (Vanderzant et al., 1982).

Initial off-odor scores for strip loin steaks packaged in each of three films and displayed at each of two temperatures are reported in Table 5. Off-odor scores of steaks overwrapped in oxygen-permeable film and evaluated immediately upon opening packages revealed that steaks displayed at 2°C had less off-odor than those displayed at 7°C in 7 of 21 comparisons; in no case did those displayed at 7°C have less off-odor than those displayed at 2°C. Comparable data for strip loin steaks packaged in either medium oxygen-barrier bags or high oxygen-barrier film revealed that display at 2°C resulted in less off-odor than display at 7°C in 11 of 21 and 12 of 21 comparisons, respectively. A noticeable gas accumulation in many medium and high oxygen-barrier vacuum packages of steaks from subprimals that had been stored for 12 or 24 days may account for the extremely high incidence of off-odor of steaks displayed at 7°C for extended periods. Steaks packaged in high oxygen-barrier film tended to have less off-odor than those packaged in medium oxygen-barrier bags, although the differences were not statistically consistent (data not presented). The more frequent isolation of small percentages of gram-negative bacteria from steaks packaged in medium oxygen-barrier bags than from comparable steaks packaged in high

Table 5 – Mean off-odor[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
	0	9.97^{c}	9.80^{c}	0	8.97^{c}	9.70^{c}	9.70^{c}	9.93^{c}
	1	9.77^{c}	9.03^{c}	5	5.40^{d}	4.80^{d}	8.33^{d}	7.90^{d}
	2	8.86^{cd}	9.00^{c}	10	3.80^{e}	2.70^{e}	6.10^{e}	5.17^{e}
0	3	9.03^{cd}	8.73^{c}	15	2.83^{ef}	1.83^{f}	5.60^{ef}	3.30^{f}
	4	7.84^{de}	6.57^{d}	20	3.30^{ef}	1.63^{f}	3.25^{g}	2.33^{fg}
	5	6.95^{ef}	4.77^{e}	25	2.33^{f}	1.77^{f}	4.53^{fg}	2.20^{fg}
	6	5.43^{f}	4.27^{e}	30	2.90^{ef}	1.53^{f}	3.43^{g}	1.29^{g}
	0	9.50^{c}	9.43^{c}	0	6.63^{c}	6.63^{c}	7.87^{c}	9.03^{c}
	1	9.67^{c}	8.47^{cd}	5	5.43^{cd}	4.03^{d}	7.90^{c}	5.60^{d}
	2	8.33^{cd}	5.30^{f}	10	3.40^{de}	3.77^{d}	5.00^{de}	2.49^{e}
12	3	9.60^{c}	7.44^{de}	15	4.80^{d}	2.04^{e}	4.70^{de}	2.33^{e}
	4	7.17^{de}	6.23^{ef}	20	4.48^{de}	1.36^{e}	4.60^{de}	1.56^{e}
	5	7.00^{de}	5.70^{f}	25	5.72^{cd}	2.00^{e}	5.36^{d}	1.76^{e}
	6	5.84^{e}	2.87^{g}	30	2.73^{f}	1.17^{e}	3.13^{e}	1.30^{e}
	0	10.00^{c}	10.00^{c}	0	8.20^{c}	8.28^{c}	9.44^{c}	9.56^{c}
	1	9.93^{c}	9.87^{c}	5	6.73^{d}	5.79^{d}	8.03^{d}	6.63^{d}
	2	8.90^{c}	7.37^{d}	10	4.16^{e}	3.33^{e}	5.57^{e}	3.50^{e}
24	3	9.33^{c}	8.47^{cd}	15	4.60^{e}	1.75^{f}	5.72^{e}	2.24^{fg}
	4	6.09^{de}	3.04^{e}	20	4.26^{e}	2.40^{ef}	4.80^{e}	2.53^{ef}
	5	5.57^{e}	4.50^{e}	25	3.36^{ef}	1.57^{f}	2.10^{f}	1.13^{g}
	6	7.30^{d}	4.80^{e}	30	2.40^{f}	1.20^{f}	2.57^{f}	1.17^{g}

[a] Means based on a 10-point scale (10 = no off-odor, 1 = abundant off-odor).
[b] Days of storage in vaccum as strip loins after arrival from the packing plant.
[c-g] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

oxygen-barrier film (Vanderzant et al., 1982) supports the observation in the present paper that steaks in high oxygen-barrier film tended to have less off-odor than those packaged in medium oxygen-barrier film. Some of the gram-negative, psychrotrophic bacteria isolated from these steaks are known as potential producers of flavor and odor defects.

Off-odor diminished somewhat upon re-evaluation of steaks, 15 min after initially opening the medium and high oxygen-barrier vacuum packages (Table 6). Display temperature differences were not consistently related to off-odor among steaks in medium or high oxygen-barrier packages, but steaks displayed at 2°C tended to have less off-odor. As subprimals are stored longer, off-odors of steaks from these subprimals increase thereby decreasing shelf-life. Also, temperature is a more important factor in determining the shelf-life of steaks from subprimals that have been stored for extended periods.

In data not presented, steaks displayed at 2°C had less ($P < 0.05$) weight-loss during display than did those displayed at 7°C in 12 of 63 comparisons. Within each packaging treatment, weight-loss increased with increased display time but the largest increase in weight-loss occurred within the PVC packaging group. No differences ($P > 0.05$)

Table 6 — Mean off-odor[a] scores for strip loin steaks packaged with medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures, evaluated 15 min after opening packages

	Storage period[b] 0 days				12 days				24 days			
	Medium oxygen-barrier		High oxygen-barrier		Medium oxygen-barrier		High oxygen-barrier		Medium oxygen-barrier		High oxygen-barrier	
	2°C	7°C	2°C	7°C	2°C	7°C	2°C	7°C	2°C	7°C	2°C	7°C
0	9.80^{c}	10.00^{c}	10.00^{c}	10.00^{c}	10.00^{c}	10.00^{c}	10.00^{c}	9.83^{c}	9.90^{c}	9.40^{c}	10.00^{c}	10.00^{c}
5	9.33^{c}	9.25^{c}	9.09^{c}	8.83^{cd}	9.91^{c}	9.59^{c}	9.17^{c}	8.00^{cd}	9.75^{c}	9.41^{c}	9.91^{c}	9.25^{cd}
10	7.91^{c}	6.50^{de}	7.41^{ef}	5.59^{d}	7.83^{d}	6.08^{d}	7.09^{c}	5.92^{de}	6.83^{de}	5.83^{d}	8.75^{cd}	5.59^{e}
15	8.70^{c}	8.23^{cd}	9.33^{c}	6.09^{d}	8.42^{cd}	4.75^{d}	6.75^{c}	4.67^{ef}	7.60^{d}	6.80^{d}	8.30^{cd}	6.80^{de}
20	8.00^{c}	5.33^{e}	8.83^{cd}	8.42^{cd}	7.20^{d}	4.80^{d}	6.30^{c}	2.80^{f}	6.92^{de}	4.92^{d}	5.91^{e}	4.41^{ef}
25	7.75^{c}	5.91^{e}	8.10^{de}	6.75^{cd}	7.63^{d}	6.00^{d}	7.87^{c}	2.87^{f}	6.41^{de}	3.17^{e}	7.00^{de}	4.17^{ef}
30	7.93^{c}	4.59^{e}	6.67^{f}	6.83^{cd}	7.67^{d}	6.33^{d}	6.50^{c}	4.17^{ef}	6.17^{e}	3.58^{e}	5.00^{e}	2.08^{f}

[a] Means based on a 10-point scale (10 = no off-odor, 1 = abundant off-odor).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-f] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

Table 7 — Mean juiciness[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier film and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
	0	5.85^{c}	5.05^{c}	0	5.30^{c}	5.00^{c}	5.05^{c}	5.55^{c}
	1	5.30^{cd}	4.85^{c}	5	5.30^{c}	4.95^{c}	4.60^{c}	5.15^{c}
	2	4.80^{cd}	5.65^{c}	10	5.15^{c}	5.50^{c}	4.80^{c}	4.70^{c}
0	3	5.15^{cd}	5.40^{c}	15	4.45^{c}	4.65^{c}	5.35^{c}	4.60^{c}
	4	5.50^{cd}	5.10^{c}	20	5.75^{c}	5.10^{c}	5.25^{c}	4.90^{c}
	5	5.60^{cd}	5.20^{c}	25	5.70^{c}	5.10^{c}	5.70^{c}	5.35^{c}
	6	3.90^{d}	4.70^{c}	30	5.35^{c}	5.50^{c}	5.60^{c}	5.00^{c}
	0	6.15^{c}	6.20^{c}	0	5.75^{c}	4.75^{c}	5.15^{c}	5.20^{c}
	1	5.45^{cd}	5.30^{cd}	5	6.35^{c}	5.45^{c}	5.65^{c}	4.85^{c}
	2	4.65^{d}	5.55^{cd}	10	6.05^{c}	5.45^{c}	4.80^{c}	4.85^{c}
12	3	5.10^{cd}	4.95^{cd}	15	4.70^{c}	5.05^{c}	4.90^{c}	5.30^{c}
	4	4.65^{d}	4.95^{cd}	20	4.90^{c}	4.90^{c}	5.05^{c}	5.00^{c}
	5	5.00^{cd}	4.85^{d}	25	5.10^{c}	5.25^{c}	5.10^{c}	5.30^{c}
	6	6.15^{c}	5.15^{cd}	30	5.65^{c}	4.75^{c}	4.95^{c}	5.50^{c}
	0	5.40^{c}	4.95^{c}	0	5.65^{c}	5.15^{d}	4.95^{c}	5.75^{cd}
	1	5.85^{c}	5.55^{c}	5	5.15^{c}	5.55^{cd}	5.40^{c}	5.40^{cd}
	2	5.30^{c}	5.30^{c}	10	4.75^{c}	5.70^{cd}	5.30^{c}	5.05^{d}
24	3	6.00^{c}	5.95^{c}	15	6.00^{c}	6.25^{c}	5.60^{c}	6.10^{c}
	4	5.15^{c}	5.30^{c}	20	5.30^{c}	5.15^{d}	4.95^{c}	5.25^{d}
	5	5.10^{c}	5.30^{c}	25	5.20^{c}	5.00^{d}	5.50^{c}	5.10^{d}
	6	5.40^{c}	5.70^{c}	30	4.90^{c}	4.75^{d}	4.90^{c}	5.25^{d}

[a] Means based on an 8-point scale (8 = extremely juicy, 1 = extremely dry).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c,d] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

in weight-loss during display were found between steaks packaged in medium oxygen-barrier bags and those packaged in high oxygen-barrier film when steaks from each treatment were compared within each display temperature (2°C or 7°C). In addition, no significant differences were observed in cooking loss percentages when strip loin steaks were compared within each packaging method for differences between display temperatures and for differences associated with increased time of retail display.

Presented in Tables 7 and 8 are mean juiciness and tenderness ratings for strip loin steaks packaged in oxygen-permeable or each of two oxygen-barrier films and displayed at different temperatures. No differences ($P > 0.05$) in juiciness were observed between steaks displayed at 2°C and those displayed at 7°C, regardless of storage period, film type, or number of days of retail display. Furthermore, juiciness did not differ ($P > 0.05$) as time of retail display increased, regardless of storage period as subprimals, temperature of display or film type. Similar results were observed for tenderness; with only two exceptions, no differences ($P > 0.05$) were found between steaks displayed at 2°C and those displayed at 7°C, regardless of storage period, display period or type of packaging film. Significant differences in tenderness of strip loin steaks in association with increasing time in the retail case were found in 2 of 6 comparisons of steaks from subprimal cuts stored 0 days prior to fabrication. The lack of differences in sensory panel tenderness ratings for the remainder of the comparisons could be due to the fact that subprimals from high quality (low and average Choice) carcasses were used, and that all subprimals were stored in vacuum packages for at least 5 days during transportation-distribution and for 12 or 24 additional days prior to cutting in these treatments; therefore, the product had undoubtedly achieved the maximum amount of tenderization expected in association with aging. In this study, the initial time interval (7 days) closely corresponds to the time period found by Smith et al. (1978) of 8–11 days for optimal tenderization, which helps explain why further aging did not result in substantial increases in tenderness. Shear force values confirm the lack of effects on tenderness of time or temperature that had been observed by the trained sensory panel (Table 9). With two exceptions, no differences ($P > 0.05$) were observed when shear force values from steaks displayed at 2°C were compared with those from steaks displayed at 7°C. Differences in shear force associated with increased display time within packaging treatment were inconsistent; tenderness increased only in the 2°C high oxygen-barrier group from subprimals cut after no additional days of storage after arrival.

Mean flavor ratings of strip loin steaks packaged in oxygen-permeable or each of two oxygen-barrier films and displayed at either 2°C or 7°C are reported in Table 10. Differences ($P < 0.05$) were found between steaks displayed at 2°C and those displayed at 7°C in only 8 of 63 comparisons. Steaks overwrapped in oxygen-permeable film and displayed at 2°C were assigned acceptable flavor scores (greater than 4.5) for 6, 6 and 4 days, respectively, for steaks from subprimals stored 0, 12 and 24 days. Steaks wrapped in oxygen-permeable film and displayed at 7°C were acceptable throughout retail display when steaks were cut from subprimals stored for 0 days, while steaks cut from subprimals that had been stored for 12 or 24 days were not significantly different ($P > 0.05$), with one exception (12 days storage; 2 days of display) through 5 days of

Table 8 — Mean tenderness[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
0	0	6.55[c]	5.85[c]	0	6.15[d]	6.65[c]	6.20[d]	6.50[c]
	1	6.50[c]	6.40[c]	5	6.95[cd]	6.25[c]	6.85[cd]	6.70[c]
	2	5.80[c]	6.80[c]	10	6.60[cd]	6.45[c]	6.85[cd]	6.75[c]
	3	6.05[c]	6.10[c]	15	6.70[cd]	6.50[c]	6.55[cd]	6.35[c]
	4	6.75[c]	6.35[c]	20	6.80[cd]	6.50[c]	6.75[cd]	6.45[c]
	5	6.00[c]	6.10[c]	25	6.45[cd]	6.35[c]	6.65[cd]	5.75[c]
	6	6.70[c]	6.70[c]	30	7.20[c]	7.05[c]	7.45[c]	6.50[c]
12	0	7.55[c]	6.60[c]	0	6.90[cd]	6.65[c]	6.30[c]	5.90[c]
	1	6.05[c]	6.65[c]	5	6.25[d]	6.55[c]	6.45[c]	6.50[c]
	2	5.85[c]	6.55[c]	10	6.90[cd]	6.75[c]	6.50[c]	6.35[c]
	3	6.70[c]	6.05[c]	15	6.40[d]	6.90[c]	6.80[c]	6.85[c]
	4	6.10[c]	6.25[c]	20	6.60[cd]	7.15[c]	6.55[c]	6.55[c]
	5	7.20[c]	7.30[c]	25	7.70[c]	7.55[c]	7.25[c]	7.50[c]
	6	6.80[c]	6.40[c]	30	7.05[cd]	6.55[c]	6.80[c]	7.00[c]
24	0	7.40[c]	5.65[c]	0	7.55[c]	6.80[c]	7.00[c]	6.75[c]
	1	6.75[c]	6.95[c]	5	7.00[c]	6.80[c]	7.35[c]	6.90[c]
	2	6.70[c]	6.10[c]	10	6.00[c]	6.40[c]	6.35[c]	6.30[c]
	3	7.00[c]	7.20[c]	15	7.10[c]	7.05[c]	6.95[c]	7.20[c]
	4	6.05[c]	6.65[c]	20	6.85[c]	7.05[c]	6.65[c]	7.20[c]
	5	6.70[c]	6.60[c]	25	6.77[c]	6.20[c]	7.05[c]	6.50[c]
	6	7.20[c]	6.80[c]	30	6.90[c]	6.90[c]	7.25[c]	6.75[c]

[a] Means based on a 8-point scale (8 = extremely tender, 1 = extremely tough).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c,d] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

retail display. Steaks from subprimals stored 0, 12 or 24 days, vacuum packaged in medium oxygen-barrier bags and displayed at 2°C did not have flavor ratings that differed significantly from those at day 0 until they had been displayed for periods of 20, 20 and 10 days, respectively; comparable data for steaks displayed at 7°C were 20, 10 and 10 days, respectively. Flavor ratings of steaks from subprimals stored 0, 12 and 24 days, vacuum packaged in high oxygen-barrier film and displayed at 2°C did not have flavor ratings that differed ($P > 0.05$) from those at day 0 until they had been displayed for periods of 15, 15 and 20 days, respectively; comparable data for steaks displayed at 7°C were 10, 10 and 10 days, respectively. Further research on packaged steaks with less time between sampling periods must be conducted to pinpoint the exact quality-life of beef strip loin steaks as determined by flavor.

Microbiological data in a companion paper (Vanderzant et al., 1982) support the observations that odor and/or flavor ratings of steaks displayed at 7°C frequently were lower than those of steaks displayed at 2°C and that off-odor incidence increased as the subprimals from which they were prepared had been stored for longer periods. For example, for steaks fabricated from loins that were stored for 0 days, aerobic plate counts (APC) of steaks packaged in PVC, high oxygen-barrier film and medium oxygen-barrier bags exceeded 10^6 cfu per cm^2 at 6, 10 and 10 days, respectively, when they were displayed at 2°C; counts of comparable steaks displayed at 7°C exceeded 10^6 cfu per cm^2 at 6, 10 and 5 days, respectively. At this level of microbial population, sensory defects often become noticeable in meats, poultry and fish. As storage of the vacuum-packaged loins was extended to 12 or 24 days, counts of steaks exceeding 10^6 cfu per cm^2 were reached sooner. When vacuum-packaged loins had been stored for 24 days at 2 ± 1°C, APC of steaks displayed in PVC, high oxygen-barrier film or medium oxygen-barrier bags exceeded 10^6 cfu per cm^2 at 4, 5 and 5 days, respectively, when steaks were displayed at 2°C; counts of comparable steaks displayed at 7°C exceeded 10^6 cfu per cm^2 at 2, 5 and 5 days, respectively.

Presented in Table 11 are mean overall palatability ratings for strip loin steaks packaged in oxygen-permeable or each of two different oxygen-barrier films and displayed at each of two temperatures (2°C and 7°C). Differences in overall palatability ratings were not consistently related to display temperature (2°C vs 7°C) for comparisons of type of package, length of storage as subprimals or time of display. Steaks overwrapped in oxygen-permeable film and displayed at either 2°C or 7°C did not have overall palatability ratings that differed from those at day 0 when they had been displayed for 6, 2 or 4 days, respectively, when steaks were fabricated from subprimals that were stored 0, 12 or 24 days after arrival. Overall palatability ratings of steaks that were vacuum packaged in medium oxygen-barrier bags and displayed at 2°C were not different ($P > 0.05$) from those of steaks on day 0 until they had been displayed for 20, 15 or 10 days, respectively, when steaks were cut from subprimals that had been stored 0, 12 or 24 days. Steaks vacuum packaged in high oxygen-barrier film and displayed at 2°C did not have overall palatability ratings that differed from those at day 0 until they had been displayed for 15, 25 or 20 days, respectively; comparable data for steaks displayed at 7°C were 10, 10 or 10 days, respectively, for

Table 9 — Mean shear force values[a] (kg) for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
0	0	3.72^{c}	3.50^{cde}	0	4.05^{e}	3.23^{c}	3.85^{d}	3.52^{c}
	1	2.74^{c}	3.90^{de}	5	3.09^{cde}	3.31^{c}	3.22^{cd}	2.53^{c}
	2	3.12^{c}	2.88^{c}	10	2.59^{cd}	2.74^{c}	2.94^{cd}	2.58^{c}
	3	3.47^{c}	3.92^{de}	15	3.44^{de}	3.04^{c}	2.66^{cd}	3.08^{c}
	4	3.34^{c}	3.77^{cde}	20	2.20^{c}	2.87^{c}	2.18^{c}	2.57^{c}
	5	4.02^{c}	4.11^{e}	25	2.74^{cd}	2.95^{c}	2.55^{c}	2.68^{c}
	6	2.91^{c}	3.03^{cd}	30	2.62^{cd}	2.43^{c}	2.51^{c}	2.30^{c}
12	0	3.28^{c}	3.55^{c}	0	2.81^{c}	3.29^{c}	2.66^{cd}	3.48^{c}
	1	3.46^{c}	2.85^{c}	5	2.96^{c}	2.54^{c}	3.26^{d}	3.03^{c}
	2	2.37^{c}	3.43^{c}	10	2.59^{c}	2.80^{c}	2.21^{c}	2.71^{c}
	3	2.75^{c}	3.19^{c}	15	2.92^{c}	2.70^{c}	2.64^{cd}	2.85^{c}
	4	3.25^{c}	2.70^{c}	20	2.68^{c}	2.39^{c}	2.75^{cd}	2.74^{c}
	5	2.91^{c}	3.49^{c}	25	2.70^{c}	2.67^{c}	2.57^{cd}	3.06^{c}
	6	3.05^{c}	2.58^{c}	30	2.71^{c}	2.69^{c}	2.82^{cd}	2.64^{c}
24	0	2.99^{c}	3.42^{c}	0	2.51^{c}	3.23^{c}	2.70^{c}	3.69^{d}
	1	3.01^{c}	3.25^{c}	5	2.35^{c}	2.74^{c}	2.82^{c}	2.94^{cd}
	2	2.99^{c}	3.40^{c}	10	2.61^{c}	3.15^{c}	2.55^{c}	2.59^{cd}
	3	2.44^{c}	2.43^{c}	15	2.29^{c}	2.13^{c}	2.57^{c}	2.48^{cd}
	4	2.75^{c}	2.77^{c}	20	2.68^{c}	2.46^{c}	2.69^{c}	2.58^{cd}
	5	2.22^{c}	2.71^{c}	25	2.45^{c}	2.72^{c}	2.60^{c}	2.77^{cd}
	6	2.50^{c}	2.38^{c}	30	2.42^{c}	2.17^{c}	2.46^{c}	2.08^{c}

[a] Means based on Warner-Bratzler shear force values from 1.27 cm cores.
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-e] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

steaks cut from subprimals that had been stored for 0, 12 or 24 days after arrival. There was a trend for overall palatability ratings of steaks packaged in oxygen-permeable, medium oxygen-barrier bags or high oxygen-barrier film and displayed at 2°C to have longer product life than those displayed at 7°C, although this was not statistically different in most comparisons.

Palatability traits were also analyzed (data not presented) directly comparing vacuum packaged strip loin steaks in medium oxygen-barrier bags with those packaged in high oxygen-barrier film within each retail display temperature (2°C and 7°C). No consistent differences were observed for juiciness, tenderness, flavor or overall palatability ratings or shear force values when steaks that were vacuum packaged in medium oxygen-barrier bags were compared to those that were vacuum packaged in high oxygen-barrier film when steaks were displayed at either 2°C or 7°C. Although no consistently significant differences were observed between medium and high oxygen-barrier packaging treatments, in most instances, steaks that were vacuum packaged in high oxygen-barrier film had higher numerical ratings for palatability traits.

Based on the data of the present study, strip loin steaks can be displayed for periods as long as 10 days. To ensure product quality through the retail marketing system and to the time of ultimate consumption, it is recommended that: (1) steaks be fabricated from relatively fresh subprimals that have not been stored for excessive periods after arrival at the retail level, (2) steaks be stored and displayed at 2°C rather than at 7°C, and (3) steaks be packaged in high oxygen-barrier film. For at least 36 hr of the 10-day maximum shelf-life period, steaks should be held in storage (not displayed for sale) to allow the reactions involving conversion of oxymyoglobin to metmyoglobin to reduced myoglobin to be completed and thereby to develop optimal lean color characteristics for such product. Longer periods of time must be allowed for this process to occur as postmortem age of subprimals increases. Although visual characteristics of strip loin steaks were found to be desirable for periods much longer than 10 days, steaks evaluated after display periods longer than 10 days tended to have off-odor and/or off-flavor (especially in steaks fabricated from subprimals that were stored for 12 or 24 days after arrival) that probably would not be acceptable to consumers. Further research should be conducted using shorter sampling periods to determine the exact shelf-life of vacuum packaged fresh beef loin steaks.

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Table 10 — Mean flavor[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
	0	5.70^{c}	5.90^{c}	0	5.40^{c}	5.50^{c}	5.85^{c}	5.90^{c}
	1	5.05^{c}	5.40^{c}	5	5.20^{cd}	5.35^{c}	5.50^{c}	5.60^{c}
	2	5.10^{c}	5.10^{c}	10	4.70^{cde}	4.95^{c}	4.35^{cd}	4.15^{d}
0	3	5.65^{c}	5.20^{c}	15	5.40^{c}	3.65^{cd}	3.60^{de}	2.85^{e}
	4	4.85^{c}	5.05^{c}	20	2.35^{def}	1.75^{de}	2.70^{ef}	1.30^{f}
	5	5.30^{c}	5.65^{c}	25	1.30^{f}	2.00^{de}	2.00^{f}	1.15^{f}
	6	4.90^{c}	5.25^{c}	30	1.90^{f}	1.20^{e}	2.00^{f}	1.05^{f}
	0	6.40^{c}	4.80^{c}	0	5.55^{c}	5.80^{c}	5.75^{c}	5.65^{c}
	1	5.70^{c}	5.65^{c}	5	5.95^{c}	4.55^{c}	5.15^{cd}	4.35^{cd}
	2	3.20^{d}	1.80^{d}	10	4.35^{cd}	2.45^{d}	5.05^{cd}	2.60^{de}
12	3	4.85^{cd}	4.70^{c}	15	3.65^{cd}	1.95^{d}	2.95^{de}	1.30^{e}
	4	5.00^{cd}	4.15^{c}	20	2.10^{d}	1.15^{d}	3.25^{de}	1.35^{e}
	5	5.15^{cd}	5.35^{c}	25	2.00^{d}	1.25^{d}	2.40^{e}	1.15^{f}
	6	4.80^{cd}	3.60^{cd}	30	1.80^{d}	1.20^{d}	2.05^{e}	1.20^{f}
	0	5.95^{c}	6.40^{c}	0	6.00^{c}	5.55^{c}	5.15^{c}	6.05^{c}
	1	5.85^{c}	5.75^{cd}	5	5.65^{c}	5.55^{c}	5.55^{c}	4.80^{c}
	2	5.15^{cde}	4.80^{cd}	10	3.65^{d}	2.15^{d}	4.15^{cd}	2.40^{d}
24	3	5.45^{cd}	4.35^{d}	15	3.25^{de}	1.10^{e}	4.30^{cd}	1.10^{d}
	4	4.50^{de}	4.55^{d}	20	2.05^{ef}	1.40^{e}	3.55^{d}	1.25^{d}
	5	3.60^{e}	4.35^{d}	25	1.97^{f}	1.00^{e}	1.45^{e}	1.00^{d}
	6	4.50^{de}	1.50^{e}	30	1.65^{f}	1.00^{e}	1.60^{e}	1.00^{d}

[a] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-f] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

Table 11 — Mean overall palatability[a] scores for strip loin steaks packaged with oxygen-permeable, medium oxygen-barrier or high oxygen-barrier films and displayed at different temperatures

Storage period (days)[b]	Display period (days)	Oxygen-permeable film 2°C	Oxygen-permeable film 7°C	Display period (days)	Medium oxygen-barrier 2°C	Medium oxygen-barrier 7°C	High oxygen-barrier 2°C	High oxygen-barrier 7°C
	0	5.85^{c}	5.65^{c}	0	5.40^{c}	5.55^{c}	5.85^{c}	5.85^{c}
	1	4.95^{c}	5.30^{c}	5	5.50^{c}	5.40^{c}	5.65^{c}	5.55^{c}
	2	4.85^{c}	5.05^{c}	10	5.00^{c}	4.65^{c}	4.35^{cd}	4.30^{d}
0	3	5.70^{c}	5.15^{c}	15	5.05^{c}	3.65^{cd}	3.55^{de}	2.80^{e}
	4	5.10^{c}	5.05^{c}	20	2.20^{d}	1.75^{de}	2.80^{def}	1.50^{f}
	5	5.00^{c}	5.60^{c}	25	1.25^{d}	2.05^{de}	1.90^{f}	1.00^{f}
	6	5.00^{c}	5.40^{c}	30	2.00^{d}	1.15^{e}	2.20^{ef}	1.05^{f}
	0	6.50^{c}	4.95^{c}	0	5.65^{cd}	5.70^{c}	5.45^{c}	5.55^{c}
	1	5.80^{cd}	5.50^{c}	5	6.10^{c}	4.45^{d}	5.35^{c}	4.45^{cd}
	2	3.15^{d}	1.90^{d}	10	4.75^{cd}	2.30^{e}	4.95^{cd}	2.60^{de}
12	3	4.95^{cd}	4.85^{c}	15	3.75^{de}	1.90^{ef}	2.85^{cde}	1.30^{e}
	4	4.75^{cd}	4.20^{c}	20	2.05^{e}	1.05^{f}	3.30^{cde}	1.35^{e}
	5	5.40^{cd}	5.45^{c}	25	1.90^{e}	1.40^{ef}	2.50^{de}	1.15^{e}
	6	5.00^{cd}	3.65^{cd}	30	1.85^{e}	1.25^{ef}	2.05^{f}	1.20^{e}
	0	6.15^{c}	5.55^{cd}	0	6.45^{c}	5.65^{c}	5.05^{cd}	5.85^{c}
	1	6.10^{c}	6.20^{c}	5	6.20^{c}	5.80^{c}	5.90^{c}	4.65^{c}
	2	5.20^{cd}	4.70^{cd}	10	3.75^{d}	2.15^{d}	4.25^{de}	2.30^{d}
24	3	5.50^{c}	4.45^{cd}	15	3.30^{d}	1.05^{e}	4.05^{de}	1.10^{d}
	4	4.75^{cd}	4.90^{cd}	20	2.15^{e}	1.40^{d}	3.20^{e}	1.15^{d}
	5	3.40^{d}	4.30^{d}	25	1.90^{e}	1.05^{e}	1.45^{f}	1.00^{d}
	6	5.05^{cd}	1.50^{e}	30	1.65^{e}	1.00^{e}	1.55^{f}	1.00^{d}

[a] Means based on an 8-point scale (8 = extremely desirable, 1 = extremely undesirable).
[b] Days of storage in vacuum as strip loins after arrival from the packing plant.
[c-i] Means in the same column bearing a common superscript letter do not differ ($P > 0.05$). Means in the same display period and packaging method underscored by a common line do not differ ($P > 0.05$).

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Centralized Packaging of Beef Loin Steaks with Different Oxygen-Barrier Films: Microbiological Characteristics

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ABSTRACT

Vacuum packaged beef strip loins (n = 72) were stored (2 ± 1°C) for either 0, 12 or 24 days before fabrication; steaks were packaged and displayed (2°C or 7°C) up to 6 days in oxygen-permeable film or up to 30 days in vacuum packages (medium or high oxygen-barrier film). For steaks displayed in oxygen-permeable film, *Pseudomonas* spp. were a considerable (25–49%) or dominant (>50%) part of the microflora. The microflora of vacuum-packaged steaks from 0 day loins was dominated by a combination of hetero- and homofermentative *Lactobacillus* spp.; when vacuum-packaged steaks were from 12 and 24 day loins, the microflora was in most cases dominated by the heterofermentative *Lactobacillus cellobiosus*.

INTRODUCTION

IN THE UNITED STATES about 60–70% of all beef presently leaving packing plants is in the form of vacuum-packaged subprimal cuts. Many reports (Baran et al., 1969; Ingram, 1962; Jaye et al., 1962; Johnson, 1974; Ordal, 1962; Pierson et al., 1970; Seideman et al., 1976a, b, c; Sutherland et al., 1975) show that vacuum packaging of meats prolongs the shelf-life as compared with that of cuts packaged in oxygen-permeable film. Carbon dioxide, generated by meat tissue enzyme and microbial activites, increases CO_2 level inside the package, retards the growth of gram-negative, aerobic, psychrotrophic bacteria, and allows development of facultative anaerobic bacteria such as *Lactobacillus*, *Leuconostoc* and *Streptococcus* spp. Inhibition of *Pseudomonas* spp. is known to occur at levels of CO_2 as low as 10% (Enfors and Molin, 1980; King and Nagel, 1975).

Among the lactic acid bacteria present on vacuum-packaged meats, *Lactobacillus* spp. are often dominant. Information on the species of *Lactobacillus* on such meats is scarce. Enfors et al. (1979) reported that *Lactobacillus* spp. on pork stored in 1 atm CO_2 at 4°C for 35 days consisted primarily of *Lactobacillus plantarum* (45%) and *Lactobacillus cellobiosus* (45%). According to Blickstad et al. (1981), *Lactobacillus xylosus* and a group of unidentified homofermentative *Lactobacillus* spp. dominated on pork stored for 40 days at 4°C in 1 atm CO_2. After storage in 5 atm CO_2 both at 4 and 14°C, *Lactobacillus* spp. constituted the total flora at both temperatures with *Lactobacillus lactis* (14°C) and *L. xylosus* (4°C) dominating.

Among *Leuconostoc* spp., *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* have been reported as a major part of the microflora of vacuum-packaged pork roasts (Christopher et al., 1979) and of beef loin steaks stored in O_2-CO_2-N_2 atmospheres (Savell et al., 1981). Savell et al. (1981) reported isolation of small numbers of *L. plantarum* from beef loin steaks stored in O_2-CO_2-N_2 atmospheres.

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Most studies concerning microbiological characteristics of vacuum-packaged beef have dealt with primal or subprimal cuts. Little information is available about the microbiological properties of vacuum-packaged beef retail cuts prepared at central processing plants and shipped to retailers for subsequent display in refrigerated cases. This report provides information on the microbiological characteristics of steaks, prepared from vacuum-packaged beef loins that were stored for 0, 12 and 24 days at 2 ± 1°C. Steaks were displayed in polyvinyl chloride (PVC) film (0–6 days) and in two types of vacuum packages (0–30 days) at both 2°C and 7°C. In addition, *Lactobacillus* and *Leuconostoc* isolates from these samples were characterized as to species. Data regarding retail appearance and palatability of these steaks are included in a companion report by Griffin et al. (1982).

MATERIALS & METHODS

Samples

The source of the beef strip loins and the preparation, packaging and display of steaks are described in a companion report by Griffin et al. (1982). Steaks were prepared from vacuum-packaged beef strip loins upon arrival at the Texas A&M Univ. Meats Laboratory or after storage at that facility at 2 ± 1°C for 12 or 24 days. After fabrication, steaks were packaged in oxygen-permeable polyvinyl chloride (PVC) film and in two types of vacuum package film (medium oxygen-barrier and high oxygen-barrier). Steaks wrapped in oxygen-permeable film were sampled after 0, 2, 4 or 6 days of display; steaks wrapped in medium and high oxygen-barrier film were sampled after 0, 5, 10, 15, 20, 25 or 30 days of display. (Data for results of 25 days of retail display are not presented because identification of isolates on agar plates was not carried out). The number of steaks examined was three per packaging treatment X display time X display temperature within each fabrication period (0, 12 and 24 days); as a result, complete microbiological data were collected for 324 individual steaks. Each steak was examined by removing a 10-cm^2 (2 mm thick) area from the top surface of the steak with a sterile scalpel. Each sample was placed in 100 ml of sterile 0.1% peptone in a Stomacher bag and was macerated in a Stomacher-400 for 1 min. Aerobic plate counts (APC) were determined by plating 0.1-ml volumes of appropriate dilutions on prepoured plates of tryptic soy agar (TSA, Difco). Plates were incubated for 3 days at 25°C. A few of each of the colony types appearing on each countable plate were picked and placed on TSA slants. Slants were incubated for 2 days at 25°C. Identity of isolates was determined by biochemical tests and identification schemes previously described by Vanderzant and Nickelson (1969). Diagnostic tests and classification schemes recently presented by Sharpe (1979) were used to further describe the *Lactobacillus* and *Leuconostoc* isolates. Each of the colony types, now identified to the generic or species level, then was expressed as a percentage of the total number of colonies appearing on the countable plate.

RESULTS & DISCUSSION

AEROBIC PLATE COUNTS (log APC/cm^2) and microbial types of steaks prepared from vacuum packaged loins that were stored for 0 days are presented in Tables 1–3. Increases in log APC of steaks stored in PVC and displayed for 6 days at 2 and 7°C were 7.11 and >7.65, respectively (Table 1). In 2 of 3 comparisons, after display, log APC of steaks displayed at 7°C were greater (0.54–1.56) than those of steaks displayed at 2°C. The initial microflora of

steaks consisted of *Micrococcus* and *Flavobacterium* spp. After refrigerated display for 2–6 days, *Pseudomonas* spp. and/or *Brochothrix thermosphacta* constituted a dominant part (50% or more) of the microflora of the steaks wrapped in PVC. No consistent differences in the microflora of steaks because of differences in display temperature (2 vs 7°C) could be detected.

Increases in log APC of steaks vacuum-packaged in high oxygen-barrier film and displayed for 30 days at 2 and 7°C were 6.16 and 6.71, respectively (Table 2). In three of five comparisons, after display, log APC of steaks displayed at 7°C were slightly greater (0.26–0.71) than those of steaks displayed at 2°C; in the other cases, differences in count were small. *Lactobacillus* spp., including heterofermenta-

Table 1—Aerobic plate counts and microflora of steaks wrapped with PVC and displayed for up to 6 days at 2°C or 7°C. Steaks were prepared from vacuum packaged loins stored for 0 days.

Display time (days)	Display temp (C)	APC[b] (log/cm^2)	Percentage distribution of microflora[a,b]						
			Het. Lac	Hom. Lac	B.t.	Mic	Flav	M-A	Ps
0	2	1.00				50.0	50.0		
	7	<1.00							
2	2	3.11	31.8		64.0				4.2
	7	4.67	0.5		61.5	2.8			35.2
4	2	5.46			19.1				80.9
	7	5.18			54.2			0.2	45.6
6	2	8.11			23.8				76.2
	7	8.65		0.7	10.6				88.7

[a] Het. Lac = Heterofermentative Lactobacillus, Hom. Lac = Homofermentative Lactobacillus, B.t = Brochothrix thermosphacta, Mic = Micrococcus, Flav = Flavobacterium, M-A = Moraxella - Acinetobacter, Ps = Pseudomonas.
[b] Each figure represents the mean of three steaks.

Table 2—Aerobic plate counts and microflora of steaks vacuum-packaged in high oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 0 days

Display time (days)	Display temp (C)	APC[b] (log/cm^2)	Percentage distribution of microflora[a,b]													
			Bac	Cor	Het. Lac	Hom. Lac	Unid. Lac	Leu	Strep	B.t	Mic	A.h	M-A	E.1	H.a	Ps	A.p.
0	2	1.18	75.0								25.0						
	7	1.18		25.0						75.0							
5	2	4.89			21.7	45.7			15.6	6.8		0.2	1.0		0.2	3.6	5.2
	7	5.60			46.1	25.8			3.5	8.7		3.2	4.6			8.1	
10	2	7.36			32.0	48.7		17.6				1.7					
	7	7.08			66.2	25.7		6.9		0.2		1.0					
15	2	7.69			35.2	2.0	60.9			1.0				0.9			
	7	7.23			80.2	3.1	16.7										
20	2	7.58			83.6	14.8	1.2	0.2		0.2							
	7	7.84			45.4	12.8	33.5	4.7				0.1				3.5	
30	2	7.34			51.9	0.4	1.3	46.0		0.4							
	7	7.89			28.6	9.1	17.1	33.0						12.2			

[a] Bac = Bacillus, Cor = Coryneform bacteria, Het. Lac = Heterofermentative Lactobacillus, Hom. Lac = Homofermentative Lactobacillus, Unid. Lac = Unidentifled Lactobacillus, Leu = Leuconostoc, Strep = Streptococcus, B.t = Brochothrix thermosphacta, Mic = Micrococcus, A.h = Aeromonas hydrophila, M-A = Moraxella - Acinetobacter, E.1 = Enterobacter liquefaciens, H.a = Hafnia alvei, Ps = Pseudomonas, A.p = Alteromonas putrefaciens.
[b] Each figure represents the mean of three steaks.

Table 3—Aerobic plate counts and microflora of steaks vacuum-packaged in medium-oxygen barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 0 days

Display time (days)	Display temp (C)	APC[b] (log/cm^2)	Percentage distribution of microflora[a,b]														
			Cor	Het. Lac	Hom. Lac	Unid. Lac	Leu	Strep	B.t	Mic	A.h	Flav	E.1	H.a	P.m	Ps	Unid. G-
0	2	2.32							36.1	63.9							
	7	2.08	41.7	8.3					16.7	8.3						25.0	
5	2	5.28		23.2	52.0		0.4		22.7		0.3					1.4	
	7	6.15		42.7	16.7		1.0	11.3	16.3		1.4					10.6	
10	2	6.57		63.2	29.7		1.7		2.7		0.9	0.9				0.9	
	7	7.45		55.9	32.7				7.7		3.0			0.5		0.2	
15	2	7.63		36.3	12.3	29.5			19.3							2.6	
	7	7.52		21.9	34.1	37.8	1.2		0.7		1.6					1.2	1.5
20	2	7.56		67.8	22.0		5.9		3.5		0.3			0.5			
	7	7.48		72.4	0.6		21.2				3.4				2.4		
30	2	7.98		18.7	25.8	33.8	13.1		0.5		1.2					6.9	
	7	7.71		20.1	40.6		8.6						0.5	30.2			

[a] P.m = Proteus morganii, Unid. G- = Unidentified gram-negative microorganisms; for other abbreviations see footnotes to Tables 1 and 2..

tive, homofermentative and unidentified lactobacilli, comprised more than 50% of the microflora of all samples displayed for 5–30 days. Heterofermentative *Lactobacillus* spp. were dominant (≥50%) in four of ten samples and constituted a considerable part (25–49%) of the microflora in five more of the ten samples. Homofermentative *Lactobacillus* spp. were a considerable part of the microflora on four of ten of the displayed samples (at 5 and 10 days of display). Other microbial types present on displayed steaks (at 5–30 days) were, *Leuconostoc* spp. (six of ten samples), *B. thermosphacta* (six of ten samples), *Aeromonas hydrophila* (five of ten samples), *Pseudomonas* spp. (three of ten samples), *Streptococcus* spp. (two of ten samples), *Moraxella-Acinetobacter* spp. (two of ten samples), *Enterobacter liquefaciens* (two of ten samples), *Hafnia alvei* (one of ten samples), and *Alteromonas putrefaciens* (one of ten samples). Differences in display temperature of steaks (2 vs 7°C) did not cause any consistent differences in the distribution of the microflora of the steaks.

Increases in log APC of steaks vacuum-packaged in medium oxygen-barrier film and displayed for 30 days at 2°C and 7°C were 5.66 and 5.63, respectively (Table 3). In two of five comparisons, after display, log APC of steaks displayed at 7°C were greater (0.87–0.88) than those of steaks displayed at 2°C; in the other cases, counts differed only slightly. *Lactobacillus* spp. were a dominant part of the microflora of all steaks displayed for 5–30 days. Other microbial types isolated from these steaks included *Leuconostoc* spp., *B. thermosphacta* and *A. hydrophila*, each in eight of ten samples. *Pseudomonas* spp. (seven of ten samples), *H. alvei* (three of ten samples), *Streptococcus* spp., *Flavobacterium* spp., *E. liquefaciens*, *Proteus morganii* and unidentified gram-negative bacteria, each in one of ten samples. No consistent differences in the distribution of the microflora of the samples because of differences in display temperature (2 vs 7°C) could be detected.

A comparison of the microflora of steaks packaged in high (Table 2) or medium oxygen-barrier (Table 3) film and displayed for 5–30 days at 2 or 7°C showed that heterofermentative *Lactobacillus* spp. were 25% or more of the microflora in nine of ten samples of steaks packaged in high oxygen-barrier film and in six of ten samples packaged in medium oxygen-barrier film, whereas homofermentative *Lactobacillus* spp. were 25% or more of the microflora in four of ten samples packaged in high oxygen-barrier film and in six of ten samples packaged in medium oxygen-barrier film. This suggests a somewhat greater activity of homofermentative *Lactobacillus* spp. on steaks packaged in medium oxygen-barrier as compared with high oxygen-barrier film. In addition, *Pseudomonas* spp., *A. hydrophila*, *Leuconostoc* spp., *B. thermosphacta*, and *H. alvei* were more frequently isolated from the medium oxygen-barrier packaged steaks than from the high oxygen-barrier packaged steaks (5–30 days); frequencies of isolation were 7 vs 3, 8 vs 5, 8 vs 6, 8 vs 6, and 3 vs 1, respectively. APC of steaks packaged in PVC, high or medium oxygen-barrier film and displayed at 2°C exceeded 10^6 cfu per cm^2 at 6, 10 and 10 days, respectively, and, when steaks were displayed at 7°C, at 6, 10 and 5 days, respectively.

Aerobic plate counts and microbial types of steaks prepared from vacuum-packaged loins which had been stored for 12 days at 2 ± 1°C are presented in Tables 4–6. Initial log APC of steaks from loins stored for 12 days at 2 ± 1°C (2.36–3.64) were slightly higher than log APC of steaks

Table 4—Aerobic plate counts and microflora of steaks wrapped in PVC and displayed for up to 6 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 12 days

Display time (days)	Display temp (C)	APC[b] (log/cm²)	Percentage distribution of microflora[a,b]									
			Cor	Het. Lac	Hom. Lac	B.t	Mic	M-A	E.1	Ps	A.p	Unid. G-
0	2	2.36	15.0	16.7	33.3	22.2		4.2			8.6	
	7	2.36	22.1	19.0			43.0	13.9			2.0	
2	2	3.81		31.3	54.1	2.3	2.6				9.7	
	7	5.67		8.9	45.1	23.8				17.5	4.7	
4	2	6.70		84.9		8.4		3.0		0.6		3.1
	7	7.83	27.2			16.9		24.2		31.7		
6	2	6.79		41.2		0.1		13.6	0.1	41.3	3.5	0.2
	7	8.82		26.4		6.7				66.9		

[a] Abbreviations are described in footnotes of Tables 2 and 3.
[b] Each figure represents the mean of three steaks.

Table 5—Aerobic plate counts and microflora of steaks vacuum-packaged in high-oxygen barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 12 days

Display time (days)	Display temp. (C)	APC[b] (log/cm²)	Percentage distribution of microflora[a,b]															
			Cor	Het. Lac	Hom. Lac	Unid. Lac	Leu	Strep	B.t	Mic	A.h	M-A	E.1	H.a	Ps	A.p	Unid. G–	Y
0	2	2.66	4.8	52.4		11.1		11.1		3.2		12.7			1.6			3.1
	7	2.66	13.6	46.3	27.4		0.9			4.2		3.0			2.4	1.9		0.3
5	2	5.89		98.3	1.1											0.6		
	7	7.00		98.1	1.4								0.2			0.2	0.1	
10	2	6.94		88.6	10.6											0.8		
	7	7.40		59.4	31.2	1.1								8.3				
15	2	6.99		99.8												0.2		
	7	7.49		38.7	43.1	1.8			0.9		0.9			14.6				
20	2	6.91		97.3									2.7					
	7	7.51		32.9	53.4		8.9							4.8				
30	2	7.52		87.8	12.2													
	7	7.93		41.0	50.6	0.5	4.6							3.3				

[a] Y = yeasts, other abbreviations are described in footnotes of Tables 2 and 3.
[b] Each figure represents the mean of three steaks.

from loins stored for 0 days (<1.00–2.32). Initial APC of steaks packaged with medium oxygen-barrier film were somewhat higher than those packaged in PVC or high oxygen-barrier film. Increases in log APC of steaks displayed for 6 days in PVC at 2 or 7°C were 4.43 and 6.46, respectively (Table 4). In all comparisons, log APC of steaks displayed for 2–6 days at 7°C were greater (1.13–2.03) than those of steaks displayed at 2°C. The initial microflora of steaks consisted of Coryneform bacteria, hetero- and homofermentative *Lactobacillus* spp., *B. thermosphacta, Micrococcus* spp., *Moraxella-Acinetobacter* spp. and *A. putrefaciens. Lactobacillus* spp. on PVC steaks comprised a dominant part (≥50%) of the microflora in three of six samples and a considerable part of the flora (25–49%) in the other three samples. In general, *Lactobacillus* spp. alone (three of six samples), *Lactobacillus* spp. plus *Pseudomonas* spp. (2 of 6 samples) or *Pseudomonas* spp. alone (one of six samples) dominated the microflora of PVC-wrapped steaks displayed for 2–6 days. Compared with steaks from loins stored for 0 days, (Table 1) the microflora of PVC steaks from loins stored for 12 days (Table 4) at 2 ± 1°C showed an increased dominance of *Lactobacillus* spp. and a reduced significance of *B. thermosphacta* and *Pseudomonas* spp. when steaks were displayed for 2–6 days. In addition to *Lactobacillus* spp., PVC-wrapped steaks displayed for 2–6 days contained *B. thermosphacta, Micrococcus* spp., *Moraxella-Acinetobacter* spp., *E. liquefaciens, Pseudomonas* spp., *A. putrefaciens* and unidentified gram-negative bacteria.

Increases in log APC of steaks vacuum-packaged in high oxygen-barrier film and displayed for 30 days at 2 or 7°C were 4.86 and 5.27, respectively (Table 5). In five of five comparisons, log APC of steaks displayed for 5–30 days at 7°C were greater (0.41–1.11) than log APC of steaks displayed at 2°C. *Lactobacillus* spp. completely dominated the microflora of the samples displayed for 5–30 days. In those steaks, heterofermentative species were dominant in seven of ten samples and made up a considerable part of the microflora in the other three samples. Homofermentative species were a dominant part of the microflora in two of ten samples and constituted a considerable part of the flora in two other samples. Other isolates from these steaks included *H. alvei* (four of ten samples), *A. putrefaciens* (four of ten samples), *Leuconostoc* spp. (two of ten samples, *E. liquefaciens* (two of ten samples), and *B. thermosphacta, A. hydrophila,* and unidentified gram-negative bacteria each in one of ten samples.

Increases in log APC of steaks vacuum-packaged in medium oxygen-barrier bags and displayed for 30 days at 2 or 7°C were 3.54 and 4.29, respectively (Table 6). In five of five comparisons, log APC of steaks displayed for 5–30 days at 7°C were greater (0.43–1.05) than those of steaks displayed at 2°C. During display, *Lactobacillus* spp., particularly heterofermentative species, became predominant. Other isolates from these steaks included *E. liquefaciens* (nine of ten samples), *A. putrefaciens* (seven of ten samples), *Leuconostoc* spp. and *H. alvei* each in five of ten samples, *B. thermosphacta, A. hydrophila* and *Pseudomonas* spp. each in three of ten samples and *Streptococcus* spp. (two of ten samples). A comparison of the microflora of steaks packaged in medium oxygen barrier vs high oxygen-barrier films and displayed for 5–30 days at both 2 and 7°C showed (a) a slightly increased dominance of heterofermentative *Lactobacillus* spp. in medium oxygen-barrier packaged steaks (dominant in nine of ten samples) as compared with steaks in high oxygen-barrier film (dominant in seven of ten samples) and (b) increased frequency of isolation of certain gram-negative species in steaks packaged in medium oxygen-barrier bags as compared to steaks in high oxygen-barrier film (for example, *E. liquefaciens*–nine vs two samples; *A. putrefaciens*–seven vs four samples; *A. hydrophila*–three vs one sample; and *Pseudomonas* spp.–three vs zero samples). APC of steaks packaged in PVC, high oxygen-barrier or medium oxygen-barrier films and displayed at 2°C exceeded 10^6 cfu per cm^2 at 4, 10 and 5 days, respectively, and, when displayed at 7°C, at 4, 5 and 5 days, respectively. No consistent differences in microflora of steaks packaged in PVC, high or medium oxygen-barrier films could be attributed to differences in display temperature (2 vs 7°C).

Aerobic plate counts and microbial types of steaks from vacuum-packaged loins which had been stored for 24 days at 2 ± 1°C are presented in Tables 7–9. Initial APC of steaks from these loins were about 2 logs higher than those of steaks prepared from loins stored for 0 days. Increases in log APC of steaks displayed for 6 days in PVC at 2 or 7°C were 5.03 and 6.00, respectively (Table 7). In three of three comparisons, log APC of steaks displayed for 2–6 days at 7°C was greater (0.84–1.43) than log APC of steaks displayed at 2°C. The initial microflora of the PVC-wrapped steaks was dominated by heterofermentative *Lactobacillus* spp.; this undoubtedly resulted from storage of the loins in vacuum packages for 24 days at 2 ± 1°C. During display of the PVC steaks for 2–6 days at 2 or 7°C, heterofermentative *Lactobacillus* spp. continued to be a part of the microflora of the steaks but their significance was greatly reduced–in only two of six samples did they comprise more than 25% of the microflora. On the other hand,

Table 6–Aerobic plate counts and microflora of steaks vacuum-packaged in medium-oxygen barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 12 days

Display time (days)	Display temp (C)	APC[b] (log/cm²)	Percentage distribution of microflora[a,b]															
			Cor	Het. Lac	Hom. Lac	Unid. Lac	Leu	Strep	B.t	Mic	A.h	Flav	M-A	E.1	H.a	Ps	A.p	Y
0	2	3.64	15.8	14.5						15.7		0.5	11.8			12.7	27.5	1.5
	7	3.46	26.8	2.1	25.0					7.6		0.3	33.7			1.3	3.2	
5	2	6.48		87.4	1.5						0.9			2.9		2.3	5.0	
	7	7.53		95.8	1.1				0.3		0.4			1.4	0.3	0.3	0.4	
10	2	6.57	0.8	89.6		2.3	0.6	0.6						0.7			5.4	
	7	7.00		95.2	0.2	1.4		1.8	0.4					0.4			0.6	
15	2	6.84		89.5	0.2		6.1				0.2			2.4			1.6	
	7	7.61		51.4	31.5	2.3	0.8		0.9					1.5	11.6			
20	2	7.20		75.9	21.7									0.1			2.3	
	7	7.63		36.4	52.1	2.3	7.6								0.4	1.2		
30	2	7.18		56.4	28.1		14.2							0.3	1.0			
	7	7.75		54.5	35.1	7.6								0.7	2.0		0.1	

[a] Abbreviations are described in footnotes of Tables 1, 2 and 5.
[b] Each figure represents the mean of three steaks.

Pseudomonas spp. which were a small part of the initial microflora (4.5–4.8%) were a dominant part of the microflora on five of six samples and made up a considerable part of the microflora of the other sample. With counts of PVC-wrapped steaks either approaching (when displayed at 2°C) or exceeding (when displayed at 7°C) 10^6 cfu per cm^2 after 2 days of refrigerated display, the high percentage of *Pseudomonas* spp. and the continued presence of *A. putrefaciens* (at 2–4 days) are of concern from the standpoint of maintaining quality. Compared with steaks fabricated from loins stored for 12 days at 2 ± 1°C, the microflora of PVC steaks from loins stored for 24 days showed an increased significance of *Pseudomonas* spp. and a reduced significance of heterofermentative *Lactobacillus* spp. In addition to *Pseudomonas* and *Lactobacillus* spp., PVC-wrapped steaks contained *B. thermosphacta, Moraxella-Acinetobacter* spp., *E. liquefaciens,* Coryneform bacteria and *A. putrefaciens.*

Increases in log APC of steaks vacuum-packaged in high oxygen-barrier film and displayed for 30 days at 2°C or 7°C were 4.72 and 4.66, respectively. In four of five comparisons, log APC of steaks displayed for 5–30 days at 7°C were slightly greater (0.10–0.98) than those of steaks displayed at 2°C; in the other case, counts were the same (Table 8). Increases in log APC of steaks packaged in medium oxygen-barrier film and displayed for 30 days at 2 or 7°C were 3.38 and 3.96, respectively (Table 9); in four of five comparisons, after display, log APC of steaks displayed at 7°C were slightly greater (0.04–0.54) than those of steaks displayed at 2°C. The microflora of steaks packaged in high or medium oxygen-barrier film from loins stored for 24 days at 2 ± 1°C was dominated by *Lactobacillus* spp., in most cases by heterofermentative species. Other isolates from samples displayed at 2 or 7°C included: from high oxygen-barrier packaged steaks, *E. liquefaciens* (eight of ten samples), *H. alvei* (six of ten samples) and *Leuconostoc* spp. and *A. putrefaciens* (each in one of ten samples); from medium oxygen-barrier packaged steaks, *E. liquefaciens* and *H. alvei* (each in eight of ten samples), *A. putrefaciens* (five of ten samples), *Leuconostoc* and *Pseudomonas* spp. (each in two of ten samples), and *A. hydrophila* and *Moraxella-Acinetobacter* spp. (each in one of ten samples). A comparison of the microflora of medium vs high oxygen-barrier packaged steaks displayed for 5–30 days at 2 or 7°C showed a greater frequency of isolation of gram-negative types such as *A. putrefaciens* (5 vs 1), *Pseudomonas* spp. (2 vs 0), *H. alvei* (8 vs 6), *A. hydrophila* (1 vs 0) and *Moraxella-Acinetobacter* spp. (1 vs 0) from steaks packaged in medium oxygen-barrier film. No consistent differences in microflora of steaks, regardless of packaging treatment, could be attributed to differences in display temperature (2°C vs 7°C). Counts of PVC-wrapped steaks displayed at 2 or 7°C exceeded 10^6 cfu per cm^2 at 4 and 2 days, respectively. Counts of steaks packaged in high or medium oxygen-barrier film exceeded 10^6 cfu per cm^2 at 5 days of display. Counts at 30 days of display of high and medium oxygen-barrier packaged steaks from loins that were stored for 24 days were only slightly different (0.04–0.46) from counts of comparable steaks from loins that were stored for 12 days at 2 ± 1°C. In most cases, counts of PVC-wrapped steaks after 6 days of refrigerated display were higher than those of steaks packaged with high or medium oxygen-barrier film and displayed for 30 days at 2°C or 7°C.

Table 7—Aerobic plate counts and microflora of steaks wrapped in PVC and displayed for up to 6 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 24 days

Display time (days)	Display temp. (C)	APC[b] (log/cm^2)	Percentage distribution of microflora[a,b]								
			Cor	Het. Lac	Hom. Lac	Unid. Lac	B.t	M-A	E.1	Ps	A.p
0	2	3.20		90.0	0.3			0.3		4.5	4.9
	7	3.45		89.7			0.8			4.8	4.7
2	2	5.61	1.8	25.7				1.8		68.9	1.8
	7	6.45		20.3		9.7	23.4			43.2	3.4
4	2	6.91		16.8		4.4	5.5			66.8	6.5
	7	8.34		29.8			0.3		14.3	55.0	0.6
6	2	8.23		17.9			1.9			80.2	
	7	9.45		20.9	0.5				16.6	62.0	

[a] Abbreviations are described in footnote of Table 2.
[b] Each figure represents the mean of three steaks.

Table 8—Aerobic plate counts and microflora of steaks vacuum-packaged in high-oxygen barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 24 days

Display time (days)	Display temp (C)	APC[b] (log/cm^2)	Percentage distribution of microflora[a,b]										
			Cor	Het. Lac	Hom. Lac	Unid. Lac	Leu	B.t	A.h	M-A	E.1	H.a	Ps	A.p
0	2	2.98	1.0	90.1						0.6			6.7	1.6
	7	3.23		86.9	0.2			0.2	0.2	1.8			5.7	5.0
5	2	6.28		100.0										
	7	7.26		94.2	2.9						0.6	2.3		
10	2	7.38		95.8	3.6						0.5			0.1
	7	7.48		63.9	17.6						4.2	14.3		
15	2	7.30		91.8	8.0						0.2			
	7	7.67		67.1	9.8							23.1		
20	2	7.42		76.5	14.5	0.8					0.2	8.0		
	7	7.42		42.8	39.2						5.4	12.6		
30	2	7.70		47.3	52.0						0.7			
	7	7.89		29.3	59.1		1.9				2.5	7.2		

[a] Abbreviations are described in footnote of Table 2.
[b] Each figure represents the mean of three steaks.

The 885 *Lactobacillus* or *Leuconostoc* isolates from this study either resembled or fit the characteristics of the following nine species: *Lactobacillus cellobiosus, Lactobacillus viridescens, Lactobacillus coryneformis, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus xylosus, Leuconostoc mesenteroides, Leuconostoc paramesenteroides* and *Leuconostoc dextranicum.* Unidentified lactobacilli were *Lactobacillus* isolates which were not further identified to the species level.

The most frequently isolated heterofermentative *Lactobacillus* sp. was cellobiose+, mannitol+ or −, produced NH_3 from arginine and CO_2 from glucose. Among the mannitol+ isolates, the sugar fermentation pattern was more like that of *L. plantarum*; among the mannitol− isolates it was more like that of *L. curvatus*. Neither *L. plantarum* nor *L. curvatus* should produce gas from glucose. *L. cellobiosus* is the only gas-producing *Lactobacillus* that ferments cellobiose and is dextran−. A majority of the present isolates are melezitose+. *Lactobacillus buchneri* is the only gas-producing *Lactobacillus* that ferments melezitose, but is also cellobiose− and amygdalin−, whereas in this study the isolates were cellobiose+ and amygdalin+. We have called these heterofermentative *Lactobacillus* isolates resembling *L. cellobiosus* and recognized two groups with the following characteristics: group 1—esculin+, NH_3 from arginine (2% glucose, at 14 days)−, mannitol+, melezitose+, melibiose+ or −, raffinose+ or − and sorbitol+; group 2—esculin+, NH_3 from arginine (2% glucose, at 3–7 days)+, mannitol−, melezitose+ or −, melibiose−, raffinose− and sorbitol−. These isolates were present on 199 of 216 vacuum-packaged steaks (high and medium oxygen-barrier film) and on 44 of 72 steaks packaged in PVC. On 39 of the vacuum-packaged steaks, these isolates constituted 25–49% of the microflora; for 128 samples, these isolates comprised 50% or more of the microflora. In 24 of the PVC-wrapped steaks these isolates made up 25% or more of the microflora.

A second heterofermentative *Lactobacillus* sp. resembled *L. viridescens* except that the isolates were cellobiose+ and galactose+. It is unlikely that these organisms are more closely related to *L. paramesenteroides* than to *L. viridescens* because of their reactions to cellobiose, lactose, mannitol, melibiose, raffinose, and xylose. This organism was isolated from 10 of 216 vacuum-packaged steaks and from 1 of 72 steaks packaged in PVC.

The most frequently isolated homofermentative *Lactobacillus* resembled *L. coryneformis*, except that the isolates were arabinose+, mannitol−, and ribose+. These isolates were present on 80 of 216 vacuum-packaged steaks, for 15 steaks they constituted between 25–49% and for 13 steaks

Table 9—Aerobic plate counts and microflora of steaks vacuum-packaged in medium oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 24 days.

Display time (days)	Display temp (C)	APC[b] (log/cm^2)	Percentage distribution of microflora[a,b]										
			Het. Lac	Hom. Lac	Unid. Lac	Leu	B.t	A.h	M-A	E.1	H.a	Ps	A.p
0	2	4.26	67.1		11.2				0.6			6.1	15.0
	7	3.88	73.5	19.3			0.2	0.1	0.1			4.5	2.3
5	2	6.90	74.6	1.5	22.9					0.3		0.4	0.3
	7	6.76	90.4	7.8				0.2		1.3			0.3
10	2	7.34	93.5	5.8						0.1	0.5		0.1
	7	7.38	75.8	8.5							15.7		
15	2	7.26	93.4	3.5	2.1					0.2	0.8		
	7	7.80	65.2	14.2					0.6	1.7	18.0		0.3
20	2	7.30	87.3	6.6	1.6	0.2					2.7	0.3	1.3
	7	7.78	42.3	22.6	24.8					4.6	5.7		
30	2	7.64	40.2	36.1		18.7				3.4	1.6		
	7	7.84	20.1	68.2						1.4	10.3		

[a] Abbreviations are described in footnote of Table 2.
[b] Each figure represents the mean of three steaks.

Table 10—Percentage of Lactobacillus spp. on steaks wrapped in PVC and displayed for up to 6 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for either 0, 12 or 24 days at 2 ± 1°C

Display time (days)	Display temp (C)	Percentage of *Lactobacillus* spp. on steaks prepared from loins stored for[a,b]										
		0 days			12 days				24 days			
		Het.[c]		Hom.[d]	Het.			Hom.	Het.		Hom.	
		L. cel. 1	L. cel. 2	L. cur.	L. cel. 1	L. cel. 2	L. vir.	L. cur.	L. cel. 1	L. cel. 2	L. cor.	Un.
0	2					16.7		33.3	26.8	63.1	0.3	
	7				1.4	17.5			18.9	70.9		
2	2	0.8	31.0		11.2		20.1	54.1	9.2	16.5		
	7		0.5			8.9		45.1	5.3	14.9		9.7
4	2				16.4	68.5			0.4	16.5		4.4
	7				1.4	25.8			1.4	28.4		
6	2				1.6	39.5				17.9		
	7			0.7	1.1	25.4			1.0	19.8	0.5	

[a] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks).
[b] L. cel. 1 = L. cellobiosus 1; L. cel. 2 = L. cellobiosus 2; L. cur. = L. curvatus; L. vir. = L. viridescens; L. cor. = L. coryneformis; Un. = Unidentified.
[c] Het. = Heterofermentative
[d] Hom. = Homofermentative

they comprised 50% or more of the microflora. They were also present in two steaks packaged in PVC.

A second group of homofermentative *Lactobacillus* sp. fit the characteristics of *L. curvatus*; these isolates were present on 44 of 216 vacuum-packaged steaks. For 12 steaks they constituted 25–49% of the microflora; for 13 steaks they comprised 50% or more of the microflora. They were present on seven of 72 steaks packaged in PVC and on six steaks they constituted 25% or more of the microflora.

Another group of homofermentative *Lactobacillus* sp. either fit or resembled closely the characteristics of *L. plantarum*. Some of the isolates were rhamnose+, others mannitol, raffinose and sorbitol–, but otherwise they adhered to the characteristics of *L. plantarum*. These isolates were present on 23 of 216 vacuum-packaged steaks; on two steaks they constituted 25–49% of the microflora, on three steaks they comprised 50% or more of the microflora.

For three of 216 vacuum-packaged steaks, isolates resembling *L. xylosus* were isolated. These isolates fit all characteristics of *L. xylosus* except that they were mannitol– and sorbitol+.

For 15 of 216 vacuum-packaged steaks, *L. mesenteroides* was isolated and this organism constituted from 0.5–25.8% of the microflora; however, none was isolated from steaks packaged in PVC.

Isolates resembling *L. paramesenteroides* were isolated from 23 of 216 vacuum-packaged steaks. They fit all characteristics of *L. paramesenteroides* except that they did not grow at 37°C. For two steaks this organism constituted 25–49% of the microflora, for four steaks they comprised 50% or more of the microflora.

An isolate with all of the characteristics of *L. dextranicum*, except that it did not ferment lactose, was recovered from one of 216 vacuum-packaged steaks.

We have refrained from calling our isolates simply "atypical" streptobacteria, betabacteria or *Leuconostoc* spp. because we feel a great need for enlarging the knowledge of specific characteristics of lactic acid bacteria from more recently investigated products such as meats, poultry and fish that have been packaged in oxygen-impermeable films or in modified gaseous atmospheres.

Information on the varous types of *Lactobacillus* and *Leuconostoc* spp. on steaks packaged with PVC, high oxygen-barrier or medium oxygen-barrier film from loins that were stored for 0, 12 or 24 days at 2 ± 1°C is presented in Tables 10–16. In PVC-wrapped steaks (Table 10) from

Table 11—Percentage of Lactobacillus and Leuconostoc spp. on steaks vacuum-packaged in high oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 0 days at 2 ± 1°C.

		Percentage of *Lactobacillus* or *Leuconostoc* spp.[a,b,c]								
		Het. Lac			Hom. Lac			Lac	Leu	
Display time (days)	Display temp. (C)	L. cel. 1	L. cel. 2	L. vir.	L. cor.	L. cur.	L. plan.	Un.	L. mes.	L. pm.
0	2									
	7									
5	2	0.2	18.1	3.5		45.7				
	7	22.2	21.4	2.5		24.5	1.3			
10	2	5.4	26.6		3.4	40.1	5.2		0.8	16.8
	7	18.1	38.4	9.8		24.6	1.0		1.7	5.2
15	2	2.0	33.3		0.5		1.5	60.9		
	7	16.7	63.5		3.1			16.7		
20	2		83.6		14.9			1.2	0.2	
	7		45.4		11.1		1.8	33.5	1.8	2.9
30	2	4.1	47.7				0.4	1.3		46.0
	7		28.6		6.1		3.0	17.1		33.0

[a] Het. Lac = Heterofermentative Lactobacillus, Hom. Lac = Homofermentative Lactobacillus, Lac = Lactobacillus, Leu = Leuconostoc
[b] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks)
[c] L. cel. 1 = L. cellobiosus 1; L. cel. 2 = L. cellobiosus 2; L. vir. = L. viridescens; L. cor. = L. coryneformis; L. cur. = L. curvatus; L. plan. = L. plantarum; L. mes. = L. mesenteroides; L. pm. = L. paramesenteroides; Un. = Unidentified.

Table 12—Percentage of Lactobacillus and Leuconostoc spp. on steaks vacuum-packaged in medium oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 0 days at 2 ± 1°C.

		Percentage of *Lactobacillus* or *Leuconostoc* spp.[a,b]									
		Het. Lac			Hom. Lac				Lac.	Leu	
Display time (days)	Display temp (C)	L. cel. 1	L. cel. 2	L. vir.	L. cor.	L. cur.	L. plan.	L. xyl.	Un.	L. mes.	L. pm.
0	2										
	7		8.3								
5	2	1.8	21.4			52.0				0.4	
	7		42.7		1.4	15.3				1.0	
10	2	5.4	43.5	14.3	8.9	16.6	2.9	1.4		1.7	
	7	7.0	44.0	4.8		32.7					
15	2	8.6	27.7		1.9	3.6	6.9		29.5		
	7	18.0	3.9		15.8	18.3			37.8	1.2	
20	2	8.6	59.2		22.0					5.9	
	7	25.4	47.0		0.6						21.2
30	2		18.7				25.4	0.4	33.8	5.5	7.6
	7		20.1		31.6		7.9	1.1		1.2	7.4

[a] L. xyl. = L. xylosus, other abbreviations are described in footnotes of Table 11.
[b] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks).

loins that were stored for 12 or 24 days, the heterofermentative *L. cellobiosus*, particularly group 2, was the most significant among the *Lactobacillus* spp. Homofermentative *L. curvatus* and *L. coryneformis* and the heterofermentative species *L. viridescens* were much less frequently isolated. *Lactobacillus* spp. were not often isolated from PVC-wrapped steaks fabricated from loins that were stored for 0 days at 2 ± 1°C. *L. cellobiosus, L. viridescens, L. coryneformis, L. curvatus, L. plantarum, L. xylosus* (medium oxygen-barrier film only), *L. mesenteroides* and *L. paramesenteroides* were isolated from steaks packaged with high and medium oxygen-barrier film that were fabricated from loins that were stored for 0 days at 2 ± 1°C (Tables 11, 12). *L. cellobiosus*, particularly group 2, constituted a considerable (25–49%) or dominant part (≥50%) of the microflora more often than did other *Lactobacillus* and *Leuconostoc* spp.

The *Lactobacillus* and *Leuconostoc* spp. isolated from steaks packaged with high and medium oxygen-barrier films that were fabricated from loins that were stored for 12 or 24 days at 2 ± 1°C (Tables 13–16) were similar to those described for comparable steaks from loins that were stored for 0 days except for the presence of *L. dextranicum* (high oxygen-barrier film, from loins stored 12 days). The heterofermentative *L. cellobiosus*, particularly group 2, was a more dominant part of the microflora of vacuum packaged steaks from loins that were stored for 12 and 24 days at 2 ± 1°C than it was for comparable steaks fabricated from loins that were stored for 0 days at 2 ± 1°C.

The results of the present study show that *Pseudomonas* spp. comprised a considerable or dominant part of the microflora of steaks displayed in PVC for 2–6 days at 2°C or 7°C. This was particularly true for the steaks cut from vacuum-packaged loins that had been stored for 0 or 24 days at 2 ± 1°C. *B. thermosphacta* was present in nearly all steaks wrapped in PVC and displayed for 2–6 days and appeared as a dominant part of the microflora in 50% of the steaks cut from loins that had been stored for 0 days.

The increased frequency of isolation of *Lactobacillus* spp. in PVC-wrapped steaks from vacuum-packaged loins that were stored at 2 ± 1°C for 12 and 24 days is not surprising; numerous studies have shown that under those conditions aerobic, psychrotrophic, gram-negative bacteria are suppressed and that facultative anaerobes such as lactic acid bacteria develop. Changes in film permeability and gaseous atmosphere in the package caused rapid changes in

Table 13—Percentage of Lactobacillus and Leuconostoc spp. on steaks vacuum-packaged in high oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 12 days at 2 ± 1°C

		Percentage of *Lactobacillus* or *Leuconostoc* spp.[a,b]							
		Het. Lac			Hom. Lac		Lac	Leu	
Display time (days)	Display temp (C)	L. cel. 1	L. cel. 2	L. vir.	L. cor.	L. cur.	Un.	L. dex.	L. pm.
0	2	6.4	46.0				11.1		
	7	11.1	33.1	2.1		27.4		0.9	
5	2	0.2	98.1		1.1				
	7	5.3	92.8		1.4				
10	2	4.9	83.7		10.6				
	7	6.3	53.1		8.3	22.9	1.1		
15	2	3.4	96.4						
	7	0.9	37.9		43.1		1.8		
20	2	5.3	92.0						
	7	1.5	31.4		53.4				8.9
30	2	5.4	82.4			12.2			
	7	0.2	40.8			50.6	0.5		4.6

[a] L. dex. = L. dextranicum, other abbreviations are described in footnotes of Table 11.
[b] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks).

Table 14—Percentage of Lactobacillus and Leuconostoc spp. on steaks vacuum-packaged in medium oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 12 days at 2 ± 1°C

		Percentage of *Lactobacillus* or *Leuconostoc* spp.[a,b]							
		Het. Lac		Hom. Lac			Lac	Leu	
Display time (days)	Display temp (C)	L. cel. 1	L. cel. 2	L. cor.	L. cur.	L. plan.	Un.	L. mes.	L. pm.
0	2	10.6	3.9						
	7	1.1	1.1		25.0				
5	2	21.9	65.5	1.5					
	7	6.9	89.0	1.1					
10	2	14.0	75.6				2.3	0.6	
	7	4.2	91.1	0.2			1.4		
15	2	20.1	69.3	0.2					6.1
	7	4.7	46.7	31.5			2.3	0.8	
20	2	4.5	71.4			21.7			
	7	0.7	35.7	33.2	19.0		2.3	6.3	1.3
30	2	2.9	53.5	28.1				8.6	5.6
	7	0.9	53.6	35.1			7.6		

[a] Abbreviations are described in footnotes of Table 11.
[b] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks).

the microflora of the steaks. This is illustrated by the differences in microflora of freshly fabricated and displayed steaks prepared from vacuum-packaged loins that were stored for 24 days at 2 ± 1°C (Table 7). The initial microflora of the steaks was dominated by heterofermentative *Lactobacillus* spp. Refrigerated display of these steaks in PVC for 2–6 days at 2 or 7°C resulted in dominance of *Pseudomonas* spp. Although initial log APC of PVC wrapped steaks from vacuum-packaged loins that were stored for 12 and 24 days at 2 ± 1°C were low (2.36–3.45 per cm^2), counts of PVC-wrapped steaks after 4 days of refrigerated display exceeded 10^6 per cm^2. *Lactobacillus* spp. clearly dominated the microflora of vacuum-packaged steaks from vacuum-packaged loins that had been stored for 0, 12 or 24 days at 2 ± 1°C. Although both hetero- and homofermentative species were isolated, heterofermentative species were more dominant on steaks from loins stored for 12 or 24 days than on these steaks from loins stored for 0 days.

Of the nine types of *Lactobacillus-Leuconostoc* spp. isolated from steaks from all three packaging treatments, *L. cellobiosus* was isolated from 92% of the vacuum-packaged steaks; in 59% of those steaks this organism constituted 50% or more of the microflora. *L. cellobiosus, L. plantarum* and *L. xylosus* have been isolated from refrigerated pork stored in 1 atm CO_2 (Blickstad et al., 1981; Enfors et al., 1979) and *L. plantarum* from beef packaged in O_2-CO_2-N_2 atmospheres (Savell et al., 1981). *L. mesenteroides* and *L. dextranicum* were reported (Christopher et al., 1979) on vacuum-packaged pork roasts. Little is known about the effect of these *Lactobacillus* and *Leuconostoc* spp. on the quality characteristics of beef and pork. Because *Lactobacillus* spp., particularly the heterofermentative types, are dominant on vacuum-packaged steaks, it is tempting to attribute loss of quality characteristics during refrigerated display (Griffin et al., 1982) to metabolic activities of these organisms. Small percentages of certain gram-negative bacteria such as *A. hydrophila, E. liquefaciens, H. alvei, Pseudomonas* spp., and *A. putrefaciens* that are known as potential producers of defects (Gill and Newton, 1978, 1979; Hanna et al., 1979; Nicol et al., 1970; Patterson and Gibbs, 1977) continue to persist in vacuum-packaged steaks. A comparison of the frequency of isolation of these types from steaks packaged in high vs medium oxygen-barrier films showed that these types were isolated more frequently from steaks wrapped with medium oxygen-barrier film (12 of 15 comparisons). This latter phenomenon may be associated with the greater gas permeability of the medium oxygen-barrier film. The importance of these gram-negative

Table 15—Percentage of Lactobacillus and Leuconostoc spp. on steaks vacuum-packaged in high oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 24 days at 2 ± 1°C

		Percentage of *Lactobacillus* or *Leuconostoc* spp.[a,b]						
		Het. Lac		Hom. Lac			Lac	Leu
Display time (days)	Display temp (C)	L. cel. 1	L. cel. 2	L. cor.	L. cur.	L. plan.	Un.	L. p.m.
0	2	46.5	43.6					
	7	36.7	50.2	0.2				
5	2	9.4	90.6					
	7	1.9	92.2	2.0		1.0		
10	2	9.8	86.0	0.4	2.8	0.4		
	7	4.6	59.3	3.6	5.7	8.3		
15	2	1.1	90.8	3.9		4.1		
	7	2.4	64.6	5.9	3.8			
20	2	6.0	70.5	14.5			0.8	
	7	2.5	40.3	36.3		2.9		
30	2		47.3	51.0	1.0			
	7		29.3	47.2	11.9			1.9

[a] Abbreviations are described in footnotes of Table 11.
[b] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks).

Table 16—Percentage of Lactobacillus and Leuconostoc spp. on steaks vacuum-packaged in medium oxygen-barrier film and displayed for up to 30 days at 2°C or 7°C. Steaks were prepared from vacuum-packaged loins stored for 24 days at 2 ± 1°C

		Percentage of *Lactobacillus* or *Leuconostoc* spp.[a,b]							
		Het. Lac			Hom. Lac			Lac	Leu
Display time (days)	Display temp (C)	L. cel. 1	L. cel. 2	L. vir.	L. cor.	L. cur.	L. plan.	Un.	L. pm.
0	2	24.9	42.1					11.2	
	7	38.6	34.9			19.3			
5	2	1.5	73.1		1.5			22.9	
	7	19.6	70.7		5.8		2.0		
10	2	3.7	89.8		5.8				
	7	1.1	72.6	2.2	8.5				
15	2	6.7	86.8		1.6	1.9		2.1	
	7	2.6	62.7		7.1	7.1			
20	2	9.4	77.8		6.6			1.6	0.2
	7	1.3	41.0		9.7	12.9		24.8	
30	2	0.1	40.1		22.2	13.3	0.7		18.7
	7		20.1		14.6	14.7	38.9		

[a] Abbreviations are described in footnotes of Table 11.
[b] Percentages are expressed in terms of the total microflora on countable plates (mean of three steaks).

bacteria in quality deterioration of vacuum-packaged steaks is not clear. The results of the present study suggest the need for information on the effect of specific *Lactobacillus* and *Leuconostoc* spp. and combinations of these lactic acid bacteria and gram-negative bacteria on the shelf-life of vacuum-packaged meat.

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PROFICIENCY TEST FOR PSP . . . From page 1043

Evaluation of individual laboratory performance in terms of repeatability of results on the duplicate samples showed that there were no significant differences between replicate analyses. This indicated that the split samples received by the collaborating laboratories were homogenous. Overall, the within laboratory variance (Sr^2) was lower than the between laboratory variance (Sb^2). The variance of the two levels of mashed potato increased with an increase in the level of toxin. For both levels measured by the collaborators, the mashed potato showed better repeatability on duplicate samples than the spiked clam used by McFarren's naturally toxic and spiked shellfish show nearly exact performance (Fig. 1).

On the basis of overall performance, it may be concluded that mashed potato is as good as natural or spiked shellfish for use as a proficiency test specimen. Since it offers some significant advantages in preparation, it is a satisfactory matrix for laboratory and collaborative studies.

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Sectioned and Formed Product Made from Emulsion-Coated Pork Tissue

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ABSTRACT

Sectioned and formed cured pork tissue was prepared by emulsion coating instead of tumbling. The characteristics and shelf life of emulsion-coated tissue were measured and compared with those of tumbled pork tissue. Both emulsion coated and tumbled pork tissue possessed similar yields (90%), maintained good sliceability and had a high overall sensory acceptance. Emulsion-coated samples were superior in flavor and textural appeal but tumbled samples had a slightly better cohesiveness score. Like tumbled samples, emulsion-coated samples were quite stable (TBA and bacterial number) in a shrunken, vacuum packaged Cryovac bag during holding in a 3°C cooler through 8 wk of storage.

INTRODUCTION

THE TUMBLING or massaging of meat tissue is rapidly gaining acceptance in the meat industry. In both processes, the meat pieces are subjected to a number of mechanical forces. In tumbling the forces are primarily impact forces developed when meat pieces fall in a rotating tumbler (Krause et al. 1978a). Massaging, on the other hand, involves primarily frictional forces between the meat pieces (Theno et al., 1977). The effects of tumbling and massaging seem to be quite similar.

Much of the economic advantage of tumbling or massaging is due to the increased cooking yield (Krause et al., 1978a) compared to normally processed ham. Rahelic et al. (1974) also found that tumbling reduced the cooking shrinkage of cured canned pork.

Macfarlane et al. (1977) compared the binding abilities of three muscle protein fractions and found that myosin showed the best binding strength in samples with salt present. Actomyosin resulted in an intermediate binding strength value and sarcoplasmic proteins had a minimal binding strength value. Ockerman et al. (1978) found cohesiveness scores of cured semimembranosus muscles were significantly increased in continuous tumbling for 30 min. This indicates that in only 30 min of tumbling, sufficient exudate is extracted from the muscle to improve the binding of adjacent surfaces. As the massage time increased, the fat and protein content of the exudate increased resulting in improved binding properties of cooked ham with addition of salt and/or phosphate (Siegel et al. 1978).

Another of the functions of meat tumbling is to aid in the distribution of curing ingredients in the product. Krause et al. (1978a, b) found that the migration of salt and nitrite was increased in the semimembranosus muscle by a tumbling treatment. Intermittent tumbling for 18 hr was found to improve external appearance, color, sliceability, taste, aroma and yield of the hams as compared to nontumbled hams held 18 hr. Tumbling was found to be most effective when the fat on the ham was trimmed to 3 mm or less.

Structure change during tumbling was examined by Rahelic et al. (1974) and Cassidy et al. (1978). They found that, during tumbling, a swelling and loosening of the structure of the sarcomere occurs. With prolonged tumbling the clarity of the striation pattern of muscles was reduced suggesting a breaking of the sarcomeres. By using a scanning electron microscope and a light microscope, Theno et al. (1976) found that muscle membranes are disrupted with nuclei migrating to and collecting in the intercellular spaces. The exudate at the binding junction between muscles appeared to be a pseudo emulsion. The microstructure of the binding junctions in massaged, sectioned and formed ham has been examined by Theno et al. (1978) and they found that binding junctions, in ham with 2 or 3% salt, were composed of areas of aligned fibers and areas with emulsion-like characteristics. In general, it was observed that alignment of fibers in the bind sites was associated with good binding or cohesiveness in the massaged product.

Theno et al. (1977) summarized the advantages and disadvantages of tumbling used in the meat industry. The advantages include acceleration of brine dispersion in cured products, enhanced release of salt soluble proteins to produce a creamy, tacky exudate, improved uniformity of cured color and texture, and reduced weight loss during cooking. The process does possess some inherent disadvantages, especially if improper processing procedure is utilized. The mechanical treatment may cause excessive muscle destruction. Insufficient treatment may result in products exhibiting poor slicing characteristics and/or poor cure distribution and color development. Massaging and tumbling are both lengthy procedures that require high initial capital inputs and increased labor.

This study was designed to investigate the effect of emulsion coating on the characteristics of sectioned and formed cured pork and to compare this with a tumbled cured pork product.

MATERIALS & METHODS

Preparation of sectioned and formed cured pork

Relatively inexpensive muscles sectioned from pork picnics were used to prepare the cured pork in this experiment. Conversion of tissue from picnics into sectioned and formed cured pork would be an alternative way to utilize the pork picnic. Also, the picnic is composed of more than 10 pieces of tissue of suitable size and, therefore, would make a good raw material to test the properties of sectioned and formed cured pork prepared by different processing techniques.

Eight pairs of pork picnics were collected and stored in a freezer (−23°C) until used. Before dissection, the picnics were permitted to thaw at 13°C for approximately 36 hours. Picnics then were sectioned and most of the visible connective tissue and fat was removed. The tricep muscle was cut into sections of approximately 200–300g, weighed, and stitch injected with cure to approximately 119% (average) of green weight with a single needle using a pickle containing by weight: 77% water, 16% salt, 3.3% sugar, 3.25% sodium tripolyphosphate, 0.37% sodium erythorbate and 0.08% sodium nitrite. The pumped picnics were allowed to drain 1 min before the weight was recorded. The picnics from the same animal were randomly assigned, one to each of the two processing procedures: tumbling or emulsion coating. Pieces of meat from one picnic were placed in a tumbler (81 x 53 cm) containing three baffles. The tumbler was intermittently (10 min per hr) rotated at 11 rpm for 18 hr in a 3°C cooler. After 18 hr of tumbling, cured tissue was

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stuffed randomly into an 8½" cm diameter, stuck fibrous, cellulose casing (Union Carbide, F 8½) and tightened by hand. The stuffed weight was recorded. The cured tissues were then smoked and cooked for approximately 6 hr according to the schedule in Table 1. When a final internal temperature of 66°C was reached, the cured and cooked tissues were taken from the smokehouse and cooled in a holding cooler (3°C) overnight.

Pumped, drained meat from the other picnic of the pair was placed in a small stainless steel container for mixing and rubbing by hand with 7.5% (by weight) of a meat emulsion for 15 minutes before stuffing. The emulsion was prepared from ground lean meat and 15% pumping pickle, by weight, which was passed twice through an 1/8" (0.32 cm) plate. Coated cured muscles were stuffed into the same type of casing (F 8½), hung in a holding cooler (3°C) for 18 hours and then subjected to the same smoking and cooking schedule (at the same time) as the tumbled cured tissue. The finished weights were obtained the following morning. The percent cooking yield was calculated by dividing the finished cooled weight by the stuffed weight and multiplying by one hundred.

Preparation of samples for sensory evaluation and shelf life

The casing was peeled from the chilled, cooked, cured tissue and the product was sliced, approximately 4 mm in thickness. Every other slice was displayed on a tray for sensory evaluation. The sensory panel was composed of eight members who had an average of 5 yr experience evaluating cured pork products. Each sample was judged for cohesiveness, color uniformity, textural appeal and overall acceptance on an 8-point scale with (1) as extremely poor and (8) as excellent, and internal color with (1) as extremely pale and (8) as extremely dark. Panelists were asked to evaluate cohesiveness by visual observation and by manipulation of the sample. The remaining half of the slices were individually packaged (vacuum to 25 inches of Hg) with a Tipper clipper Model AZ4100L, into a clear B-540 barrier (Cryovac®), bag (6" x 12") and shrinkage was obtained by dipping the package into a hot water bath of 91°C for 2 sec. The packed products were then displayed on a metal grill shelf in a holding cooler (3°C) under incandescent lighting. At 0, 2, 4, 6, and 8 wk of storage, slices were randomly chosen for color measurement by reflectance using the Spectronic 20 (Ockerman, 1980), TBA values were determined (Ockerman, 1980) and microbial (anaerobic and total plate) counts were made (Ockerman, 1980). For proximate analysis (Ockerman, 1980), four slices of product from each casing were randomly chosen and cut into small pieces and then mixed and ground through 1/2" (0.32 cm) plate twice. The well mixed, ground samples were used to determine moisture, fat, protein, ash, NaCl and $NaNO_2$ content (Ockerman, 1980).

Analysis of Variance procedure was used to test significant level of moisture, fat, protein, NaCl, ash, nitrite, cohesiveness, internal color, color uniformity, flavor, textural appeal and overall acceptance. Least Squares and Maximum Likelihood General program for Fixed Models of Harvey (1968) was used to compute significant test level between storage and treatments for TBA value, color change, log of aerobic microbial count and log of anaerobic microbial count.

RESULTS & DISCUSSION

Sectioned and formed product production

The emulsion prepared by grinding lean meat with pickle was sticky. The 7.5% emulsion used was determined to be sufficient in preliminary investigation to form a sticky layer on the muscle pieces and was chosen for this experiment. Immediately after injection of pickle the meat surface was somewhat difficult to coat with the emulsion. After mixing and rubbing the meat with the emulsion for 15 min, the emulsion adhered and a thin coating was obtained.

Due to variation in pickle retention capacity of the muscle, it is difficult to maintain the same amount of pickle in all samples. Pickle retention ranged from 16–22% for both treatments, with an average of 19.13% for coating tissue and 18.88% for tumbling tissue (not significant $p < 0.05$). The difference in pickle retention between two picnics of the same pair ranged from 0–2%.

Table 1—Smokehouse schedule.

	Wet bulb setting	Dry bulb setting	Time
Drying		54.4°C	30 min
Smoking	54.4°C	68.5°C	1 hr
Smoking	65.6°C	73.9°C	1.5 hr
Cooking	78.9°C	85.0°C	to 66°C internal temp (approx 7 hr)

Cooking yield, product composition and sensory properties

Percent cooking yields were 89.61 ± 0.51 and 89.96 ± 0.59 for coated and tumbled sectioned and formed product, respectively. No significant ($p < 0.05$) weight difference between the two processing procedures was found and it should be pointed out that yields in both cases were not exceptionally high. Tissue from both processing procedures had a very uniform pink appearance; there was an absence of water pockets found on the packaging film; the surface was dry and smooth; and, the products had a very acceptable sliceability characteristic.

The increase in cooking yield obtained by the tumbling process was not significant ($p < 0.05$) when compared with that of the coated tissue. Emulsion-coated tissues were also covered by solubilized protein that denatures and immobilizes water transport during cooking. This would suggest that, in the 90% cooking yield range, surface coated protein that is obtained by either tumbling or manually coating will result in approximately the same yield.

Proximate composition of cured tissue prepared by the two processes is shown in Table 2. Except for ash, there were no significant differences.

Sensory Panel Evaluation

Effects of tumbling and coating on the sensory evaluations are shown in Table 3. Tissue from both processes resulted in good cohesiveness. The tumbling process, however, gave a small but significant increase ($p < 0.05$) in cohesiveness scores. However, the coated tissues exhibit good cohesiveness and sliceability and could probably be further improved by using lean meat of low collagen content in the emulsion preparation for coating.

Table 2—Proximate composition of cured cooked products

	Tumbling	Coating
Moisture (%)	72.16 ± 1.22	72.07 ± 1.16
Fat (%)	3.47 ± 0.93	3.56 ± 0.82
Protein (%)	18.66 ± 0.80	18.53 ± 0.86
Ash (%)	4.51 ± 0.23**	4.77 ± 0.25
NaCl (%)	3.37 ± 0.27	3.42 ± 0.24
$NaNO_2$ (ppm)	72.71 ± 17.34	68.00 ± 18.62

** $p < 0.01$.

Table 3—Effect of processing on cohesiveness, internal color, color uniformity, flavor, textural appeal and overall acceptability

	Tumbling	Coating
Cohesiveness[a]	6.81 ± 0.46*	6.20 ± 0.30
Internal color[b]	5.30 ± 0.41	5.08 ± 0.55
Color uniformity[a]	4.81 ± 0.52	4.76 ± 0.63
Flavor[a]	5.86 ± 0.37*	6.21 ± 0.25
Textural appeal[a]	5.62 ± 0.46*	6.25 ± 0.33
Overall acceptance[a]	5.82 ± 0.32	6.07 ± 0.18

[a] Scale, 1 = extremely poor, 8 = excellent.
[b] Scale, 1 = extremely pale, 8 = extremely dark.
* $p < 0.05$.

The coating process gave a slightly lighter internal color while the tumbling process resulted in better color uniformity although in both evaluations the difference was not significant. Muscles sectioned from picnics are quite different in color intensity so that cured sectioned and formed tissue prepared from picnics resulted in a lack of color uniformity in both processes.

Coated cured tissue resulted in significantly ($p < 0.05$) better flavor and textural appeal than tumbled cured tissue. The tumbling process will improve the tenderness of cured ham; however, improved tenderness may not always be desired by consumers (Ockerman et al., 1978). Combined attributes will affect the overall acceptance. No significant difference was found in average overall acceptance scores.

Shelf life

Effect of tumbling and emulsion coating on the rancidity of cured tissue was monitored by determination of TBA during the 8-wk storage period (Table 4). Tissue from both the coating process and the tumbling process was very stable in the shrunken Cryovac® bag and gave TBA values of less than 0.15. Neither type of processing nor the storage period resulted in a significant ($p < 0.05$) change in TBA values. Low temperature and shrunken vacuum packaging were expected to provide good storage conditions for the cured products. The low fat content of the cured pork tissue and mild light (average 800 lux) conditions probably contributed to the stability of the product (Table 4).

Table 4—Effect of processing on the TBA value change during storage

Storage period[a]	TBA	
	Tumbling[b]	Coating[b]
0 wk	0.11 ± 0.09	0.10 ± 0.08
2 wk	0.15 ± 0.05	0.13 ± 0.06
4 wk	0.11 ± 0.05	0.09 ± 0.05
6 wk	0.13 ± 0.04	0.12 ± 0.03
8 wk	0.12 ± 0.02	0.12 ± 0.03

[a] Effect of storage was nonsignificant, $p < 0.05$.
[b] Effect of processing was nonsignificant, $p < 0.05$.

Table 5—Effect of processing on the color change during storage

Storage[a] period (wk)	Reflectance ratio[bc]		Panel color score[bd]	
	Tumbling	Coating	Tumbling	Coating
0	1.91 ± 0.08	1.88 ± 0.07	3.12	3.01
2	2.00 ± 0.13	2.00 ± 0.06	3.42	3.42
4	1.97 ± 0.10	2.05 ± 0.08	3.32	3.59
6	2.12 ± 0.16	2.10 ± 0.15	3.82	3.76
8	2.16 ± 0.23	2.09 ± 0.15	3.96	3.72

[a] Storage time effect was nonsignificant, $p < 0.05$.
[b] Processing effect was nonsignificant, $p < 0.05$.
[c] Reflectance ratio = % Reflectance at 650 nm/% Reflectance at 570 nm
[d] Panel color score = −3.34 + 3.38 (% Reflectance at 650 nm/% Reflectance at 570 nm). 1 = Completely inferior; 2 = Faded; 3 = Slightly faded; 4 = Average; 5 = Excellent; 6 = Superior.

Table 6—Effect of processing on anaerobic and total microbial count during storage

Storage[b] period (wk)	Anaerobic (log)[a]		Total count (log)[a]	
	Tumbling	Coating	Tumbling	Coating
0	0 ± 0	0.09 ± 0.25	2.17 ± 0.51	2.06 ± 0.82
2	0.03 ± 0.09	0.13 ± 0.23	1.90 ± 0.53	1.94 ± 0.53
4	0.99 ± 1.65	0.13 ± 0.35	2.44 ± 1.09	1.80 ± 0.44
6	0.70 ± 1.24	0.34 ± 0.91	2.47 ± 1.06	1.98 ± 0.89
8	1.77 ± 2.76	1.65 ± 2.57	3.30 ± 1.69	3.07 ± 2.16

[a] No significant effect due to processing, $p < 0.05$.
[b] No significant effect due to storage time, $p < 0.05$.

The effects of processes on the color change of cured pork tissue during storage are shown in Table 5. Based on reflectance measurement, both finished products from the tumbling and coating processes had scores that indicate a slightly faded color. It is interesting to note that the average color of the cured tissue improved during storage. However, there is no significant ($p < 0.05$) difference in color between the two processes with storage time. Visually, color was very acceptable throughout the storage period.

Anaerobic and total plate counts of cured pork tissue during storage are shown in Table 6. Most cured pork tissue had very low anaerobic microbial counts; however, a few samples had a high anaerobic count in the last two weeks of storage, which resulted in a large standard deviation. This variation was probably due to lack of total destruction of the organism during smoking and cooking.

Most of the cured and sectioned and formed sample had a total count of less than 300 organisms per gram. Some of the samples had a high total count in the last storage period. No significant ($p < 0.05$) difference in total count was found in this experiment between samples prepared from the coating process or the tumbling process. The large standard deviation indicated that smokehouse heating to an internal temperature of 66°C may not be enough for products to be stored for up to 8 wk at 3°C.

CONCLUSIONS

SECTIONED AND FORMED CURED PORK tissue was prepared by 7½% emulsion-coating and compared with similar cured product that was tumbled. Cooking yield of the two processing techniques was similar and in the 90% range. Tumbling produced significantly higher cohesiveness scores although both treatments formed tight bonds between adjacent tissues. Flavor and texture scores were rated slightly higher for the coated tissue. Both products had very satisfactory flavor and texture characteristics. Internal color, color uniformity and overall acceptance were not significantly different for the two treatments. Vacuum packaged, refrigerated, stored products had low TBA values through 8 weeks of storage without significant difference between treatments. Storage time and processing treatment did not significantly affect color as measured by reflectance. Anaerobic and total plate counts increased during storage but were relatively low at the end of 8 wk and not significantly different for treatments. These results would suggest that satisfactory sectioned and formed cured pork products can be produced by emulsion coating.

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Ham Massaging. Effect of Massaging Cycle, Environmental Temperature and Pump Level on Yield, Bind, and Color of Intermittently Massaged Hams

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ABSTRACT

Commercially available hams (7.69–9 kg) were open face boned, closely trimmed of fat and then stitch pumped with various levels of pickle to determine the efficacy of pump level, massage cycle, or temperature of the ham during massaging on cook shrink, USDA yield, bind, and cured color intensity and uniformity. On a constant time basis, continuous massaging appeared superior to intermittent massaging and shorter rest periods were superior to longer rest periods where intermittent massaging was used. High pump levels (30, 35%) gave correspondingly higher cook shrink losses and lower bind values (adhesion) than low pump levels. But the 35% pump level gave the best combination of cured color intensity, uniformity and yield yet still provided sufficeint bind for slice durability. Ideal environmental massaging temperatures appeared to be between 4.4°C and 10°C for maximizing cured color development and yield. Bind was reduced at 10°C; however, no problem in slice durability was experienced.

INTRODUCTION

IN RECENT YEARS there has been much interest in the use of massagers and/or tumblers to increase rate of curing, produce consistently high yields, and promote slice durability in processed hams. Weiss (1974) distinguished between massaging and tumbling on the basis of the type of energy imparted by the two methods. Massaging was associated with frictional energy while tumbling was associated with impact and frictional energy.

Cassidy et al. (1978) characterized the disruption of cell membranes, and disorganization of nuclei associated with tumbling while Rejt et al. (1978), Theno et al. (1978) and Seigel et al. (1978a) noted similar cellular destruction due to massaging. Cassidy et al. (1978) indicated that an advantage in cell disruption was obtained in intermittent tumbling over continuous tumbling when compared on a tumbling time constant basis. However, Krause et al. (1978a) failed to confirm that intermittent tumbling would significantly ($P < 0.05$) improve yield.

Krause et al. (1978b) working with pork muscles established the existance of an increased rate of cure migration due to tumbling and theorized that this was due to cellular destruction which enhanced cure migration both between muscle bundles and fibers and within them.

Solomon et al. (1980) showed that curing solution was absorbed by the outside layer of pork muscle during the first hour of continuous tumbling and that movement from outside to inside occurred in the next 23 hr but equilibration had not occurred within 24 hr. They further indicated that absorption within the muscles during a curing period without tumbling was almost as great as during intermittent tumbling. The long equilibration time suggests a need for injection of curing solutions even though massaging or tumbling was employed.

Siegel et al. (1976) showed that as the massaging time of hams increased the level of protein increased in the exudate and that this increase was more pronounced in the presence of salt and phosphate. Macfarlane et al. (1977) compared the relative binding ability of crude myosin, actomyosin and sarcoplasmic proteins and concluded that myosin and actomyosin contributed the most to adhesion or bind of meat particles. In 1979 Siegel and Schmidt showed that salt and phosphate increased the solubilization of myosin and enhanced the binding of meat pieces together as protein content of the exudate increase from 0 to 8%. Siegel et al. (1978a, b) also attributed an increase in bind to a relative increase in solubilization of actin and myosin caused by the addition of phosphate. They also pointed out that massaging distributed the protein exudate on the muscle surfaces enhancing adhesion.

Knipe et al. (1981) showed that tumbling temperature significantly affected internal color, tenderness, and yield of hams. However, only two temperatures were compared (3°C and 25°C). One was near curing cooler temperatures (2–4°C) and the second was extremely warm (25°C).

Acton (1972) demonstrated the effect of product internal temperature on yield in poultry meat loaves and suggested that in order to maximize yield the final internal temperature should be kept low, 55–62°C. Rust and Olsen (1973) indicated that the protein exudate when cooked, formed a seal around the hams which helped lock in juices and improve yield.

Gillett et al. (1981) in a previous report on continuously massaged hams determined the effect of massage time, percentage of pickle pump and massage temperature on color, yield and bind in water added hams.

It was the purpose of this study to determine the effect of intermittent massaging, pickle pump level, and environmental massaging temperature upon the yield, bind (adhesion) and color of water added hams.

EXPERIMENTAL

Ham preparation

Ham preparation varied with the purpose of the experiment. Part 1 was designed to examine the effect of massage cycle, Part 2 to study the effect of pump level and Part 3 to evaluate the effect of massaging temperature. The treatment groups are listed in Table 1.

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Table 1–Ham treatment groups

Treatment	Rest period min/hr on 18 hr cycle	Pump level	Environmental temperature
1	0	30%	4.4°C
2	10	30%	4.4°C
3	20	30%	4.4°C
4	30	30%	4.4°C
5	20	20%	4.4°C
6	20	25%	4.4°C
7	20	30%	4.4°C
8	20	35%	4.4°C
9	20	30%	–9°C
10	20	30%	4.4°C
11	20	30%	10°C

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Part 1. Effect of massage cycle

Forty fresh hams (7.69–9 kg green weight) were open face boned and closely trimmed. The external shank muscles were then removed and the hams were randomly assigned to four treatment groups to determine the effect of rest period length (massage cycle) on selected ham quality characteristics. All these hams were stitch pumped with a 5 prong needle (Griffith pump and scale) to 30% of the trimmed weight of the hams with a pickle containing water, salt, sugar, sodium tripolyphosphate (Curafos), sodium erythorbate (Merck), and sodium nitrite (Table 2–30% pump level). Boneless pickled hams were then intermittently massaged in a Knud-Simonsen Industries Ltd. (KSI) massager 50 kg for 18 hr at 5 rpm in a 4° ± 1°C cooler with rest periods of 0, 10, 20 and 40 min/hr.

Part 2. Effect of pump level

Forty hams were boned and trimmed as in Part 1, then stitch pumped as above except four levels of pump were employed as shown in Table 2 (20, 25, 30 and 35%). Hams were then intermittently massaged for 18 hr at 4.4°C and 5 rpm using a cycle with a 20 min rest period/hr (20 min forward-10 min rest, 20 min reverse-10 min rest).

Part 3. Effect of environmental massaging temperature

Thirty hams were boned, trimmed and stitch pumped as in Part 1, then intermittently massaged as in Part 2 except the massaging was conducted in coolers at three different temperatures (−0.9°C, 4.4°C, 10°C).

Stuffing

Massaged hams were stuffed approximately 24 hr following pumping into size 7 Easy Peel, heavy prestuck, Fibrous casing (Union Carbide) via a Smeco press. Ham rolls were pressure packed to a recommended diameter of 111 ± 1 mm using a Tipper Press Tie (The Dover Corp.) with 2327 mm Hg pull up pressure.

Thermal processing

All hams were smoked for 7 min in a Drying System Co. smokehouse using a Kartridg Pak (The Kartridg Pak Co.) smoke generator at 400°C and a sawdust feed setting of 7. Dry bulb temperature was programmed to raise from 60°C to 82°C over a 1-hr cycle. Wet bulb was programmed to maintain 40% relative humidity throughout the cycle. Smokehouse temperature was held at 82°C until an internal ham temperature of 67°C was reached (approximately 6 to 6½ hr). Cooked product was showered in tap water for 30 min before being held overnight in a 4° ± 1°C cooler.

Table 2—Pickle formulas for various pump levels

Ingredient (%)	Pump level (% of Pickle)			
	20%	25%	30%	35%
Water	81.46	85.17	87.64	89.42
Salt	12.10	9.68	8.07	6.91
Sucrose	3.30	2.64	2.20	1.88
SOdium tripolyphosphate	2.75	2.20	1.83	1.57
Sodium erythorbate	0.30	0.24	0.20	0.17
Sodium nitrite	0.086	0.069	0.057	0.049

Cook shrink and USDA yield determination

Batch weights were taken on the raw stuffed hams and cooked chilled hams for each of the seven treatments. Batch weights were necessary because massaging tore the hams and reconstitution was impossible. Cook shrink was calculated using the following formula:

$$\% \text{ Cook Shrink} = \frac{\text{Stuffed wt} - \text{Cooked wt}}{\text{Stuffed wt}} \times 100$$

The USDA yield was calculated based on the meat proximate analysis of two ham samples where: USDA yield = (% moisture + % salt) − (3.79 × % protein) + 100%. (USDA regulations currently do not include the % salt for calculation of added water in hams.)

Instron bind determinations

Tensile strength values were determined as described by Gillett et al. (1978) using the Instron Universal Testing Machine with a Slice Holding Accessory. Determinations were made on 10 randomly selected 6 mm thick slices from two ham rolls of each treatment. Bind values were reported in g/cm^2.

Visual color panel analysis

Color intensity and color uniformity were evaluated by a 10-member laboratory panel. Both characteristics were evaluated on a scale of 1–10, where 1 was least desirable (lightest or least uniform) and 10 was most desirable (darkeest or most uniform).

Statistical analysis

The least significant difference method (LSD) was used as a method of multiple mean comparison following a significant F ratio on a nested analysis of variance (Ostle, 1963; Bennett and Franklin, 1954). Regression analysis of the linear, quadratic and cubic effects were evaluated as described by Brownlee (1953).

RESULTS & DISCUSSION

FOR CLARITY of presentation the results and discussion will be divided into the effect of (1) massage cycle, (2) pump level, and (3) temperature.

Part 1. Effect of massaging cycle

Shrinkage and USDA yield. Effect of massaging cycle on

Table 3—Effect of massaging cycle on shrink, yield, mean proximate composition and various ham characteristics[a,b]

	Massaging cycle (min/hr)														
	On 60	Off 0	On 25	Off 5	On 25	Off 5	On 20	Off 10	On 20	Off 10	On 10	Off 20	On 10	Off 20	LSD P < 0.05
	(%)		(%)				(%)				(%)				
Cook shrink	10.1		11.5				12.6				13.3				
USDA yield[c]	112.3		105.9				109.4				104.7				
Fat	6.15c		7.22a				5.86d				6.83b				0.27
Protein	16.20c		17.85a				17.10b				17.85a				0.32
Water	71.72a		71.02b				71.04b				70.03c				0.69
Ash	3.92b		3.76bc				4.15a				3.69c				0.19
Instron bind[d] (g/cm^2)	283a		492a				435b				386c				25.2
Color intensity[e]	6.8a		6.3a				6.1ab				5.4b				1.1
Color uniformity[e]	7.9a		7.4a				7.3a				5.9b				1.0

[a] Means on the same line not bearing the same letter are significantly different (P < 0.05) (According to Ostle, 1963).
[b] Ten hams were pumped 30%, massaged using various rest periods for 18 hr. at 4.4°C and 4 rpm.
[c] USDA Yield = (% Water + % Salt) — (3.79 x % Protein) + 100; 3.79 = Protein multiplier factor for water-added hams.
[d] Bind values (tensile strengths) were determined on ten 6 mm slices.
[e] Color intensity (redness) and uniformity scores were determined by a 10-member panel on a scale of 1—10, where 1 was lightest or least uniform and 10 the reddest or most uniform.

shrink or yield and proximate composition and characteristics of hams pumped 30% and massaged for 18 hr at 4.4°C are shown in Table 3. Cook shrink was lowest on the continuously massaged cycle and increased as the length of rest period in the cycle increased. USDA yields calculated from the proximate composition and a protein multiplier factor generally decreased overall as the rest period increased with the exception of the cycle with a 10 min/hr rest period which deviated from this trend.

Proximate composition of hams. The mean fat content of the massaged hams varied by only 1.36% or less. However, this represented a significant difference ($P < 0.05$) between groups in fat content. There was significantly more water and less protein in the continuously massaged hams than in the intermittently massaged hams. The massage cycle with the longest rest period (40 min/hr) had a high protein content (17.85%) and significantly less water retained than all other cycles. This might be expected since there was likely less myofibular protein extracted due to less agitation and therefore less water would be held yielding a higher protein content. Ash content of the hams varied by less than 0.5%.

Bind values. Adhesion of the meat within slices was expressed as Instron bind in g/cm^2. Bind values approaching 385 g/cm^2 provided sufficient adhesion for slice durability. The continuously massaged hams and the cycle with the shortest rest period 10 min/hr were significantly higher in bind values than the cycles with longer rest periods (20 or 40 min/hr). Siegel et al. (1978a, b) attributed the binding of adjacent meat pieces to the presence of a protein exudate. Theno et al. (1978) suggested a minimum of 8 hr of continuous massaging to realize the greatest benefit from massaging. They demonstrated an increase in protein content of the exudate as massaging continued. Apparently the cycles with longer massaging periods in this study provided more protein for the exudate than those with shorter massage periods and thus produced stronger adhesion. This was likely produced by enhanced brine dispersion which Theno et al. (1978) described as the major function of massaging.

Color intensity and uniformity evaluations. The massaging cycles with the longest massaging periods received the highest scores for desirable color intensity and uniformity. Theno et al. (1978) and Cassidy et al. (1978) indicated that tumbling caused an increase in muscle sarcolemma disruption. While Rejt et al. (1978) showed a similar effect from massaging. Krause et al. (1978b) theorized that the disruption of the sarcolemma increased the migration of curing ingredients between and into muscle fibers. This was verified by Solomon et al. (1980) on vacuum and nonvacuum tumbled hams. The increased desirability in color intensity and uniformity associated with cycles having longer massage periods in this study was likely related to a more rapid and uniform distribution of curing ingredients in the ham in agreement with the theory of Krause et al. (1978b).

The continuously massaged hams in this study were the most desirable in color intensity and uniformity, had the highest yields, and had optimal bind values. It therefore appears that continuous massaging may be preferable to intermittent massaging. The data indicated that on a time constant basis there was no advantage to intermittent massaging. This may appear contrary to the results of Cassidy et al. (1978) who reported a beneficial effect due to intermittent tumbling. However, their data were based on procedures where tumbling time, rather than total time, was held constant and tumbling rather than massaging was also employed.

When a time constant basis is used as the method of comparing massaging cycles, the massaging and rest periods vary inversely and total time is constant; however, when a massage constant basis is used, the total processing time increases with longer rest periods while massaging time is constant.

Table 4 illustrates that six additional hours are required on the intermittent cycle conducted on a massage constant basis compared to a time constant basis. In choosing between the use of intermittent massaging or continuous massaging it is obvious that a longer holding time is required for intermittent than continuous massaging. However, since some cure migrates during rest periods the length of massaging time can be reduced accordingly. The energy costs saved by the shorter massaging times must be compared with increased energy costs required for longer holding periods. The equipment will be committed for longer periods of time and a reduction in production volume would occur using intermittent massaging compared with continuous massaging. Cost comparison and utilization of equipment would be different with tumbling systems where vats are tumbled in sequence. However, these comparisons would still be valid in systems where vats of hams can be tumbled simultaneously.

Part 2. Effect of pump level on shrinkage

Table 5 summarizes the effect of pump level on shrinkage, yield, proximate composition and characteristics of intermittently massaged hams. It is interesting to note that the higher the pump level the higher the corresponding cook shrink obtained. There was no particular advantage obtained in yield when pump level exceeded 30%.

Proximate composition. When pumped excessively one may speculate that protein is often purged from the meat with a resultant loss of moisture-holding capacity. However, diagonal stitching, tandem pumping, decreasing the needle size while increasing the number of injection needles, reducing pump pressure while increasing dwell time are known to reduce purge. Under the conditions of this test the protein level in the hams varied inversely with pump level. All pump levels except the 30 and 35% were significantly different ($P < 0.05$) in protein content. The highest moisture level was retained in the 25% pump treatment. A 20% pump level was too low to obtain sufficient moisture retention as evidenced by the fact that it was significantly lower in moisture than all other ham treatment groups (68.26%). Ash content between pump levels was not significantly different ($P > 0.05$).

Bind values. Adhesion of meat particles decreased significantly ($P < 0.05$) as the pump level increased in the hams. However, there was sufficient bind to insure slice durability at all levels of pump tested. The presence of water between the muscle fibers likely reduced the adhesion of muscle fibers and hence the tensile strength of the slices.

Cure color intensity and uniformity. Subjective scores on the desirability of cure color intensity and uniformity indicated that the 20% pump level did not produce hams with sufficient cure color nor was the cured color uniform. The 25% level provided sufficient pickle to yield a desirable level of cure color intensity but color uniformity was

Table 4—Comparison of time constant vs massage constant basis

	Time constant, intermittent cycle (20 min rest/hr)	Time or Massage constant Continuous cycle	Massage constant, Intermittent cycle (20 min rest/hr)
Massaging time	12 hr	18 hr	18 hr
Rest time	6 hr	0 hr	6 hr
Total time	18 hr	18 hr	24 hr

scored significantly lower than on the 30 and 35% pump levels.

Overall, the 35% pump level gave the best combination of cured color intensity and uniformity. Yield was high at 35% and although bind was lowest at that pump level it was sufficient to provide excellent sliceability. Pump levels from 25–35% seemed to perform adequately.

Part 3. Effect of temperature

Shrink and USDA yield. Hams intermittently massaged at −0.9°C had the lowest USDA yield and highest cook shrink while those massaged at 4.4 and 10°C had low cook shrink and high USDA yields (Table 6). Apparently there was an advantage to massaging hams between 4.4 and 10°C to maximize yield rather than at lower temperatures. A higher temperature (25°C) according to Knipe et al. (1981) did not produce as high a yield as temperatures approaching curing cooler temperatures (3°C) on tumbled hams.

Proximate composition. Hams varied in fat by 1.4% or less between the three temperature treatment groups. However, the hams massaged at 10°C had significantly more fat than the other groups. Differences in protein were nonsignificant while moisture levels varied directly with USDA yield. The hams massaged at 10°C retained more moisture than the other two temperature groups ($P < 0.05$). Ash content was not significantly different between any of the temperature groups.

Bind values. Adhesion of ham slices was highest (435 g/cm^2) on the hams massaged at 4.4°C closely followed by those processed at −0.9°C (See Table 6). Hams processed at 10°C had significantly less bind 385 g/cm^2) than hams massaged at lower temperatures. All treatment groups had sufficient bind to insure satisfactory sliceability of the product.

Color intensity and uniformity. Temperature did not affect the uniformity of cure color. Temperature variations did however significantly affect cure color intensity ($P < 0.05$). Cure color intensity increased with each increase in temperature. This is in agreement with the work of Knipe et al. (1981) on tumbled hams. They obtained significantly higher color scores for hams tumbled at 25°C than those at 3°C.

In the range of temperatures used in this study it appeared that a tumbling temperature between 4.4 and 10°C would be most ideal. Warm temperatures generally promoted cure color development, increased yield and made the meat more flexible and thus reduced voids. Bind was low at 10°C but considerably higher at 4.4°C. Temperatures between 4.4 and 10°C may provide a suitable compromise between bind and other characteristics such as yield and color.

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Table 5–Effect of pump level[a] on cook shrink, yield, mean proximate composition and characteristics of intermittently massaged hams[b,c]

	Pump level				
	20	25	30	35	LSD
	(%)	(%)	(%)	(%)	$P < 0.05$
Cook shrink	9.1	11.7	12.6	14.5	–
Overall yield[d]	109.2	110.2	111.1	111.1	–
USDA yield[e]	106.2	110.2	109.4	109.6	–
Fat	9.02a	5.42c	5.86b	5.99b	0.44
Protein	19.75a	17.30b	17.10bc	16.95c	0.74
Water	68.26c	72.57a	71.04b	70.03b	1.04
Ash	3.94a	3.00a	4.15b	4.13a	N.S.
Instron bind[f] (g/cm^2)	494a	474b	435c	413d	17.28
Cure color intensity[g]	5.2c	7.2a	6.1b	7.0a	0.90
Color uniformity[g]	3.8c	6.1b	7.1a	7.4a	0.98

[a] Pump level based on percent green boneless trimmed weight.
[b] Ten hams from each treatment were massaged intermittently (20 min forward—10 min rest, 20 min reverse—10 min rest) for 18 hr at 4.4°C and 5 rpm.
[c] Means on the same line not bearing the same letter are significantly different ($P < 0.05$) (According to Ostle, 1963).
[d] Overall yield = [(Cooked wt)/(Boneless fresh wt)] x 100.
[e] USDA Yield = (% Water 3 % Salt) — (3.79 x % Protein) + 100; 3.79 = Protein multiplier factor fo water-added hams.
[f] Bind values (tensile strengths) were determined on ten 6 mm slices.
[g] Color intensity and uniformity were determined by a 10-member panel on a scale of 1—10, where 1 was the lightest or least uniform and 10 the reddest or most uniform in color.

Table 6–Effect of temperature on cook shrink, yield, mean proximate composition and characteristics of intermittently massaged hams[a,b]

	Temperature			
	−0.9°C	4.4°C	10°C	LSD
	(%)	(%)	(%)	$P < 0.05$
Cook shrink	14.4	12.6	12.7	–
USDA yield[c]	108.2	109.4	110.6	–
Fat	6.00b	5.80b	7.20a	0.36
Protein	17.00a	17.10a	17.50a	N.S.
Water	70.34b	71.40b	72.83a	0.74
Ash	4.10a	4.20a	4.00a	N.S.
Instron bind (g/cm^2)[d]	426a	435a	385b	12.76
Cure color intensity[e]	3.4c	6.1b	7.6a	0.89
Color uniformity[e]	7.5a	7.3a	7.4a	N.S.

[a] Ten hams from each treatment were pumped 30% and massaged intermittently (20 min rest/hr) for 18 hr at 5 rpm at various temperatures.
[b] Means on the same line not bearing same letter are significantly different ($P < .05$) according to Ostle, 1963.
[c] USDA yield = (% Water + % Salt) — (3.79 x % Protein) + 100; 3.79 = protein multiplier factor for water-added hams.
[d] Bind values (tensile strengths) were determined on ten 6 mm slices.
[e] Color intensity and uniformity were determined by a 10-member panel on a scale of 1—10, where 1 was the lightest or least uniform and 10 the reddest or most uniform in color

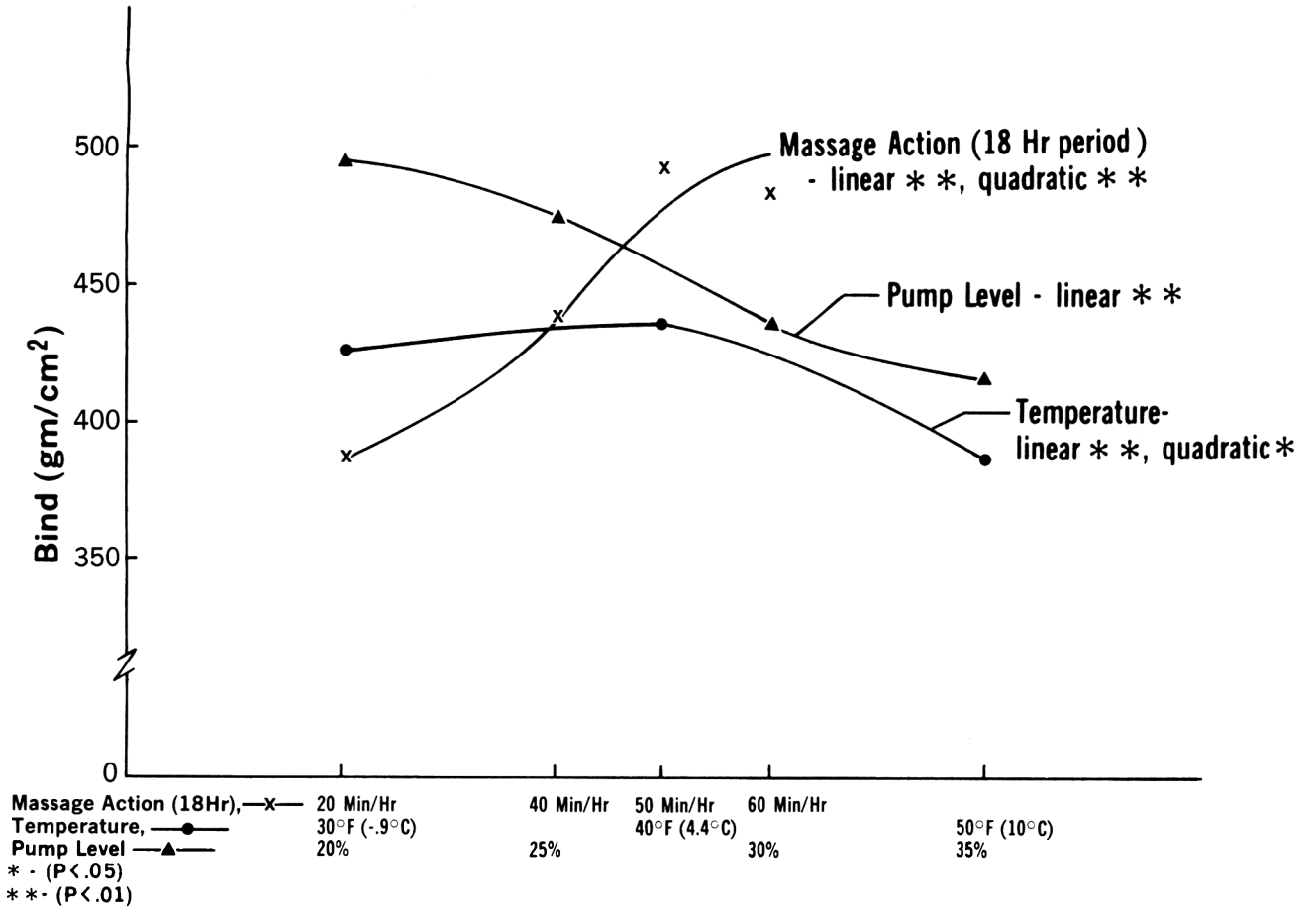

Fig. 1—Effect of massaging cycle, pump level, and environmental massage temperature upon bind (adhesion).

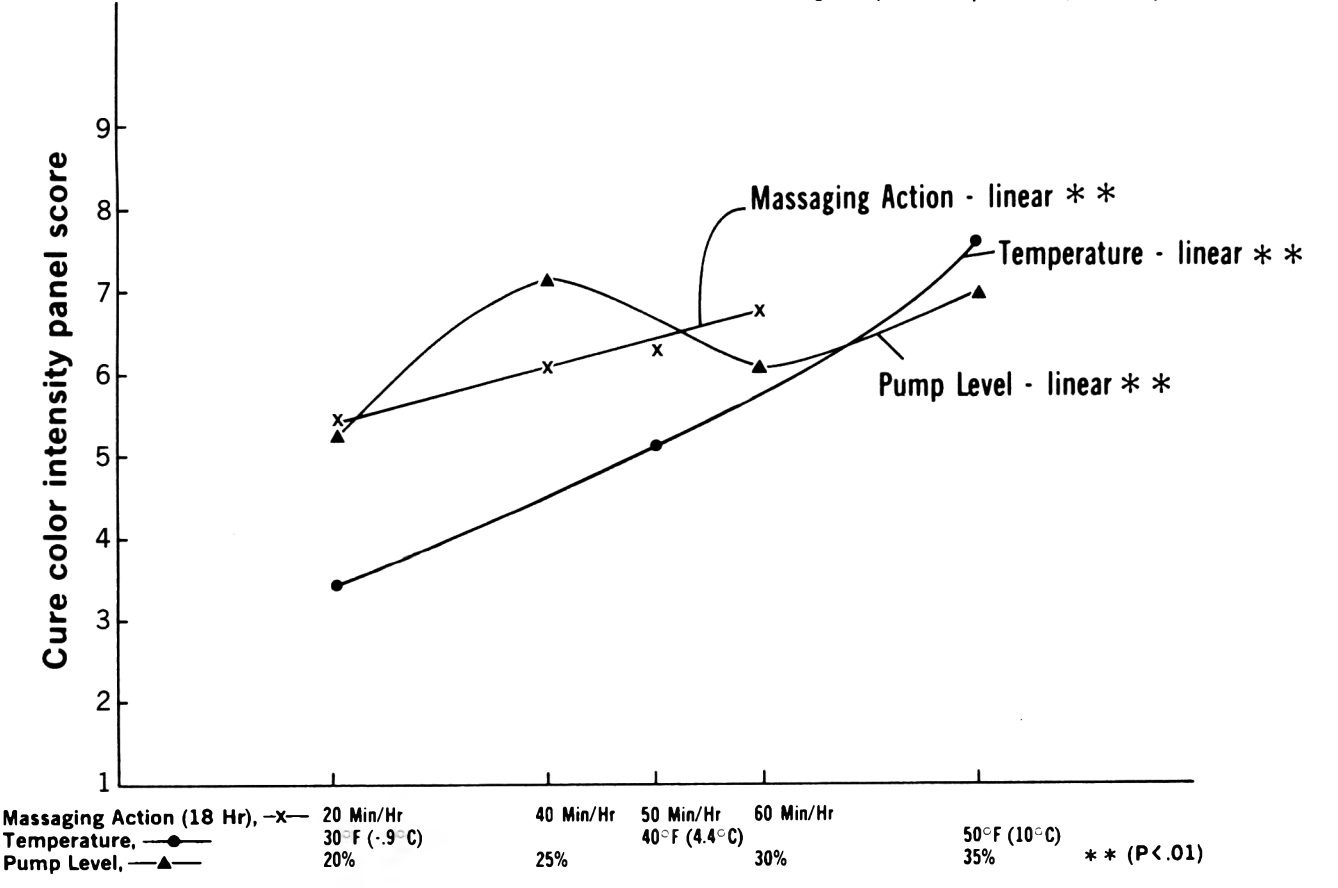

Fig. 2—Effect of massaging cycle, environmental massage temperature, and pump level upon cured color intensity.

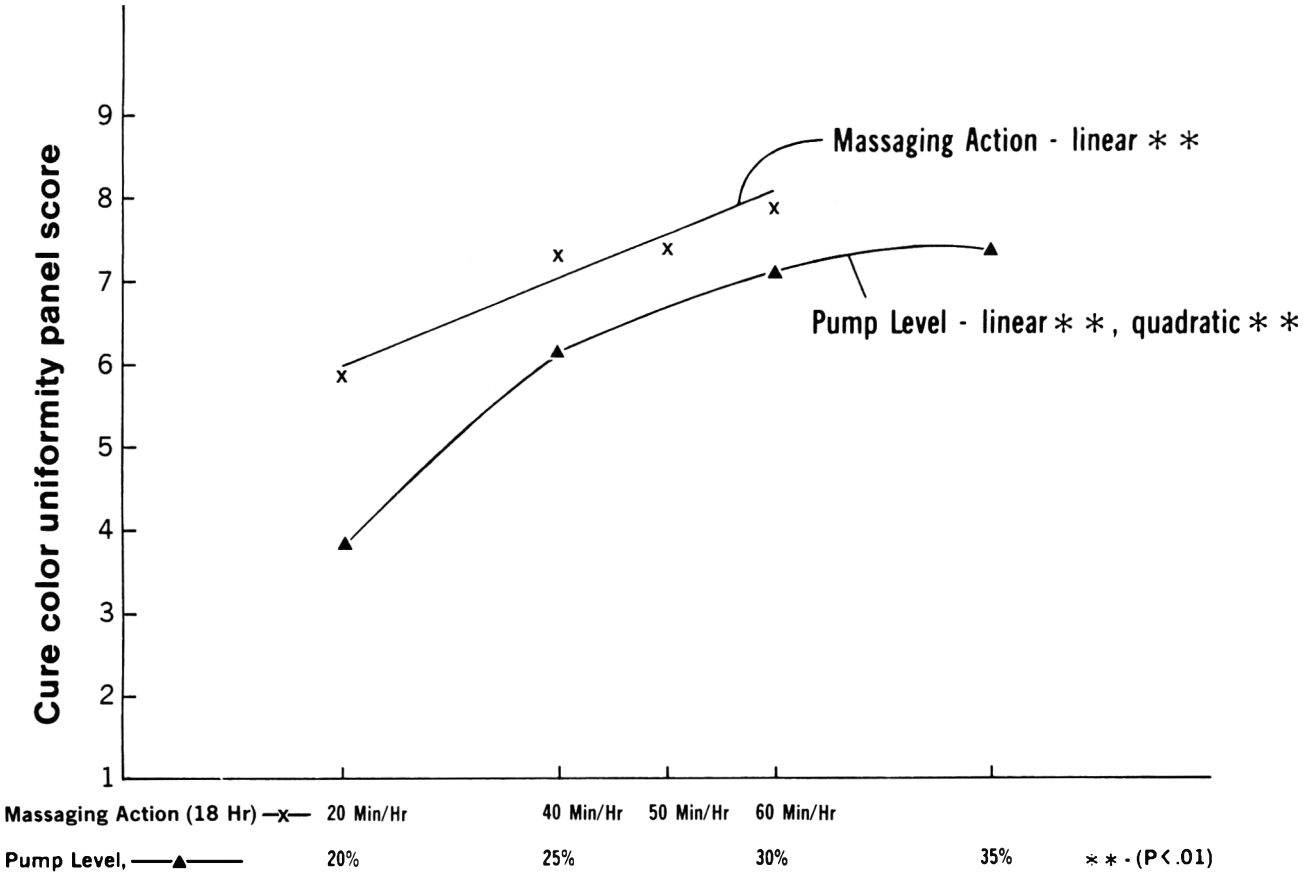

Fig. 3—Effect of massaging cycle, pump level, and environmental massage temperature upon bind (adhesion).

SUMMARY AND CONCLUSIONS

FIG. 1 SUMMARIZES the effect of factors identified in this study which influenced bind. Note that as the action time increased or rest period declined in the massaging cycles the bind increased in a linear and quadratic manner. Therefore, actual massaging time in a cycle should be long in comparison to the rest period to promote bind—such cycles also reduce cook shrinkage. Continuous massaging appears to be a better practice than intermittent massaging, provided ham tearing is not excessive.

Bind decreased in a linear manner as pump levels increased, suggesting that more problems can be expected with adhesion of meat particles and slice durability as pump levels rise. Apparently, cook shrinkage also increases as pump levels rise.

The effect of high environmental massaging temperature (10°C) can be considered detrimental to bind; however, since high temperatures also reduce voids and enhance cure color intensity, relatively high temperature massaging (7°C) is still recommended.

Fig. 2 identifies three factors which influenced cure color intensity in a linear manner. Increasing environmental massaging temperature, higher pump level and using cycles with longer action periods and shorter rest periods, all promoted intense cure color. Increasing the action interval in the massaging cycles caused a linear increase in color uniformity of hams while temperature had no effect (Fig. 3). Increasing pump levels promoted a linear and quadratic effect on color uniformity. As high pump levels were reached the effect on color uniformity diminished. Pump levels near 35% gave good yield and cure color intensity, excellent color uniformity, and provided sufficeint bind for slice durability under the conditions of this study.

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Cook/Chill Foodservice Systems with Conduction, Convection and Microwave Reheat Subsystems. Nutrient Retention in Beef Loaf, Potatoes and Peas

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ABSTRACT

Percent thiamin in beef loaf and peas and percent ascorbic acid in potatoes were determined at all process stages in a simulated hospital cook/chill foodservice system. Retention of nutrients in experimental products after reheating by conduction, convection and microwave radiation was compared. Points in food product flow causing largest nutrient losses were: beef loaf, precooking; peas, reheating; and potatoes, 24-hr chilled storage. Statistical analyses indicated no significant difference in nutrient retention of experimental products due to method of reheating.

INTRODUCTION

THE U.S. HEALTH CARE INDUSTRY is estimated to contain 27,960 foodservice units (Wintergreen, 1981). When compared to conventional foodservice systems, newer types of foodservice systems used in health care settings such as cook/chill or cook/freeze foodservice systems require further processing for the production of hot entrees. Further processing may involve chilled or frozen storage, portioning for meal assembly, distribution of meals from production to service facilities and reheating, a second heat process.

Accurate evaluation of nutrient content of food served to patients is a priority goal of physicians and dietitians in health care settings. Accurate nutritional information on foods served in cook/chill and chill/freeze foodservice systems, however, is not currently available. Tables of food composition such as the publication by Pennington and Church (1980) or the publication by Watt and Merrill (1963) are frequently used by dietitians to determine nutrient composition of food served to clients. Lachance et al. (1973) emphasized the discrepancy which exists between values for nutrient composition of food in such handbooks and values for nutrient composition of food at point of service in involuntary foodservice settings such as hospitals. These authors state that nutritional losses in processing, storage and reheating of the served foods were not taken into account when the handbook was put together. This perspective is as valid in the 1980's (Pennington and Church, 1980) as it was in the 1960's (Watt and Merrill, 1963).

Reheating food in microwave ovens in cook/chill foodservice systems has been shown to cause loss of nutrients. Dahl and Matthews (1980) determined that beef loaf prepared in a simulated cook/chill foodservice system and reheated by microwave energy 20, 50, 80 or 110 sec lost 5–10% of original thiamin content (before precooking) due to reheating. Bobeng and David (1978b), using beef loaf of the same formulation, reported a 12.5% loss in thiamin due to 90 sec of microwave reheating. Similar data are needed for other food products prepared in cook/chill foodservice systems.

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Reheating foods in health care settings may be accomplished using one of several methods. In a recent study of short-term care hospitals in New York City, Fransese (1981) determined that 15% of hospitals use a foodservice system in which food is reheated by microwave energy while 4% of hospitals have implemented a system in which food is reheated by conduction. Other reheating systems for health care units currently available involve the use of forced-air convection ovens for reheating food.

The main purpose of this study was to observe and compare the effect of conduction, convection and microwave reheating on nutrient retention of hot entree items processed according to procedures used in hospital cook/chill foodservice systems. In addition, data from this study could provide information on nutrient losses resulting from chilled storage, portioning and distribution of food in a cook/chill system. Results from this study could be used by health care personel to increase the accuracy of nutrient calculations for clients and by foodservice administrators to determine methods of processing for optimal nutrient retention in food.

METHODS & MATERIALS

LABORATORY SIMULATION of a cook/chill foodservice system was used in this study to control parameters of time and temperature of processing and selection of equipment. The present study concentrated on three reheating subsystems of a cook/chill foodservice system (Fig. 1). The three subsystems differed by the method of reheating: conduction, convection or microwave radiation. The experiment, as explained below, was replicated four times; 48 portions of each product were prepared for each replication; duplicate samples were obtained for testing at each designated point in food product flow (Fig. 1).

Experimental products used included beef loaf, mashed potatoes and peas. Beef loaf was chosen because it had a compact consistency which facilitated temperature measurement. Potatoes and peas were chosen because when served with beef loaf, they are a typical example of the reheated portion of a meal served in a hospital cook/chill system.

Formulation and preparation techniques for beef loaf were comparable to those described by Dahl and Matthews (1980). Thiamin content of beef loaf and peas and ascorbic acid content of potatoes were used as indicators of nutritional quality since they are among the least stable of vitamins (Tannenbaum, 1976). Extensive losses of thiamin and ascorbic acid have been reported to occur in foods as a result of various processing operations similar to those used in foodservice operations (Chung et al., 1981; Tannenbaum, 1976; Lachance, 1975). Ang et al. (1975) showed that thiamin and ascorbic acid were unstable in reheated food in a cook/freeze foodservice setting.

In this study, 100-g portions of precooked beef loaf, mashed potatoes or peas, prepared in four laboratory simulations of a hospital cook/chill foodservice system, were reheated in one of three reheat subsystems. Each subsystem represented a method of reheating commonly used by hospitals which operate cook/chill systems. Each reheat subsystem was characterized by reheating equipment, plates and plate covers. Components of reheat subsystems are listed in Table 1.

Conduction reheating

Conduction rehreating was completed using the 3M Integral Heating Food Service System (Table 1). The 3M system was designed to reheat refrigerated or chilled meals on a large volume basis

and consists of two elements: heating/serving dishes with covers and the heating cabinet. The heating/serving dish converts electric energy to heat through the use of thin-coat carbon composition resistors fused to the bottom of each dish. Dishes for the system are in two parts. The interior of the dish is porcelain ceramic and the outer section is a polysulfone plastic shell on which metal button-like contact points for the electric current are placed. The heating cabinet or Food Service Module which supplies electrical energy holds 24 dishes on 12 permanent chrome plated shelves. Time of reheating was 18 min. Time of holding was 12 min. Times for heating and for holding were predetermined by the manufacturer.

Convection reheating

Convection reheating was completed using an airline type, ducted, forced-air convection oven (Table 1). Twelve meals were reheated at a time in one oven. Time of reheating (30 min) and oven temperature for reheating (177°C/350°F) were based on recommendations of the manufacturer.

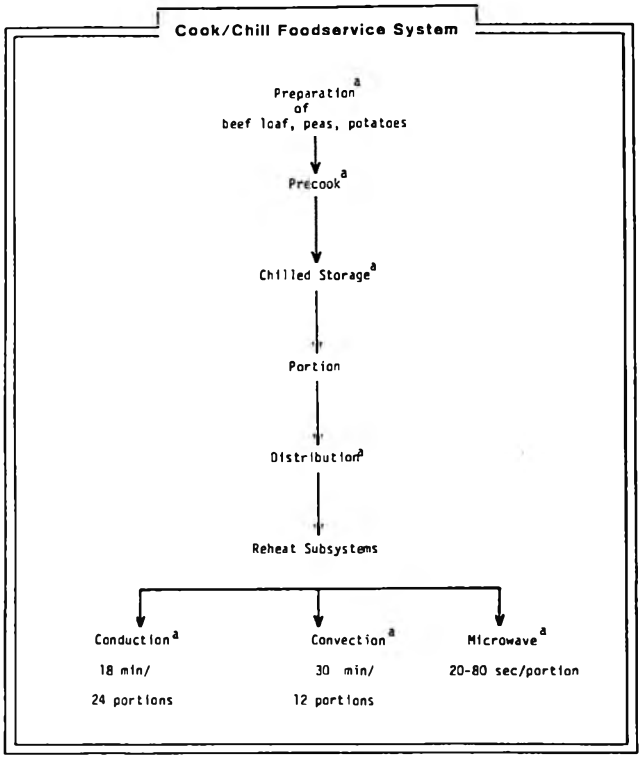

Fig. 1—Cook/chill foodservice system with optional reheat subsystems: Product flow and experimental design for nutrient assay of beef loaf, potatoes and peas. [a]Samples of product obtained for nutrient assay following process step indicated.

Microwave reheating

Microwave reheat was completed using a 2450 MHz microwave oven. The microwave oven was operated at 208V. Energy output of the microwave oven was 1368.5 ± 52.5 Watts (N = 20). Manufacturer's input energy rating for the microwave oven was 2860 Watts. Time of microwave reheating for each product was re-evaluated before each simulation and was based on number of seconds to achieve an internal end temperature of 74°C/165°F (Bobeng and David, 1978a).

Beef loaf

Beef loaf (60% ground beef, 26% fresh whole milk, 9% fresh whole eggs, 4% packaged bread crumbs, and 1% salt) was prepared following time-temperature recommendations of the Hazard Analysis Critical Control Point Model for Hospital Cook/chill Systems (Bobeng and David, 1978a). After combining 28.26 kg of beef loaf mixture in a mixer (Model H 600, Hobart Corporation, Troy, OH), 1 kg of mixture was placed in each of 24 aluminum pans (No. 409, Kaiser Aluminum, Chicago, IL). Pans of loaf mixture were precooked in a forced-air convection oven (Model No. 186C-2, Market Forge Co., Everett, MA; Energy Input Rating, 15.3 kw) operating at 162.8°C/325°F. After precooking, loaves were drained 10 min at ambient temperature (22.28°C/72.1°F) on a wire rack, weighed, placed in a clean aluminum pan and covered with aluminum foil. After chilled storage for 24 hr, crust-ends of loaves (60 ± 10g) were removed and loaves were divided into 6 portions of 100 ± 1g each. Samples of beef loaf to be used for analysis of thiamin were randomly chosen from among portions adjacent to the crust. Portions of beef loaf located toward the end of the loaf adjacent to the crust were believed to contain minimum amounts of thiamin because they had achieved higher temperatures when compared to center slices. Portions of beef loaf to be reheated weighed 100 ± 1g and measured approximately 6.4 cm wide x 11.4 cm long x 1.3 cm thick.

Portions of beef loaf to be used for thiamin assay were individually placed on appropriate plates (Table 1), covered with matching plate covers (Table 1), and stored 2 hr to simulate distribution of food from the central kitchen of a hospital to galleys in patient areas.

Following simulated distribution, 12 portions of beef loaf in appropriate containers were placed in the ducted forced-air convection oven to reheat for 30 min (at 350°F) while 24 portions of beef loaf in appropriate containers were placed in the Food Service Module to reheat for 18 min. Meanwhile, for the microwave reheat subsystem, one portion of beef loaf was selected randomly and removed from the refrigerator. The portion was placed in the center of the microwave oven and was reheated for the predetermined period of time. Time of microwave reheating was monitored with a stopwatch. After reheating, portions of beef loaf to be used for thiamin assay were immediately frozen in sterile plastic bags until analyzed.

Peas

Eight kilograms of individually quick frozen peas (nine, 2-lb boxes) were removed from frozen storage (≤20°C/−4°F) and placed in refrigerated storage (≤7°C/45°F) for 24 hr to defrost. To

Table 1—Cook/chill foodservice systems: Equipment paramenters for three reheat subsystems

Reheat subsystem	Oven	Plate	Plate Cover
Conduction	Heating cabinet Food Service Module 3M Co., St. Paul, MN	Insulated 8" ceramic dishes with electrical resistors. Heating/serving dishes 3M Co., St. Paul, MN	Plate covers, 8" plastic 3M Company, St. Paul, MN
Convection	Ducted forced-air convection oven Crimsco Inc., Kansas City, MO	9" China plates Shinango China, New Castle, PA	Polysulfone covers, Kover UPS. Kendrick A. Johnson, Minneapolis, MN
Microwave	Microwave oven Model M-312T Hobart Corp., Troy, OH	9" Paper plates SS-9DP Sweetheart Cup Div. of Maryland Cup Corp. Owings, MD	9" Plastic lid, LS-9DP. Sweetheart Cup Div. of Maryland Cup Corp., Owings, MD

precook, half of the peas were placed in the steam insert of a stainless steel pan (46 cm wide x 66 cm long x 15 cm high; 18 in x 26 in x 6 in) and filled with 100 ml of water. The pan was covered with aluminum foil and the peas were steamed for approximately 13 min until the temperature of peas was ≥74°C/165°F. After storage for 24 hr at ≤7°C/45°F in a pan of similar size, peas were portioned and reheated using the method previously described for beef loaf.

Potatoes

Eight kilogram sof instant mashed potatoes (16% granulated potatoes, 72% water, 11% whole fresh milk, 1% butter and 1% salt) were reconstituted according to directions of the manufacturer (Frosty Acres Instant Mashed Potatoes, Frosty Acres Brands, Inc., Atlanta, GA). Reconstituted potatoes were stored 24 hr at ≤7°C/45°F in a 46 cm wide x 66 cm long x 15 cm high (18 inch w x 26 inch l x 6 inch h) stainless steel pan and covered with aluminum foil. Potatoes to be assayed for ascorbic acid and/or reheated were handled using a method similar to the one previously described for beef loaf.

Nutrient retention

Thiamin content of beef loaf and peas was determined at the following points in food product flow: at preparation, after precooking, after 24-hr chilled storage, after distribution and after three types of reheating (Fig. 1) according to the automated method described by Kirk (1974).

Ascorbic acid content of potatoes was determined at identical points in food product flow except not at preparation because potatoes had not yet been reconstituted. Ascorbic acid content was determined using the method described in AOAC (45.054) (1975). Two samples of product were obtained at each sampling point and duplicate assays were run on each sample.

Percent retention of nutrient in the experimental product was adjusted to account for weight losses during processing according to the recommendations of Murphy et al. (1975).

Formula One: Calculation of Nutrient Retention

$$\%\ \text{Retention of Nutrient} = \frac{\text{Nutrient content per g of (precooked, stored or reheated) food x g of food after (precooking, storage or reheating)}}{\text{Nutrient content per g of food before (precooking, storage or reheating) x g of food before (precooking, storage or reheating)}} \times 100$$

The calculation of nutrient retention in formula one is based on the assumption that solids such as fat, protein, vitamins, and minerals are contained in liquid processing losses which result from cooking and handling (Murphy et al., 1975).

Weight loss

Weight loss of experimental products was used to calculate percent retention of thiamin and ascorbic acid according to formula one. In this study, calculations for weight after processing were based on 100g of raw product (or rehydrated product for potatoes). Thus, weight loss after each step in food product flow was subtracted from 100g. One hundred grams of product at preparation were used as the basis for calculation because results could be reviewed as grams or as percent, and because results could be more easily compared with other studies on cook/chill systems. Grams of weight loss were calculated for each sample of beef loaf assayed for thiamin during each stage in food product flow in four replications of the present study.

Statistical analysis

Analysis of variance on the data was carried out using a randomized complete block design (Steel and Torrie, 1980) and the differences in average values due to the treatment were identified using Duncan's multiple range test (Duncan, 1951). Statistical analysis was accomplished using the program titled, MSU STAT System Version 4 on a cyber 750 CDC computer.

RESULTS

Cook/chill foodservice system

Mean time/temperature parameters for processing beef loaf, peas and potatoes are shown in Tables 2, 3, and 4. Data for time and temperature of processing beef loaf in the present study are similar to those reported for hospital cook/chill foodservice systems by Bobeng and David (1978b) and by Dahl and Matthews (1980). Data for time and temperature parameters of beef loaf, peas and potatoes from preparation through distribution obtained in this study did comply with recommendations in the HACCP model for hospital cook/chill foodservice systems (Bobeng and David 1978a), except for the following.

Temperature of experimental products rose an additional 7–8°C beyond 7.2°C during portioning and distribution. Time of portioning and handling together, however, was less than 4 hr as recommended by Longree (1972) and the FDA *Food Service Sanitation Manual* (USDHEW, 1976) Portioning at ambient temperature (21–24°C) probably accounted for the rise in temperature.

Reheat subsystems

Conduction reheat per portion of beef loaf took the least time when compared to convection or microwave reheat (Table 2). Mean internal end point temperatures (EPT) of beef loaf after reheating did comply with HACCP guidelines (74–77°C) except for microwave reheat where the mean EPT was 79 ± 2°C (Table 2). Mean EPT's of peas reheated by conduction and microwave radiation (Table 3)

Table 2—Cook/chill foodservice system: Percent thiamin retention in beef loaf reheated by conduction, convection, and microwave radiation

Food product flow	Cook/chill foodservice system (N=8)			Thiamin retention				
		Temp (°C)		Replication (N=2)				
	Time	Begin	End	1	2	3	4	X̄±S.D.
	Units			Percent				
Preparation	76 + 9 min	5 + 2	9 + 3	100 (1.03)[a]	100 (0.80)	100 (0.82)	100 (0.96)	100±0 (0.90)
Precook	54 + 4 min	10 + 4	71 + 5	77.6	81.6	78.3	63.5	75.3+8
Chilled storage	24 hr	57 + 7	4 + 1	71.6	85.6	76.3	67.5	75.3+8
Portion	63 ± 24 min	4 + 1	15 + 3	—[b]	—	—	—	—
Simulated distribution	120 min	15 + 3	8 + 2	70.5	89.4	62.9	63.5	71.6+12
Reheat[c]								
Conduction	18 min/24 portions	8 + 2	74 + 4	49.7	80.5	71.8	73.7	68.1+13
Convection	25 min/12 portions	7 + 2	75 + 9	51.7	75.9	68.4	72.7	67.1+11
Microwave radiation	68 + 2 s/portions	7 + 1	79 + 2	58.6	72.5	56.8	64.5	63.7+7

[a] Micrograms of thiamin per gram of product
[b] No measurement taken
[c] N=8

and of potatoes (Table 4) reheated by conduction were also 76°C while the mean EPT of potatoes reheated by convection was less than the 74°C recommended in the HACCP model.

Weight after processing. Liquid processing losses from handling and cooking often contain water-soluble nutrients such as thiamin (Tannenbaum, 1976). Weight losses of experimental products (Table 5) were used to calculate percent retention of thiamin in beef loaf according to the recommendations of Murphy et al. (1975) in formula one.

Beef loaf prepared in this study had the identical formulation to beef loaf prepared in the study by Dahl and Matthews (1980). Weight losses of beef loaf in this study were identical to weight losses of beef loaf reported by Dahl and Matthews (1980) except for the reheat subsystem. Mean weight retention of beef loaf reheated by microwave radiation for 68 sec in this study was comparable to weight of beef loaf processed 80 sec in a microwave oven reported by Dahl and Matthews (1980). This difference may be partially explained by differences in raw products.

Mean weight losses of beef loaf reheated by microwave radiation were approximately 9% greater than losses of beef loaf reheated by conduction or convection. Other authors have reported consistently higher losses of weight for meat products processed by microwave radiation when compared to other methods (Causey and Fenton, 1951; Cipra et al., 1970; Janicki and Appledorf, 1974). Weight retention of beef loaf microwave reheated in this study (63%) was comparable to 64% weight retention of beef loaf microwave reheated 80 S by Dahl and Matthews (1980). Convection reheat caused greater mean weight loss in peas or in potatoes than did either conduction or microwave radiation (Table 5). A possible explanation for this high weight loss was the greater length of time to reach 176°C/348°F required for convection reheat as well as the speed of air circulation within the heating chamber. Microwave reheated peas had the greatest weight retention among three reheat subsystems; figures for weight retention of microwave and conduction reheated potatoes were comparable. It was concluded that weight loss in reheat subsystems are product/reheat subsystem related rather than dependent on the subsystem alone.

Thiamin content per gram of product as analyzed. Nutrient content in μg/g of experimental product are in Table

Table 3—Cook/chill foodservice system: Percent thiamin retention in peas reheated by conduction, convection and microwave radiation

Food product flow	Cook/chill foodservice system: Time	Temp (°C): Begin	Temp (°C): End	Thiamin retention: Replication (N=2) 1	2	3	4	$\bar{X}$ ± S.D.
	Units			Percent				
Preparation	24 hr	−20 ± 0	−0.4 ± 1	100 (2.45)[a]	100 (2.38)	100 (2.48)	100 (2.63)	100 ± 0 (2.49)
Precook (steam)	44 ± 10	−0.4 ± 1	75 ± 2	84.5	107.8	102.5	90.7	96.4 ± 11
Store	24 hr	67 ± 3	6 ± 1	83.5	85.0	96.7	98.7	91.4 ± 8
Portion	76 ± 26 min	6 ± 1	14 ± 3	—[b]	—	—	—	—
Simulated distribution	120 min	18 ± 3	7 ± 2	84.4	97.1	93.2	95.4	92.5 ± 6
Reheat[c]								
Conduction	18 min/24 portions	9 ± 2	86 ± 3	79.3	91.5	87.3	82.0	85.0 ± 6
Convection	20 min/12 portions	10 ± 2	76 ± 3	83.4	90.9	81.7	87.3	85.8 ± 4
Microwave radiation	20 ± 1 s/portion	8 ± 1	79 ± 3	81.4	91.8	92.9	75.9	85.5 ± 8

[a] Micrograms of thiamin per gram of product or 100%
[b] No measurement taken
[c] N = 8

Table 4—Cook/chill foodservice system: Percent ascorbic acid retention in potatoes reheated by conduction, convection and microwave radiation

Food product flow	Cook/chill foodservice system: Time	Temp (°C): Begin	Temp (°C): End	Ascorbic Acid Retention: Replication (N=2) 1	2	3	X ± S.D.
	Units			Percent			
Precook (Reconstituted)	50 ± 5 min	—	64 ± 0	100.0 (0.76)[a]	100.0 (0.73)	100.0 (0.73)	100.0 ± 0 (0.74)
Store	24 hr	67 ± 6	9 ± 2	41.3	46.8	83.9	57.3 ± 2
Portion	72 ± 8 min	9 ± 2	15 ± 3	—[b]	—	—	—
Simulated distribution	120 min	15 ± 3	7 ± 2	24.8	16.0	16.1	19.0 ± 5
Reheat[c]							
Conduction	18 min/24 portions	7 ± 2	79 ± 8	21.1	14.1	31.0	22.1 ± 8
Convection	25 min/12 portions	7 ± 2	62 ± 13	20.0	16.5	16.2	17.6 ± 2
Microwave radiation	77 sec/portions	7 ± 2	77 ± 6	14.2	13.0	18.5	15.2 ± 3

[a] Micrograms of thiamin per gram of product or 100%
[b] No measurement taken
[c] N = 6

7. Nutrient content per gram of experimental product was used to calculate nutrient retention in experimental products according to formula one.

As expected, among beef loaf, peas, and potatoes, peas contained the largest amount of thiamin per gram of products (Table 6).

Percent thiamin retention in beef loaf. Values for percent thiamin retention in beef loaf was adjusted for processing losses and are in Table 2. Percent retention of thiamin in beef loaf was considered 100% at preparation. Precooking beef loaf in a convection oven to a mean EPT of 71°C resulted in a 25% mean loss of thiamin (Table 2). Beef loaf of identical formulation prepared by Bobeng and David (1978b) (Table 6) showed a loss of only 12.5% thiamin after precooking. Losses in this study were probably greater than losses reported by Bobeng and David (1978b) because EPT of beef loaf prepared by Bobeng and David was 60–62°C while in the present study mean EPT of beef loaf after precooking was 71°C (Table 2).

Chilled storage of beef loaf did not result in loss of thiamin. Time of holding of beef loaf during simulated distribution and portioning resulted in a mean loss of 4% thiamin. Losses during storage, portioning and distribution of beef loaf were minimal when compared to losses resulting from precooking.

In this study, reheating beef loaf resulted in a loss of thiamin regardless of method. Microwave reheating resulted in lowest mean percent thiamin retention for beef loaf as well as lowest percent retention in three out of four replications. Percent retentions of thiamin for conduction and convection reheat were comparable (Table 2). ANOVA indicated no significant differences among values for percent thiamin retained in beef loaf. Lowest values for percent thiamin in beef loaf after microwave reheating may be partially attributed to the higher weight loss of beef loaf (Table 5) which was microwave reheated.

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Table 5—Beef loaf, peas and potatoes: Figures for weight retention used to calculate thiamin content

Product and food product flow	Replication 1	2	3	4	$\bar{X}$ ± S.D.
Beef loaf	← Weight in grams →				
Preparation	100.0	100.0	100.0	100.0	100.0 ± 0.0
Precook	79.9	81.6	72.1	77.2	77.7 ± 4.1
Store	78.9	80.6	71.1	76.2	76.7 ± 4.2
Simulated distr.	78.9	80.6	71.1	76.2	76.7 ± 4.2
Reheat					
Conduction	69.2	77.6	68.4	75.3	72.6 ± 4.5
Convection	72.6	77.4	65.9	75.1	72.7 ± 4.9
Microwave radiation	67.1	63.1	58.2	63.8	63.1 ± 3.7
Peas					
Preparation	100.0	100.0	100.0	100.0	100.0 + 0.0
Precook	90.0	90.0	90.0	90.0	90.0 + 0.0
Store	88.0	88.0	88.0	88.0	88.0 + 0.0
Simulated distr.	88.0	88.0	88.0	88.0	88.0 + 0.0
Reheat					
Conduction	78.5	79.2	79.0	79.9	79.2 + 0.6
Convection	77.9	79.4	73.7	79.2	77.6 + 2.7
Microwave radiation	85.8	84.0	83.1	84.0	84.2 + 1.1
Potatoes					
Precook (Reconstitution)	100.0	100.0	100.0		100.0 + 0.0
Store	99.0	99.0	99.0		99.0 + 0.0
Simulated distr.	99.0	99.0	99.0		99.0 + 0.0
Reheat					
Conduction	92.1	93.6	94.8		93.5 + 1.4
Convection	96.00	86.1	85.1		89.1 + 6.0
Microwave radiation	85.44	96.0	93.1		91.5 + 5.5

Table 6—Beef loaf, peas and potatoes. Thiamin per gram as analyzed

Product and food product flow	Replication 1	2	3	4	$\bar{X}$ ± S.D.
Beef Loaf	← μg thiamin/g of producta →				
Preparation	1.03	0.80	0.82	0.96	0.9 ± 0.1
Precook	1.00	0.80	0.89	0.79	0.9 ± 0.1
Store	0.94	0.85	0.88	0.85	0.9 ± 0.0
Simulated Distribution	0.92	0.89	0.78	0.80	0.9 ± 0.1
Reheat					
Conduction	0.74	0.83	0.86	0.94	0.8 ± 0.1
Convection	0.73	0.79	0.85	0.93	0.8 ± 0.1
Microwave radiation	0.90	0.92	0.80	0.97	0.9 ± 0.1
Peas					
Preparation	2.45	2.38	2.48	2.63	2.5 + 0.1
Precook	2.30	2.85	2.83	2.65	2.7 + 0.3
Store	2.38	2.30	2.73	2.95	2.6 + 0.3
Simulated Distribution	2.35	2.63	2.63	2.85	2.6 + 0.2
Reheat					
Conduction	2.48	2.75	2.75	2.70	2.7 + 0.1
Convection	2.63	2.73	2.75	2.90	2.8 + 0.1
Microwave radiation	2.33	2.60	2.78	2.38	2.5 + 0.2
Potatoes					
Precook	0.78	0.73	0.73		0.8 ± 0.0
Store	0.32	0.35	0.62		0.4 ± 0.2
Simulated Distribution	0.19	0.12	0.12		0.1 ± 0.0
Reheat					
Conduction	0.17	0.11	0.24		0.2 ± 0.1
Convection	0.18	0.11	0.16		0.2 ± 0.0
Microwave radiation	0.13	0.13	0.13		0.1 ± 0.0

[a] No adjustment for weight loss from processing

Table 7—Comparison of percent thiamin retention among three studies of cook/chill foodservice systems using identical formulations for beef loaf

Food Product Flow	Present Study	Boberg and David (1978b)	Dahl and Matthews (1980)
	Mean % retention of thiamin in beef loaf		
Preparation	100.0 (0.9)[a]	100.0 (0.8)	100.0 (0.9)
Precook	75.3	87.5	–[b]
Store	75.3	75.0	–
Portion	–	–	–
Distribute	71.6	–	79.7
Reheat			
Conduction	68.0	–	–
Convection	67.0	–	–
Microwave	63.0[c]	62.5[d]	71.8[e]

[a] μg thiamin per gram of beef loaf
[b] No measurement reported
[c] 68 sec; End point temperature (EPT) = 79°C.
[d] 90 sec; EPT = 74-75°C.
[e] 80 sec; EPT = 77°C.

Regardless of differences in EPT of precook, values for percent thiamin of beef loaf in this study were similar to values reported by Bobeng and David (1978b) (Table 7). Values reported by Dahl and Matthews (1980) were consistently higher than those reported in this study or in the study by Bobeng and David (1978) possibly indicating the effect of initial differences in raw materials.

Percent thiamin retention in peas. Precooking peas to 75°C resulted in a mean loss of 4% thiamin in pes compared to 25% loss due to precooking in beef loaf (Table 3). Storage, portioning and distribution showed an additional mean loss of 4% in thiamin content of peas.

Reheating resulted in a mean loss of 7% thiamin regardless of method. Analysis of variance indicated no significant differences in percent thiamin in peas due to reheat method. Mean thiamin losses due to reheating in peas were under 10% of values after preparation in all replications.

Percent ascorbic acid retention in potatoes. Maximum ascorbic acid content of potatoes was 0.74 g/g of product or 100% of values after precooking (Table 4). Storage of potatoes, both during 24 hr chilled storage (at ≤7°C) and 2 hr distribution (at ≤7°C), resulted in higher losses of ascorbic acid than did chilled storage of beef loaf(Table 2) or chilled storage of peas (Table 3) when thiamin was considered. Possible explanation of the decrease of ascorbic acid during storage of potatoes was the length of chilling from 67°C to 9°C. Each batch of potatoes prepared in this study was chilled in one pan measuring 30.4 cm wide x 61.0 cm long x 15.2 cm high.

Table 8—Percent of the Recommended Dietary Allowance supplied by 100 grams of experimental product during food product flow in a Cook/chill foodservice system

Food product flow	Nutrient and product		
	Thiamin in beef loaf	Thiamin in peas	Ascorbic acid in potatoes
	% of RDA[a] for US Males 19—21 yr		
Preparation	6[b,c]	17[b]	NA[d]
Precook	6	18	0.0125
Reheat			
Conduction	6	18	0.0040
Convection		18	0.0050
Microwave	6	17	0.0058

[a] Recommended Dietary Allowances (National Academy of Sciences, 1980)
[b] The RDA recommends 1.5 g/day of thiamin for US males aged 19—21 yr.
[c] Micrograms of nutrient per gram of experimental product (Table 2, 3, or 4) times 100g divided by RDA recommendation for that nutrient US males 19—21 yr) times 100 equals percent RDA for US males listed in this table.
[d] The RDA recommends 60g/day of ascorbic acid for US males aged 19—21 yr.

Although chilling time of potatoes was not calculated in this study, Dahl and Matthews (1980) reported chilling time of 8 hr for 5.68 kg of instant mashed potatoes stored in a pan of similar length and width. Center internal temperature of potatoes in the pan did not ever reach ≤7°C/45°F even after 24 hr of chilled storage. Such temperature recordings have microbiological implications.

Nineteen percent of original ascorbic acid present in potatoes remained after portioning and distribution. Handling as well as storage time and temperature appear to affect ascorbic acid content of potatoes in this study. Microwave reheating potatoes resulted in an additional mean loss of 4%. Potatoes that were reheated by a conduction subsystem to 79°C did not demonstrate additional loss of percent ascorbic acid. Analysis of variance indicated no significant differences among values for percent thiamin retained by potatoes after reheating. Losses of ascorbic acid in potatoes due to reheating were less than 10% of precook values in all replications.

DISCUSSION

STATISTICALLY SIGNIFICANT DIFFERENCES did not exist among values for percent retention of thiamin or ascorbic acid in beef loaf, peas and potatoes when conduction, convection and microwave radiation were used as reheat methods in a simulated cook/chill foodservice system. When nutrient retention is considered, use of conduction, convection or microwave reheat subsystems appears to have resulted in similar nutrient quality in food. On the other hand, Ang et al. (1975) concluded that microwave ovens which required shorter heating times tended to retain greater amounts of heat-liable nutrients. In contrast, mean values for percent thiamin retained in beef loaf and for percent ascorbic acid retained in potatoes were lowest of three reheat subsystems when reheated by microwave energy (Tables 2, 3, and 4).

Differences in nutrient retention of foods reheated in three subsystems would appear to have little or no effect on the diet of an average person. Table 8 shows the nutrient contribution of beef loaf, peas, or potatoes to the Recommended Daily Allowance of a US Male (National Academy of Science, 1980). Data in Table 8 indicate that only a 1% change in contribution to the daily nutrient recommendations was caused by differences among reheat methods in this study. Thus, reheat methods in cook/chill systems may be considered comparable when nutrient retention in beef loaf, potatoes or peas of similar formulation is considered.

Discrepancies exist between values for nutrient intake at point of service which are published in books of nutrient composition (Pennington and Church 1980; Watt and Merrill, 1963) and those calculated from samples of experimental products in the present study (Table 9). For example, micrograms of ascorbic acid per gram of potatoes was 0.30 at reheat in this study but handbooks of food composi-

Table 9—Comparison of nutrient values determined in the present study and those in two commonly used handbooks of food preparation

Food product flow	Present study			Watt and Merrill (1963)			Pennington and Church (1980)		
	Beef loaf	Peas	Potatoes	Beef loaf	Peas	Potatoes	Beef loaf	Peas	Potatoes
	μg thiamin/g		μg ascorbic acid/g	μg thiamin/g		μg ascorbic acid/g	μg thiamin/g		μg ascorbic acid/g
Preparation	0.9	2.49	—	0.90	1.45	—	—	—	—
Precook	0.87	2.66	0.75	0.90	—	0.70	0.70	2.70	.60
Store	0.88	2.59	0.57	—	—	—	—	—	—
Portion	—	—	—	—	—	—	—	—	—
Distribution	0.85	2.62	0.32	—	—	—	—	—	—
Reheat (serve)	0.86	2.64	0.30	—	—	—	—	—	—

tion (Table 9) would suggest that the actual value at service was twice the amount or 0.6g ascorbic/g of potatoes. Other authors comment on the importance of re-evaluating tables in handbooks of food composition for use in foodservice settings (Lachance et al., 1973; Dahl and Matthews, 1980).

Based on their study, Dahl and Matthews (1980) recommended calculation of maximum 10% loss in thiamin of beef loaf due to microwave reheating in cook/chill foodservice systems with similar characteristics of food product flow. In the present study, loss of nutrients due to reheating averaged: beef loaf, 6%; peas, 7%; and potatoes, 2%. In addition, loss of nutrient during chilled storage averaged: beef loaf, 0%; peas, 5%; and potatoes, 43% (Tables 2, 3, and 4). Also, losses of nutrients during portioning and distribution averaged; beef loaf 4%; peas, 0%; and potatoes, 38%. Nutrient losses recorded in the present study indicate that although the adjustment factor of 10% for nutrient loss suggested by Dahl and Matthews (1980) would be adequate to cover reheat losses of thiamin in beef loaf, other nutrient losses during additional steps in food product flow in cook/chill systems may necessitate even greater adjustment factors. Research is needed to determine appropriate nutrient adjustment factors for foods prepared in cook/chill and cook/freeze foodservice systems.

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Destruction of Trichina Larvae in Beef-Pork Loaves Cooked in Microwave Ovens

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ABSTRACT

Beef-pork loaves containing *Trichinella spiralis* larvae were cooked in two household ovens (2450 MHz). Loaves were molded into ring (R), oval (O) or oblong (L) shapes. After cooking, internal temperatures were measured at five locations in each loaf. Cooking losses were 11–28% and were greatest in L loaves. Longer cooking time was required for L loaves to attain the same degree of doneness as of R and O loaves. Variations in energy distribution patterns within each oven were evident in the percentage of underdone and overdone meat and in the temperatures in each of the duplicate loaves. Bioassays indicated that infective trichinae remained in 8 of the 30 samples tested. Five of the positive bioassays were found in samples from L loaves, two in O loaves, and one in R loaves.

INTRODUCTION

THE DRAMATIC INCREASE in microwave ovens in American homes reflects a high interest in quick meals and in time saving and energy efficient appliances. Because 54% of the married women working have children, time is becoming a major factor and products are needed that save time and energy. Estimates indicate that by 1985 microwave ovens will be in 50% of all homes (Boutin, 1978; Anonymous, 1980). In a survey, Gast et al. (1980) found that the predominant use of microwave ovens is reheating and (or) defrosting (36%). Also types of meat, prepared at least once in the microwave oven by the 400 respondents, were ground beef (65%), beef cuts (57%), chicken (57%) and pork (46%).

Special attention must be given to the uneven distribution of power that results in hot and cold spots in the microwave oven cavity (Ring and David, 1975; Ohlsson, 1976). According to Van Zante (1973) cooking power in a microwave oven is not distributed evenly from both a two- and three-dimensional consideration. In 1978, Ohlsson and Risman stated that nonuniform distributions, with hot and cold spots with seemingly unpredictable patterns occur because microwaves, like other electromagnetic waves, are subject to refractions and reflections at the boundaries between dissimilar materials.

To adjust to this shortcoming and to equalize the food temperature, some manufacturers recommend rotating the pan 1/4 or 1/2 turn during cooking, removing the food for short rest periods or letting food stand for 5 or 10 min after cooking. Such procedures detract from the convenience image and prolong cooking time; also, some users may not read or follow directions. Some manufacturers have models that feature a turntable on the floor of the oven that rotates food while it is cooking on the premise that the uneven cooking effects will be eliminated or noticeably reduced by the constant movement. Tests made on ovens with turntables (Consumers' Union, 1981) indicated some improvement, but even these ovens did only a fair job of cooking evenly inasmuch as the open-faced cheese sandwiches near the outer edge of the turntable cooked faster than those in the center.

Some oven models have a temperature probe, thermoprobe, that works well with liquids that can be stirred, but in solid or dense foods (of different sizes and shapes) that have hot and cold spots of unknown location, the difficulty lies in deciding where the one thermoprobe should be placed during cooking. Thermoprobes in 12 ovens were tested for accuracy at 150°F by Consumers' Research (1979). Only three gave correct readings, seven were satisfactory (within ± 4°F), and two deviated more than ± 4°F. Consumers' Union (1981) had 23,000 readers reply to a questionnaire for owners of microwave ovens, and of those who have an oven with a thermoprobe, very few said they used it often.

Although the mean annual incidence of trichinosis has decreased markedly, it remains a public health problem. During the last decade, the annual mean of cases reported was less than 150. But in 1975, 284 cases were reported. Of the 135 cases reported in 1979, the major source of infection in 93 (74%) cases was pork, mainly in sausage (CDC, 1980). Because cattle are herbivorous, they are not a natural reservoir of *Trichinella spiralis;* but ground beef was identified as the probable source of infection in 34 cases in 1975 and five in 1979. Adulteration of beef may occur through use of a common grinder or through intentional mixing of beef and pork. A survey (CDC, 1976) in 12 states showed pork adulteration in 6% of 231 ground beef samples and in 8% of the 136 retail markets surveyed.

Meat loaf recipes in which pork (30–50%) is included as one of the ingredients can be found in many cookbooks; for example, Rombauer (1974), Better Homes and Gardens (1976) and Claiborne (1970). Also some supermarkets sell a mixture of ground beef and pork labeled "meat loaf meat."

The exact thermal death point of *T. spiralis* has not been established. It is likely that killing of the larvae depends on rate of heat penetration. Carlin et al. (1969) reported that digestion tests and bioassays were negative on samples from trichina-infected rib and loin roasts cooked in conventional ovens at 200°, 325° and 350°F to end-point temperatures of 140°, 145°, 150° or 160°F. In roasts cooked at 200°F, relatively few trichinae/gram were found in the bioassay tests of those cooked to 130°F, and none in those cooked to 135°F. On the other hand, for roasts cooked at 350°F to the same end-point temperatures, 130° and 135°F, both the digestion tests and bioassays indicated samples contained viable trichinae. Leon Crespo and Ockerman (1977) concluded that the reason microwave heating was not as effective as conventional heating in destroying some microorganisms was because the time to reach the designated internal temperature was much faster for microwave cooking, thus having less killing effect. No studies have been published on the effects of microwave cooking on trichinae in ground pork. The objective of this research was to determine the effect of power settings, cooking time

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and shape of loaf on evenness of cooking, temperature variations and survival of *T. spiralis* in beef-pork loaves cooked in microwave ovens from two manufactures. Cooking losses, internal temperatures at five locations and amount of underdone and overdone meat were measured. Our purpose did not include a determination of the thermal death point of trichina larvae.

EXPERIMENTAL

GROUND CHUCK (not less than 80% lean) was purchased fresh from a supermarket in quantities sufficient for the 8–10 loaves cooked each day. Lean ground pork from hogs, experimentally infected with 250,000 *T. spiralis* larvae 6 wk or more before slaughter, was obtained from the Iowa State Univeristy Veterinary Medical Research Institute (VMRI).

Each meat loaf contained ground chuck, 450g; trichina-infected, ground pork, 230g; reconstituted milk, 120g; dry bread crumbs, 60g; whole eggs, 100g; and salt, 6g. Ingredients were combined in a Kitchen Aid mixer (model K-5A) by manual and mechanical mixing as described by Ziprin and Carlin (1976).

Oven-proof clear glass is the best material for use in microwave ovens; changing the geometry of the food load by varying dish shapes may cause different results both in cooking time and in product (Van Zante, 1973). Thus, meat loaves were made in three shapes in the appropriate Pyrex pan, namely: (1) ring (R), meat mixture was molded around a Pyrex custard cup (2½-in. diameter) placed in the center of a 9-in. pie plate; (2) oval (O), meat mixture molded in a 8½ x 4½ x 3 in. loaf pan and then unmolded into a 10 x 6 x 2 in. pan; and (3) oblong loaf (L), molded and cooked in the 8½ x 4½ x 3 in. loaf pan. Sixteen loaves were made for each of the three shapes, or a total of 48 loaves.

The two houshold ovens (2,450 MHz) used were obtained from two manufacturers: Oven A (650 watts) had five power settings, and oven B (625 watts) had three power settings.

Because three shapes of loaves were to be studied, preliminary experiments were necessary to determine the cooking times required at each power setting. After the 60 meat loaves were cooked for various periods of time at different power setting in the preliminary study, measurements were made of final internal temperatures at five locations, cooking losses, amounts of underdone and overdone meat and appearance of the loaves were evaluated.

For the present study, three of the five power settings on Oven A and one of the three on Oven B were selected. Approximate power ratings as determined by Decareu's method described by Van Zante (1973) were: Oven A: HIGH, 595 watts, MEDIUM HIGH, 490 watts and MEDIUM, 370 watts; Oven B: HIGH, 575 watts. The cooking times used at each power setting are listed in Table 1. Replicate loaves were made for the shorter and shortest cooking times. Only a single loaf was made at the maximum cooking time because preliminary experiments showed these loaves were overcooked and dried out. Hard meat often constituted 25–50% of the loaf, and temperatures were 150-169°F in the small amounts of underdone meat. A total of 33 loaves was tested in Oven A and 15 in Oven B.

Before cooking, each loaf weighed 956g, and the initial temperature was 50–54°F. Temperature was measured with iron constantan thermocouples (encased in hypodermic needles) and a recording potentiometer (Honeywell Electronik 16).

The position of the pan in the oven was exactly the same for all loaves. When loaves were removed from the oven, the top surface had areas that were rare, medium-well-done and overdone (very dry). Three thermocouples were inserted in the rare area at depths of ¼, ½ and1 in. for R loaves and ½, 1 and 1½ for O and L loaves. A fourth thermocouple was placed in a medium-well-done area, and a fifth in an overdone area. The position of each thermocouple was recorded in a diagram (the shape of each loaf) in the data book. The five temperatures were recorded. After 1 min, the second reading was taken for each thermocouple.

After the temperatures were recorded, weights of loaf, pan and drip, necessary for calculation of cooking losses, were obtained. Probably, during these measurements, the internal temperature of the loaves continued rising. Preliminary experiments indicated internal temperatures 10 min after loaves were removed from the microwave ovens increased 15–25°F. Each loaf was cut into several sections, and samples of the rare meat were transferred to a labeled sample bag and weighed. The weights necessary for calculation of underdone and overdone portions of the loaves were obtained.

Bioassays by rat inoculation were carried out on 18 composite samples of duplicate loaves and on 12 single loaf samples. For the bioassays, the 100g cooked meat samples were finely ground, then digested in 1% peptin-1% HCl solution for 4 hr (Zimmermann, 1974). After the residue was allowed to settle in a graduated cone and was washed free of digestive fluid to remove all acid, the final

Table 1—Total cooking losses and underdone and overdone meat in beef-pork loaves as influenced by ovens, power settings and cooking times

	Shape of loaf											
	Ring				Oval				Loaf			
Power setting	Cooking time min	Total cooking loss %	Underdone meat %	Overdone meat %	Cooking time min	Total cooking loss %	Underdone meat %	Overdone meat %	Cooking time min	Total cooking loss %	Underdone meat %	Overdone meat %
						Oven A						
HIGH	11	13	8	4	13	14	16	19	17	21	11	30
		13	12	8		16	15	15		20	11	29
	13	18	5	8	17	20	4	20	22	26	5	40
MEDIUM HIGH	12	11	15	3	16	15	13	12	20	19	16	22
		13	19	4		15	18	12		18	16	24
	14	13	10	4	18	18	10	16	24	23	8	28
		15	10	5		18	7	18		23	7	30
	16	16	3	7	20	20	4	21	26	25	3	40
MEDIUM	17	12	13	3	20	13	22	5	26	16	8	27
		13	17	1		13	18	7		17	15	15
	20	17	3	4	24	16	8	9	34	23	1	25
						Oven B						
HIGH	13	14	13	10	17	18	14	21	20	22	10	36
		14	8	8		17	12	20		23	11	33
	15	16	8	13	20	24	8	26	22	28	4	43
		16	3	13		27	0	23		28	10	42
	17	20	2	16	22	27	0	48	24	27	2	49

wash was siphoned down to 5 ml total volume and administered to two rats by stomach tube. The rats were sacrificed 5 wk later and examined for trichinae by using the digestive process. Samples of rat tissue were finely ground and digested for 4 hr in a 1% pepsin-1% HCl solution. After settling for 30 min in a Baermann funnel, a portion of the digestive fluid and residue was drawn into a Syracuse watch glass and examined microscopically for viable trichina larvae.

RESULTS & DISCUSSION

COOKING LOSSES were similar and often identical for duplicate loaves (Table 1). Most of the total cooking losses were caused by evaporation. Drip losses, for all loaves regardless of cooking treatment, usually were 4–6%. An increase in cooking time at each power setting caused an increase in cooking losses, but the increase was greater in loaves cooked at HIGH than in those cooked at MEDIUM HIGH or MEDIUM. For loaves cooked in oven A, regardless of power settings or cooking times, the range in cooking losses for R and O loaves was 11–20%, and for L loaves, 16–26%. Hence, shape of loaf had a greater effect on cooking losses than did the other variables studied.

Loaves that received the same cooking treatment did not always have the same percentage of underdone and overdone meat (Table 1). Increasing the cooking time decreased the amount of underdone meat and increased the overdone meat considerably, especially for L loaves and for all loaves cooked in oven B. The percentage of underdone meat in most loaves did not seem to be affected by shape of loaf, but the percentage of overdone meat was least in R loaves (1–16%) and greatest in L loaves (15–49%).

The range in temperatures among the three thermocouples placed at different depths in the rare area (summarized in Table 2) indicated great variability between loaves that received the same treatment. For example in the L loaf cooked for 20 min in Oven B, temperatures were 126, 134 and 192°F, and for the duplicate loaf, 138, 132 and 169°F at ½, 1 and 1½ in. deep, respectively; or for duplicate R loaves cooked for 11 min in Oven A, temperatures were 147, 153 and 136 or 138, 131 and 154°F at depths of ¼, ½ and 1 in., respectively. Katz (1977) states that variations in energy distribution patterns occur; not only between brands of ovens for the same power setting, cooking time and food load, but also at times within the same model of one brand. According to Schiffmann (1979), the fanlike stirrer does mix up the pattern or even it out, but it is only partially effective, resulting in areas of concentration of energy or no energy. Also, each oven has a different pattern that changes as the product reaches different temperatures. Such variations, and the many uncontrollable factors that can affect the internal temperatures in loaves, make it difficult, if not impossible, to draw conclusions or make recommendations regarding power settings and cooking times–to say nothing of determining the effect of microwaves on viability of *Trichinella spiralis* in pork.

For duplicate loaves cooked in oven A, the difference between the low and high temperatures within underdone areas varied from 1–17°F, but for those cooked in oven B, the difference in temperature was from 1–23°F (Table 2). As expected, increasing the cooking time at each power setting increased both the low and high temperatures within the rare area. Shape of loaf affected the location of the low and the high temperatures in the rare meat. Examination of the data on location of the temperatures measured at the different depths in the 16 loaves cooked for each shape revealed that for R loaves, 56% of the low temperatures were ¼ in. below the top surface and 38% were ½ in. below; for O loaves, 56% at ½ in. and 44% at 1 in.; and for L loaves, 75% at 1 in. and 19% at 1½ in. For high temperatures, 31% were at ½ in. and 56% at 1 in. for R loaves; 94% were at 1½ for O loaves; and 19% at ½ in. and 75% at 1½ in. for L loaves. The location of the rare area in the O and L loaves was not in the center but was about 1/3 of the way into the loaf from the end of the pan. The meat in the corners of the loaf pans was very overcooked, hard and dry. Temperature readings taken in the overdone areas of the meat loaves

Table 2–Range[a] in temperatures at various depths in the underdone meat in beef-pork loaves cooked in two microwave ovens at various power settings for various cooking times

	Shape of loaf					
	Ring		Oval		Loaf	
Power setting	Cooking time min	Temp. range °F	Cooking time min	Temp. range °F	Cooking time min	Temp. range °F
			Oven A			
HIGH	11	136 – 153 131 – 154	13	118 – 156 135 – 185	17	126 – 152 117 – 130
	13	149 – 156	17	146 – 169	22	146 – 156
MEDIUM HIGH	12	142 – 154 144 – 148	16	136 – 161 126 – 159	20	116 – 130 119 – 124
	14	138 – 150 135 – 157	18	140 – 170 135 – 160	24	137 – 158 140 – 149
	16	152 – 160	20	154 – 186	26	151 – 156
MEDIUM	17	129 – 139 133 – 137	20	137 – 141 138 – 144	26	136 – 140 132 – 140
	20	149 – 157	24	151 – 167	34	162 – 184
			Oven B			
HIGH	13	128 – 150 138 – 144	17	124 – 148 125 – 158	20	132 – 169 126 – 192
	15	134 – 160 149 – 153	20	147 – 172 170 – 200	22	144 – 154 123 – 156
	17	151 – 178	22	162 – 200	24	157 – 184

[a] Thermocouples inserted into the rare area on the surface of cooked loaves at depths of ¼, ½ and 1 in. for R loaves and ½, 1 and 1½ for O and L loaves.

indicated that, in R loaves, the range was 182–210°F with an average of 202°F, and in L loaves, the range was 184–211°F with an average of 208°F.

The shape of loaf had more effect on physical qualities of the meat loaves than any of the variables investigated. Although the mass (956g) was the same for all loaves, the geometry was different: doughnut-shaped, oval and oblong. L loaves required from 6–14 min longer cooking time at each power setting to reach temperatures in the rare areas comparable to those found in R loaves that were cooked for shorter periods of time. L loaves had greater cooking losses and more overdone meat that was higher in temperature than did R loaves. There also was variability in the various parameters measured in loaves treated alike. This was particularly noticeable in the low temperatures recorded for loaves that received the same cooking treatment (Table 2).

Results of the bioassay, summarized in Table 3 for 18 composite samples of duplicate loaves and 12 single samples, indicated that infective larvae were present in 8 of the 30 samples tested. With the exception of the L loaf cooked for 24 min in oven B, all of the positive samples were from loaves cooked for the shorter times at each power setting. Low temperatures in these loaves were 116–144°F. Shape of loaf significantly affected the persistence of infective trichinae inasmuch as five positives were found in samples from L loaves, two from O loaves, and one from R loaves.

When evaluating the data, one should keep in mind that the temperatures given in Table 2 for the loaves do not necessarily represent the temperature of the samples used in the bioassays. Two temperatures were recorded for each of the five thermocouples inserted into each loaf. Only the second temperature has been reported, but increases of 0–10°F occurred between the first and second readings taken 1 min apart. The bioassay samples were removed some 3 or 4 min after the temperature readings were taken because, during this time, data had to be obtained on the whole loaf for calculation of cooking losses. If cooking continued while the loaves were weighed, the temperatures given in Table 2 would not represent the temperature of the loaves at the time the bioassay samples were taken. A second reason relates to the bioassay sampling procedure. Loaves were cut open and samples of the rare meat removed, but the meat may not have been in the same location where temperatures were obtained. Furthermore, in making the composite sample from loaves treated alike the amount of rare meat taken from each loaf might not have been the same. If our objective had been to determine the thermal death point of trichina larvae, our procedure would have been different.

Table 3–Results of bioassays[a] for viable trichinae in composite samples of duplicate beef-pork loaves as influenced by ovens, power settings and cooking times

	Shape of Loaf					
	Ring		Oval		Loaf	
Power setting	Cooking time min	Bio-assay	Cooking time min	Bio-assay	Cooking time min	Bio-assay
			Oven A			
HIGH	11	–	13	+	17	+
	13	–[b]	17	–[b]	22	–[b]
MEDIUM HIGH	12	–	16	–	20	+
	14	–	18	–	24	–
	16	–[b]	20	–[b]	26	–[b]
MEDIUM	17	+	20	–	26	+
	20	–[b]	24	–[b]	34	–[b]
			Oven B			
HIGH	13	–	17	+	20	–
	15	–	20	–	22	+
	17	–[b]	22	–[b]	24	+[b]

[a] +, meat loaf sample cuased trichina infection in rats; –, did not cause infection.
[b] Bioassay of one meat loaf.

One possible solution to the problem of areas of rare meat and low temperatures that result from cold spots or uneven cooking in microwave ovens is to use higher power settings and (or) increase the cooking time, but our results showed that five of the positive samples were cooked at the HIGH setting (two in oven A and three in oven B). Increasing the cooking time resulted in increased amounts of hard and dry meat, thus, the possibility exists that the loaf will be removed to prevent this, and the amount of rare meat in the loaf will be increased.

Another solution for equalizing the food temperature is turning the pan (1/4 or 1/2 turn) during cooking or removing the food for short rest periods or letting the food stand for 5 or 10 min after cooking. Zimmermann and Beach (1982) reported that infective trichinae persisted in five of the 28 roasts cooked in six microwave ovens by following complete recommendations of the manufacturers for cooking time, power, turning and standing. Some ovens have a turntable to rotate the food during cooking. Zimmermann and Beach (1982) reported that, although a microwave oven with a turntable was used, infective trichinae remained in a 6-lb pork roast cooked 51 min and a standing time of 15 min.

Microwave ovens are available with thermoprobes, but tests by Consumers' Research (1979) indicated that only three of the 12 thermoprobes in the ovens gave correct readings at 150°F. Furthermore, Zimmermann and Beach (1982) found that a roast contained infective trichinae after being cooked in a microwave oven with use of a thermoprobe and even though complete manufacturer's recommendations were followed.

The uneven cooking and presence of cold spots in the microwave ovens caused areas of rare meat and raises serious questions as to the safety of ground meat loaves, not only in regard to viability of trichinae, but also to destruction of bacteria. Ground meat often has large numbers of bacteria present. Fung and Cunningham (1980) reported that significant numbers of *Escherichia coli* survived in hamburger patties cooked in microwave ovens. They also found approximately a 30-fold difference in survival between the center (0.7%) and the edge (20%) of the patties.

This study has indicated that infective trichinae are found in beef-pork loaves after cooking in microwave ovens. Manufacturers are constantly striving to improve the energy distribution in the oven cavity that is critical to even cooking. Recent improvements, such as rotating trays and thermoprobes, have increased the versatility of the oven, but have not solved the problem. Until a new technology is developed to eliminate the uneveness of cooking, the only recommendation is not to cook pork in a microwave oven.

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Relationship of USDA Maturity Groups to Palatability of Cooked Beef

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ABSTRACT

In comparison to carcasses of B, C or E maturity, carcasses of A maturity produced broiled steaks which: (a) had higher ($P < 0.05$) palatability ratings in 62 to 86% of comparisons, (b) were decidedly less variable (C.V.), (c) were more likely to be assigned high (≥ 6.00) and less likely to be assigned low (≤ 2.99) sensory panel ratings, and (d) were more likely to have low (≤ 3.63 kg) and less likely to have high (≥ 6.35 kg) shear force values. Position within the A or A + B maturity group(s) explained 0–4% (loin steaks) and 10–18% (round steaks) of the observed variation in overall palatability ratings and/or shear force values.

INTRODUCTION

THE OFFICIAL United States Standards For Grades of Carcass Beef (USDA, 1975) specify that quality of lean in beef is evaluated by considering its marbling and firmness as observed in a cut surface in relation to carcass evidences of maturity. Maturity of a carcass is determined by evaluating the size, shape and ossification of bones and cartilages – especially the split chine bones – and the color and texture of the lean flesh; the standards recognize five different maturity groups – A, B, C, D and E in order of increasing maturity (USDA, 1975). USDA grade standards assume that advances in carcass maturity result in decreases in palatability of cooked beef. There are reports of substantial differences in tenderness of beef from very youthful vs very mature (for example, A vs E maturities) beef carcasses (Romans et al., 1965; Goll et al., 1965; Walter et al., 1965; Breidenstein et al., 1968; Schmidt et al., 1970; Berry, 1972; Prost et al., 1975), but among youthful carcasses (for example, A vs B maturities) research suggests much less difference in tenderness (McBee and Wiles, 1967; Breidenstein et al., 1968; Covington et al., 1970; Norris et al., 1971; Berry, 1972; Berry et al., 1974; Tatum et al., 1980). Bouton et al. (1978) reviewed the literature on this subject and reported three categories of conclusions – that age had positive, negative or no effects on beef tenderness – with considerable support for each effect; their own research in this regard suggested that results of age/tenderness studies depend in part on the age range studied and upon the muscle(s) studied.

The present study evaluates the relationship of carcass maturity to cooked beef palatability using carcasses selected from several commercial plants and across the complete range of maturity recognized in USDA (1975) beef grade standards.

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EXPERIMENTAL

BEEF CARCASSES (N = 1005) were selected from 8 packing plants in 6 states. Carcasses were chosen by concensus of a committee of 3 persons highly trained in beef carcass grading (1 selector was from Texas A&M Univ.; 2 were from the Standardization Branch, Livestock Division, AMS-USDA). Each member of the committee independently assessed the overall maturity and marbling of each carcass. Evaluations were made in percentages (e.g., A^{40} and $Small^{60}$) and the average of the 3 selector's evaluations was used as the basis for selection. However, a carcass was not used if any selector's score for marbling or maturity was more than ±40% of a degree from the average score for either trait. Carcasses usually were selected on the second day postmortem; evaluations were made on sides ribbed about 1 hr prior to their selection.

The number of carcasses from which loins or rounds were obtained in each maturity-marbling cell is shown in Table 1. The experimental design was based on (a) degree of marbling (Moderately Abundant through Practically Devoid) in the surface of the longissimus dorsi muscle (12th-13th ribs) and (b) maturity score, in 4 of the 5 overall carcass maturity groups (A, B, C and E) as described in the Official United States Standards For Grades of Carcass Beef (USDA, 1973). Although carcasses were selected on the basis of USDA (1973) grade standards, maturity groups and maturity X palatability relationships discussed in this paper are based on maturity as described in USDA (1975) grade standards. Maturity traits were scored using a 500-unit scale ($100 = A^{00}$; $599 = E^{99}$). An overall skeletal maturity score and an overall lean (longissimus dorsi muscle) maturity score were combined to obtain a score for overall carcass maturity.

Each of the 32 large selection cells in the experimental design (Table 1) was further divided into 9 subcells (based on thirds of maturity groups and thirds of marbling scores); in each selection cell, 10% of the carcasses were in each of the outside subcells while the remaining 20% of the carcasses were in the center subcell. Loins were obtained from all 1005 of the carcasses.

Rounds (from 347 of the 1005 carcasses) also were used for sensory panel and shear force evaluations (Table 2). For rounds, each of the 32 large selection cells was further divided into 9 subcells (based on thirds of maturity groups and thirds of marbling scores); 11–17% of the rounds were in each of the 9 subcells. A loin or a hindquarter from each carcass was shipped via refrigerated truck to the Texas A&M Univ. Meat Laboratory where it was stored at 1 ± 1°C. On the 10th–14th day postmortem, loins and/or rounds were cut into steaks. At that time, each wholesale short-loin was faced to remove dried muscle surfaces and the bone-in, strip-loin was removed and cut into steaks. Two steaks, 1.27 cm in thickness, were removed and used for other studies. The remaining portion of the strip-loin was cut into at least 8 bone-in, 2.8 cm thick, steaks; each steak was trimmed to remove external fat in excess of 0.65 cm, double-wrapped in polyethylene-coated paper, frozen (–34°C) and stored (–23°C).

At the time of cutting, 4 of the anterior 8 steaks from each strip-loin were assigned to Texas A&M Univ.; the remaining 4 of the 8 steaks were assigned to either Iowa State Univ., Colorado State Univ. or the Meat Science Research Laboratory (MSRL) of USDA. Allocation of these 8 steaks was completely randomized; at the conclusion of carcass selection, each cooperator had been assigned 4 steaks from each of 335 strip-loins. Frozen steaks were air-shipped to each cooperator in plastic-foam and corrugated fiberboard containers; shipment time did not exceed 12 hr.

Table 1 — Number of carcasses in each maturity-marbling cell from which loins or rounds were obtained

Marbling score	Overall maturity score A	B	C	E	TOTAL
Moderately abundant	34[a](12[b])	31 (9)	19 (9)	18 (9)	102 (39)
Slightly abundant	43 (12)	30 (9)	21 (9)	21 (9)	115 (39)
Moderate	58 (18)	41 (12)	20 (9)	21 (9)	140 (48)
Modest	60 (18)	43 (12)	22 (9)	21 (9)	146 (48)
Small	61 (18)	42 (11)	21 (9)	19 (9)	143 (47)
Slight	61 (18)	42 (12)	21 (9)	21 (9)	145 (48)
Traces	45 (12)	32 (9)	20 (9)	20 (9)	117 (39)
Practically devoid	37 (12)	18 (9)	22 (9)	20 (9)	97 (39)
TOTAL	399 (120)	279 (83)	166 (72)	161 (72)	1005 (347)

[a] Number of carcasses from which loins were obtained.
[b] Number of carcasses from which rounds were obtained.

Table 2 — Mean sensory panel ratings for loin (longissimus dorsi muscle) steaks from carcasses assigned to each lean maturity, skeletal maturity or overall maturity group

USDA trait and maturity group	Number of carcasses	Sensory panel rating[f] Flavor	Juiciness	Amount of connective tissue	Tenderness	Overall palatability
Lean maturity						
Calf	4	5.67[a]	4.90[d]	7.01[a]	6.55[a]	5.82[a]
A	547	5.64[a]	5.06[d]	6.53[a]	5.82[a]	5.44[a]
B	174	5.39[a]	5.15[cd]	6.21[b]	5.55[a]	5.19[a]
C	127	4.89[a]	5.23[bc]	5.75[c]	4.90[b]	4.58[b]
D	55	4.18[b]	5.43[a]	4.85[d]	4.14[c]	3.73[c]
E	98	4.01[b]	5.34[ab]	4.69[d]	3.98[c]	3.52[c]
Skeletal maturity						
A	361	5.72[a]	5.02[d]	6.68[a]	5.99[a]	5.57[a]
B	214	5.45[b]	5.11[cd]	6.37[b]	5.63[b]	5.26[b]
C	194	5.21[c]	5.17[bc]	6.07[c]	5.32[c]	4.94[c]
D	85	5.07[c]	5.31[ab]	5.60[d]	4.90[d]	4.67[d]
E	151	4.08[d]	5.35[a]	4.69[e]	3.95[e]	3.56[e]
Overall maturity						
A	384	5.72[a]	5.02[d]	6.66[a]	6.00[a]	5.57[a]
B	221	5.42[b]	5.12[c]	6.36[b]	5.61[b]	5.24[b]
C	239	5.17[c]	5.23[b]	5.93[c]	5.15[c]	4.85[c]
E	161	4.10[d]	5.35[a]	4.69[d]	3.98[d]	3.58[d]

[abcde] Means in the same column and for the same USDA trait (e.g., lean maturity) bearing a common superscript letter are not significantly different ($P > 0.05$).
[f] 8 = extremely desirable in flavor, extremely juicy, no connective tissue, extremely tender and extremely desirable in overall palatability; 5 = slightly desirable in flavor, slightly juicy, slightly tender, slight amount of connective tissue and slightly desirable in overall palatability; 4 = slightly undesirable in flavor, slightly dry, slightly tough, moderate amount of connective tissue and slightly undesirable in overall palatability; 1 = extremely undesirable in flavor, extremely dry, abundant connective tissue, extremely tough and extremely undesirable in overall palatability.

Texas A&M Univ. and each cooperator (Iowa State Univ., MSRL-USDA, Colorado State Univ.) selected 10 sensory panel members by use of the training-testing procedure of Cross et al. (1978). Ten panelists at each location were selected whose F-ratios were in close agreement with those of 2 experts at each location and whose session and day effects were minimal; 2 additional panelists, at each location, were used as alternates as needed. Following 4 months of intensive training and practice, the trained panels at all 4 locations began sensory evaluation of loin steaks.

Groups of 10 loin steaks (2 steaks from each of 5 loins) were thawed (6°C) for 24 hr, copper-constantan thermocouples were inserted and steaks were cooked to an internal temperature of 70°C on Farberware Open-Hearth electric broilers. During cooking, internal temperature of each steak was monitored every 60 sec – cooking time varied from about 20–35 min. Steaks were cooked such that a pair of steaks from the same loin reached the desired internal temperature about every 4 min. Two warm samples (9 sq cm) from the longissimus dorsi (LD) muscle of each steak were presented to each panelist at the same time. The 2 samples were independently scored and then mentally averaged by each panelist for flavor, juiciness, tenderness, amount of connective tissue and overall palatability by use of 8-point rating scales (8 = extremely desirable in flavor, extremely juicy, extremely tender, no connective tissue and extremely desirable in overall palatability; 1 = extremely undesirable in flavor, extremely dry, extremely tough, an abundant amount of connective tissue, and extremely undesirable in overall palatability, respectively). Warner-Bratzler shear force was measured in duplicate on 4 cores (1.27 cm in diameter) which were removed (parallel to the orientation of the muscle fibers) from the dorsal half of 1 of the 2 steaks after it had cooled to 25°C. For loin steaks from a given carcass, panel ratings and shear force values obtained by Texas A&M University and by the applicable cooperator were averaged.

Rounds were fabricated to produce a top round (semimembranosus, SM), a bottom round (biceps femoris, BF) and an eye of round (semitendinosus, ST). Each muscle (SM, BF, ST) was faced to re-

move dried lean surfaces and 4 steaks (2.8 cm in thickness) were cut from each muscle. Inidividual steaks were wrapped in polyethylene-coated freezer paper, frozen (−34°C) and stored (−23°C). The same sensory panel that evaluated loin steaks at Texas A&M Univ. also evaluated the palatability of cooked semimembranosus muscles; round muscles were not evaluated by any of the cooperators.

Groups of 10 top round steaks (two steaks from each of five rounds) were thawed (6°C for 24 hr), cooked to an internal temperature of 70°C on Farberware Open-Hearth electric broilers and cut into 9 sq cm pieces. Two pieces from each top round steak were scored by each panelist for flavor, juiciness, tenderness, amount of connective tissue and overall palatability in the manner and by use of the 8-point rating scales described above. Bottom round steaks and eye of round steaks were cooked in exactly the same manner as were top round steaks and used for shear force determinations. Warner-Bratzler shear force was measured in duplicate on 4 cores (1.27 cm in diameter) which were removed (parallel to the orientation of the muscle fibers) from one half of 1 of the 2 top round steaks and from each of the bottom round and eye of round steaks after the steaks had cooled to 25°C.

Regression analysis, analysis of variance and mean separation (Duncan, 1955) analysis were used to analyze the data; these analyses were performed using the Statistical Analysis Systems procedures of Barr et al. (1976). In addition, certain sensory panel ratings and shear force values were sorted into relative-magnitude groups and evaluated without use of statistical inference.

RESULTS & DISCUSSION

MEAN SENSORY PANEL ratings for loin and top round steaks and mean shear force values for loin, top round, bottom round and eye of round steaks are presented in Tables 2, 3 and 4. When carcasses were assigned to maturity groups solely on the basis of color and texture of the lean, there were differences ($P < 0.05$) in favor of steaks from carcasses of A, as compared to B, maturity in only 2 of 14 palatability traits; when carcasses were assigned to maturity groups on the basis only of skeletal characteristics, there were differences ($P < 0.05$) in favor of steaks from carcasses of A, as compared to B, maturity in 12 of 14 palatability traits. Skeletal maturity (12 of 14 comparisons) was also more effective than lean maturity (8 of 14 comparisons) in identifying differences ($P < 0.05$) in cooked beef palatability between A and C maturity carcasses, but skeletal and lean maturity were equally effective in identifying differences ($P < 0.05$) in cooked beef palatability in comparisons of A vs E (12 of 14 vs 12 of 14), B vs C (7 of 14 vs 6 of 14), B vs E (12 of 14 vs 12 of 14) and C vs E (11 of 14 vs 12 of 14) maturity carcasses. When carcasses were assigned to maturity groups on the basis of overall maturity score, there were differences ($P < 0.05$) in palatability in favor of steaks from carcasses of: (a) A, as compared to B, maturity in 11 of 14 comparisons; (b) A, as compared to C, maturity in 12 of 14 comparisons; (c) A, as compared to E, maturity in 12 of 14 comparisons; (d) B, as compared to C, maturity in 11 of 14 comparisons; (e) B, as compared to E, maturity in 12 of 14 comparisons, and (f) C, as compared to E, maturity in 12 of 14 comparisons (Tables 2, 3 and 4).

Carcasses of A maturity (lean, skeletal or overall maturity) produced steaks that were more palatable ($P < 0.05$) than did more mature carcasses in 74.6% (94 of 126 possible comparisons) of comparisons of mean sensory panel ratings or shear force values (Tables 2, 3 and 4); comparable percentages for B and C maturity groups were 71.4 (60 of 84) and 83.3 (35 of 42), respectively. Researchers who have found little or no difference in palatability between steaks from A vs B (or equivalent age) carcasses include Walter et al. (1965), Romans et al. (1965), Breidenstein et al. (1968), Berry et al. (1974), Hawrysh et al. (1975) and Tatum et al. (1980).

Table 3 — Mean sensory panel ratings for top round (semimembranosus muscle) steaks from carcasses assigned to each lean maturity, skeletal maturity or overall maturity group

USDA trait and maturity group	Number of carcasses	Sensory panel rating[f]				
		Flavor	Juiciness	Amount of connective tissue	Tenderness	Overall palatability
Lean maturity						
Calf	1	5.13[a]	4.00[b]	6.50[a]	5.75[a]	5.00[a]
A	178	4.83[a]	5.07[b]	5.51[a]	5.36[a]	4.79[a]
B	57	4.44[a]	5.12[b]	4.91[a]	4.68[a]	4.21[a]
C	40	4.35[a]	5.08[b]	4.35[b]	4.00[a]	3.82[a]
D	26	3.56[ab]	5.35[ab]	3.75[c]	3.49[b]	2.95[b]
E	45	3.27[b]	5.42[a]	3.48[c]	3.33[b]	2.76[b]
Skeletal maturity						
A	110	4.91[a]	5.07[b]	5.80[a]	5.68[a]	5.01[a]
B	61	4.61[b]	5.11[ab]	5.14[b]	4.93[b]	4.48[b]
C	76	4.57[b]	5.10[ab]	4.81[c]	4.61[b]	4.24[b]
D	31	4.12[c]	5.16[ab]	4.30[d]	3.93[c]	3.63[c]
E	69	3.39[d]	5.33[a]	3.53[e]	3.32[d]	2.79[d]
Overall maturity						
A	117	4.87[a]	5.06[b]	5.77[a]	5.64[a]	4.95[a]
B	62	4.68[ab]	5.13[b]	5.12[b]	4.94[b]	4.52[b]
C	96	4.44[b]	5.09[b]	4.66[c]	4.39[c]	4.07[c]
E	72	3.40[c]	5.36[a]	3.52[d]	3.32[d]	2.79[d]

[abcde] Means in the same column and for the same USDA trait (e.g., lean maturity) bearing a common superscript letter are not significantly different ($P > 0.05$).

[f] 8 = extremely desirable in flavor, extremely juicy, no connective tissue, extremely tender and extremely desirable in overall palatability; 5 = slightly desirable in flavor, slightly juicy, slightly tender, slight amount of connective tissue and slightly desirable in overall palatability; 4 = slightly undesirable in flavor, slightly dry, slightly tough, moderate amount of connective tissue and slightly undesirable in overall palatability; 1 = extremely undesirable in flavor, extremely dry, abundant connective tissue, extremely tough and extremely undesirable in overall palatability.

Table 4 — Mean shear force values for loin (longissimus dorsi muscle), top round (semimembranosus muscle), bottom round (biceps femoris muscle), and eye of round (semitendinosus muscle) steaks from carcasses assigned to each lean maturity, skeletal maturity or overall maturity group

USDA trait and maturity group	Shear force value (lb)[e]							
	Loin (LD)		Top round (SM)		Bottom round (BF)		Eye of round (ST)	
	N	Mean	N	Mean	N	Mean	N	Mean
Lean maturity								
Calf	4	7.36[a]	1	8.54[a]	1	9.00[a]	1	8.98[a]
A	547	8.20[a]	178	10.71[a]	178	13.63[a]	177	12.30[a]
B	174	8.49[a]	57	12.61[b]	56	14.87[a]	57	13.73[b]
C	127	10.29[b]	40	13.77[c]	40	16.85[b]	40	14.84[bc]
D	55	10.98[bc]	26	16.01[d]	26	18.95[bc]	26	16.76[d]
E	98	11.73[c]	45	16.27[d]	45	21.04[c]	45	15.97[cd]
Skeletal maturity								
A	361	7.95[a]	110	10.16[a]	110	12.18[a]	109	11.40[a]
B	214	8.69[b]	61	11.92[b]	60	14.96[b]	61	13.05[b]
C	194	9.10[bc]	76	12.27[b]	76	15.49[bc]	76	14.14[c]
D	85	9.63[c]	31	14.13[c]	31	17.14[c]	31	15.16[c]
E	151	11.50[d]	69	16.19[d]	69	20.80[d]	69	16.44[d]
Overall maturity								
A	384	7.93[a]	117	10.25[a]	117	12.57[a]	116	11.60[a]
B	221	8.67[b]	62	11.85[b]	61	14.35[b]	62	12.86[b]
C	239	9.42[c]	96	12.84[c]	96	15.98[c]	96	14.50[c]
E	161	11.43[d]	72	16.20[d]	72	20.85[d]	72	16.42[d]

[abcd] Means in the same column and for the same USDA trait (e.g., lean maturity) bearing a common superscript letter are not significantly different ($P > 0.05$).
[e] Mean force in pounds required to shear a 1.27 cm core of cooked muscle.

Steaks from more youthful (lean, skeletal or overall maturity) carcasses were ($P < 0.05$) more desirable in mean flavor ratings in 75.0% (27 of 36 comparisons) of comparisons with steaks from more mature carcasses (Tables 2, 3 and 4); comparable percentages were 0.0 (0 of 36) for juiciness, 97.2 (35 of 36) for amount of connective tissue, 86.1 (31 of 36) for tenderness, 86.1 (31 of 36) for overall palatability and 90.3 (65 of 72) for shear force values. Advancing overall maturity was associated with increases ($P < 0.05$) in juiciness in certain comparisons (B vs A, C vs A, C vs B for loin steaks; E vs A, E vs B, E vs C for both loin and top round steaks). McBee and Wiles (1967) found that steaks from B maturity carcasses were more juicy than those from A maturity carcasses; however, Berry (1972) reported no difference in juiciness among steaks from A, B, C, D or E maturity carcasses.

These data suggest that, across the complete range of maturity involved in the USDA system (A to E), principles involved in assigning USDA maturity scores to beef carcasses are effective in segmenting carcasses into groups which will produce steaks differing in flavor, tenderness and overall palatability. Tuma et al. (1962), Walter et al. (1965) and McBee and Wiles (1967) found significant differences between steaks from A vs B maturity carcasses in either flavor or tenderness. Other researchers who have reported differences among steaks from either A vs. E or A and B vs. D and E (or equivalent ages) carcasses include Tuma et al. (1962) and Walter et al. (1965) for tenderness; Romans et al. (1965) for tenderness and flavor; Breidenstein et al. (1968) for tenderness, shear force and overall palatability; Goll et al. (1965) and Norris et al. (1971) for shear force value, and, Berry et al. (1974) for amount of connective tissue.

Coefficients of variation for overall palatability ratings and shear force values for steaks from carcasses in each overall maturity group are presented in Table 5. Coefficients of variation for overall palatability ratings were almost twice as high for steaks from C and E carcasses as for loin steaks and top round steaks from carcasses of A maturity. These data suggest that the consistency with which overall maturity score predicts eating satisfaction in loin and top round steaks decreases as maturity increases. There was very little difference in consistency of shear force values between steaks from A vs B vs C vs E carcasses for the top round, bottom round and eye of round steaks. For loin, top round, bottom round and eye of round steaks (collectively), assurance (based on coefficients of variation) that steaks would be consistent in tenderness was greatest for carcasses of A maturity.

Table 5 — Coefficients of variation for overall palatability ratings and shear force values for steaks from carcasses in each overall maturity group

USDA overall maturity group	Overall palatability rating		Shear force value			
	LD	SM	LD	SM	BF	ST
A	13.8	14.7	29.8	22.2	23.4	18.7
B	18.1	17.9	36.4	20.8	27.7	18.0
C	22.1	24.1	37.3	22.7	26.4	20.0
E	22.3	25.8	29.8	22.0	27.9	18.8

Percentage incidence of loin steaks in specified sensory panel rating and shear force value ranges, stratified by USDA overall maturity group, is presented in Table 6; comparable data for round steaks are presented in Table 7. Data in Tables 6 and 7 were used to develop Tables 8 and 9, respectively, to facilitate evaluations of usefulness of USDA overall maturity scores for segregating carcasses into groups differing in percentage incidence of steaks that had mean sensory panel ratings and/or shear force values in certain desirability or acceptability ranges. To simplify discussion

Table 6 — Percentage incidence of loin (longissimus dorsi muscle) steaks in specified sensory panel rating and shear force value ranges, stratified by USDA overall maturity group

Range in palatability ratings	USDA overall maturity group: Flavor rating A	B	C	E	Juiciness rating A	B	C	E
7.00 to 8.00	1.4	–	–	–	–	–	–	–
6.00 to 6.99	34.9	27.6	22.2	.7	4.1	5.6	8.2	9.9
5.00 to 5.99	51.5	49.5	42.8	11.9	49.6	56.1	62.4	70.2
4.00 to 4.99	10.8	16.8	22.2	42.4	42.4	35.5	26.3	19.2
3.00 to 3.99	1.4	5.2	11.3	38.4	3.9	2.8	3.1	0.7
2.00 to 2.99	–	0.9	1.5	6.6	–	–	–	–
1.00 to 1.99	–	–	–	–	–	–	–	–

	Amount of connective tissue rating A	B	C	E	Tenderness rating A	B	C	E
7.00 to 8.00	29.6	15.0	7.2	–	12.2	6.5	3.1	0.7
6.00 to 6.99	60.4	61.2	52.1	6.0	44.0	38.3	32.5	3.3
5.00 to 5.99	8.6	20.1	33.0	33.8	31.0	29.9	27.8	13.2
4.00 to 4.99	1.1	3.7	7.2	37.1	10.0	16.4	23.7	30.5
3.00 to 3.99	0.3	–	0.5	20.5	2.2	6.1	9.3	31.8
2.00 to 2.99	–	–	–	2.6	0.6	2.3	3.1	19.2
1.00 to 1.99	–	–	–	–	–	0.5	0.5	1.3

	Overall palatability rating A	B	C	E	Range in shear force (lb)	Shear force value A	B	C	E
7.00 to 8.00	0.6	0.5	–	–	Less than 5.01	5.7	1.8	3.8	–
6.00 to 6.99	29.1	25.2	16.0	–	5.01 to 8.00	52.4	52.5	38.1	13.6
5.00 to 5.99	51.2	43.9	36.6	3.3	8.01 to 11.00	32.3	28.5	33.4	37.9
4.00 to 4.99	15.5	19.6	27.3	27.8	11.01 to 14.00	7.8	11.8	13.0	31.1
3.00 to 3.99	3.3	7.5	16.5	43.0	14.01 to 17.00	1.0	2.8	7.6	11.8
2.00 to 2.99	0.3	2.8	3.1	23.2	17.01 to 20.00	0.6	0.9	2.5	3.2
1.00 to 1.99	–	0.5	0.5	2.7	20.01 to 23.00	0.2	1.3	1.2	1.2
					23.01 to 26.00	–	0.4	0.4	0.6
					More than 26.01	–	–	–	0.6

of those results, we arbitrarily classified steaks that had mean sensory panel ratings of 6.00 or higher and/or mean shear force values of 3.63 kg or lower as "very desirable" and steaks that had mean sensory panel ratings of 2.99 or lower and/or mean shear force values of 6.35 kg or higher as "very undesirable." We further arbitrarily classified steaks that had mean sensory panel ratings of 4.00–8.00 and/or mean shear force values of 4.99 kg or lower as "acceptable" and steaks that had mean sensory panel ratings of 1.00 to 3.99 and/or mean shear force values of 5.00 kg or higher as "unacceptable."

Carcasses of A maturity produced "very desirable" loin steaks 1.17, 1.50 and 8.02 times as often as did carcasses of B, C or E maturity, respectively; carcasses of A maturity produced "acceptable" loin steaks 1.04, 1.10 and 1.60 times as frequently as did carcasses of B, C or E maturity, respectively. Carcasses of A maturity produced "very desirable" round steaks 2.98, 3.26 and 5.71 times as often as did carcasses of B, C or E maturity, respectively, and produced "acceptable" round steaks 1.42, 1.50 and 3.12 times, respectively, as frequently as did carcasses of B, C or E maturity (Tables 8 and 9). Carcasses of A maturity produced steaks that were only 0.19, 0.12 and 0.03 times as likely to be "very undesirable" (loin steaks), 0.37, 0.22 and 0.10 times as likely to be "very undesirable" (round steaks), 0.51, 0.33 and 0.18 times as likely to be "unacceptable" (loin steaks) and 0.62, 0.46 and 0.29 times as likely to be "unacceptable" (round steaks) as were those from carcasses of B, C or E maturity, respectively (Tables 8 and 9).

Data in Tables 6, 7, 8 and 9 can be interpreted in terms of incidence (1.00 equals 100%) of steaks in certain palatability groupings (e.g., "very desirable") as it relates to allowing carcasses from progressively more mature groups to be included in a maturity, or eventually a grade, grouping. Incidence of "very desirable" loin steaks would change from 0.46, to 0.42, to 0.38, to 0.30 if carcasses of progressively greater maturity (A; to A plus B; to A plus B and C; to A plus B, C and E, respectively) were combined with those of A maturity; comparable changes in incidence by such inclusion would be 0.14, 0.09, 0.08 and 0.06 for "very desirable" round steaks. Incidence of "very undesirable" loin steaks would change from 0.005 to 0.01 to 0.02 to 0.05 if carcasses of progressively greater maturity (A; to A plus B; to A plus B and C; to A plus B, C and E, respectively) were combined with those of A maturity; comparable changes in incidence by such inclusion would be 0.05, 0.18, 0.14 and 0.23 for "very undesirable" round steaks.

Coefficients of determination ($r^2 \times 100$) for USDA overall maturity score (by percentages within maturity group or groups) and palatability traits, in certain maturity ranges, are presented in Table 10. Differences in USDA overall maturity score were associated with 0.0% (A maturity) 2.3% (B maturity), 6.8% (C maturity), 3.1% (E maturity), 3.8% (A + B maturity), 33.5% (C + E maturity) and 37.6%

Table 7 – Percentage incidence of top round (semimembranosus muscle) steaks in specified sensory panel rating ranges and of top round (semimembranosus muscle), bottom round (biceps femoris muscle) and eye of round (semitendinosus muscle) steaks in specified shear force value ranges, stratified by USDA overall maturity group

Range in palatability ratings	USDA overall maturity group							
	Flavor rating				Juiciness rating			
	A	B	C	E	A	B	C	E
7.00 to 8.00	–	–	–	–	–	–	–	–
6.00 to 6.99	3.4	–	4.2	–	7.7	8.1	10.4	12.5
5.00 to 5.99	42.7	38.7	26.0	5.6	52.1	61.3	50.0	59.7
4.00 to 4.99	44.4	46.8	43.8	19.4	37.6	25.8	35.4	26.4
3.00 to 3.99	8.6	11.3	19.8	44.4	2.6	4.8	4.2	1.4
2.00 to 2.99	.9	3.2	6.2	29.2	–	–	–	–
1.00 to 1.99	–	–	–	1.4	–	–	–	–
	Amount of connective tissue rating				Tenderness rating			
	A	B	C	E	A	B	C	E
7.00 to 8.00	2.6	–	–	–	–	–	–	–
6.00 to 6.99	37.6	12.9	5.2	–	37.4	9.7	7.4	–
5.00 to 5.99	48.7	46.8	29.2	4.2	40.9	41.9	24.2	2.8
4.00 to 4.99	11.1	33.9	44.8	27.8	20.0	30.7	31.6	26.4
3.00 to 3.99	–	6.4	20.8	44.4	.9	14.5	29.5	27.8
2.00 to 2.99	–	–	–	20.8	.8	3.2	7.3	34.7
1.00 to 1.99	–	–	–	2.8	–	–	–	8.3
	Overall palatability rating				Top round			
					Shear force value			
	A	B	C	E	Range in shear force (lb)	A	B	C	E
7.00 to 8.00	–	–	–	–	Less than 5.01	–	–	–	–
6.00 to 6.99	6.8	1.6	2.1	–	5.01 to 8.00	12.0	–	2.1	2.8
5.00 to 5.99	42.7	33.9	15.6	–	8.01 to 11.00	53.8	41.9	26.0	–
4.00 to 4.99	43.6	38.7	36.5	8.3	11.01 to 14.00	27.4	38.7	40.6	18.0
3.00 to 3.99	6.0	24.2	33.3	36.1	14.01 to 17.00	5.2	14.6	25.0	44.5
2.00 to 2.99	.9	1.6	12.5	45.8	17.01 to 20.00	0.8	4.8	3.2	20.8
1.00 to 1.99	–	–	–	9.8	20.01 to 23.00	0.8	–	3.1	9.7
					23.01 to 26.00	–	–	–	1.4
					More than 26.01	–	–	–	2.8
	Bottom round				Eye of round			
	Shear force value				Shear force value			
Range in shear force (lb)	A	B	C	E	Range in shear force (lb)	A	B	C	E
Less than 5.01	0.9	–	–	1.4	Less than 5.01	–	–	–	–
5.01 to 8.00	0.9	3.2	2.1	2.8	5.01 to 8.00	–	1.6	–	–
8.01 to 11.00	32.4	20.1	8.2	–	8.01 to 11.00	45.7	19.3	10.4	–
11.01 to 14.00	41.1	27.9	18.8	2.8	11.01 to 14.00	49.1	48.4	39.6	23.6
14.01 to 17.00	16.3	26.2	34.4	15.2	14.01 to 17.00	1.7	24.2	33.4	38.9
17.01 to 20.00	7.6	14.7	16.7	23.6	17.01 to 20.00	1.8	6.5	12.5	25.0
20.01 to 23.00	–	6.4	11.5	22.2	20.01 to 23.00	1.7	–	4.1	9.7
23.01 to 26.00	0.8	–	8.3	13.9	23.01 to 26.00	–	–	–	2.8
More than 26.01	–	1.5	–	18.1	More than 26.01	–	–	–	–

(A – E maturity) of the observed variability in overall palatability ratings for loin steaks and with 17.9% (A maturity), 2.2% (B maturity), 21.6% (C maturity), 3.2% (E maturity), 14.0% (A + B maturity), 42.5% (C + E maturity) and 53.4% (A – E maturity) of the observed variability in overall palatability ratings for top round steaks. Carpenter (1974) reviewed the pertinent literature and concluded that maturity is of minor importance in its contribution to palatability of meat derived from fed cattle that are generally less than 30 months of age or within the A maturity group by USDA standards. Hawrysh et al. (1975) studied beef of an age-origin range comparable to that of the A and B maturity groups; they found no difference in texture, aroma, flavor, tenderness or overall palatability attributable to maturity when trained or consumer panels evaluated LD muscles, but ST muscles from older animals were less tender and more flavorful than those from more youthful animals.

In the present study (Table 10), the relationship between overall maturity score and flavor, amount of connective tissue, tenderness and overall palatability of both loin and top round steaks and between overall maturity score and shear force values of loin, top round, bottom round and eye of round steaks were greatly dependent upon the range in maturity included in the test population. As the range in maturity of the test population increased (from A; to A + B or C + E; to A – E), ability of USDA overall maturity scores (Table 10) to account for observed

Table 8 — Percentage incidence of loin (LD) steaks that had mean sensory panel ratings and mean shear force values in certain ranges (categories) stratified by USDA overall maturity group

Category[a]									
Mean sensory panel rating	Mean shear force value	USDA overall maturity group	Sensory panel rating					Shear force value	Composite of all panel ratings and shear force values[b]
			Flavor	Juiciness	Amount of connective tissue	Tenderness	Overall palatability		
I	I	A	36.3	4.1	90.0	56.2	29.7	58.1	45.7
I	I	B	27.6	5.6	76.2	44.8	25.7	54.3	39.0
I	I	C	22.2	8.2	59.3	35.6	16.0	41.9	30.5
I	I	E	0.7	9.9	6.0	4.0	0.0	13.6	5.7
II	II	A	0.0	0.0	0.0	0.6	0.3	1.8	0.4
II	II	B	0.9	0.0	0.0	2.8	3.3	5.4	2.1
II	II	C	1.5	0.0	0.0	3.6	3.6	11.7	3.4
II	II	E	6.6	0.0	2.6	20.5	25.9	17.4	12.2
III	III	A	98.6	96.1	99.7	97.2	96.4	90.4	96.4
III	III	B	93.9	97.2	100.0	91.1	89.2	82.8	92.4
III	III	C	87.2	96.9	99.5	87.1	79.9	75.3	87.7
III	III	E	55.0	99.3	76.9	47.7	31.1	51.5	60.2
IV	IV	A	1.4	3.9	0.3	2.8	3.6	9.6	3.6
IV	IV	B	6.1	2.8	0.0	8.9	10.8	17.2	7.6
IV	IV	C	12.8	3.1	0.5	12.9	20.1	24.7	12.3
IV	IV	E	45.0	0.7	23.1	52.3	68.9	48.5	39.8

[a] For mean sensory panel ratings (flavor, juiciness, amount of connective tissue, tenderness and overall palatability): Category I was 6.00 or higher, Category II was 2.99 or lower, Category III was 4.00 to 8.00 and Category IV was 1.00 to 3.99. For mean shear force value: Category I was 3.63 kg or lower, Category II was 6.35 kg or higher, Category III was 4.99 kg or lower and Category IV was 5.00 kg or higher.
[b] Computed by averaging percentages for flavor, juiciness, amount of connective tissue, tenderness, overall palatability and shear force value.

Table 9 — Percentage incidence of round steaks that had mean sensory panel ratings (for SM) and mean shear force values (for SM, BF and ST; combined) in certain ranges (categories) stratified by USDA overall maturity group

Category[a]									
Mean sensory panel rating	Mean shear force value	USDA overall maturity group	Sensory panel rating					Shear force value[b]	Composite of all panel ratings and shear force values[c]
			Flavor	Juiciness	Amount of connective tissue	Tenderness	Overall palatability		
I	I	A	3.4	7.7	40.2	37.4	6.8	4.6	13.7
I	I	B	0.0	8.1	12.9	9.7	1.6	1.6	4.6
I	I	C	4.2	10.4	5.2	7.4	2.1	1.4	4.2
I	I	E	0.0	12.5	0.0	0.0	0.0	2.1	2.4
II	II	A	0.9	0.0	0.0	0.8	0.8	12.2	4.9
II	II	B	3.2	0.0	0.0	3.2	1.6	33.0	13.4
II	II	C	6.2	0.0	0.0	7.3	12.5	50.7	22.3
II	II	E	30.6	0.0	23.6	43.0	55.6	82.9	50.2
III	III	A	90.5	97.4	100.0	98.3	93.1	48.6	78.1
III	III	B	85.5	95.2	93.6	82.3	74.2	28.7	64.6
III	III	C	74.0	95.8	79.2	63.2	54.2	16.3	51.9
III	III	E	25.0	98.6	32.0	29.2	8.3	2.3	25.0
IV	IV	A	9.5	2.6	0.0	1.7	6.9	51.4	21.9
IV	IV	B	14.5	4.8	6.4	17.7	25.8	71.3	35.4
IV	IV	C	26.0	4.2	20.8	36.8	45.8	83.7	48.1
IV	IV	E	75.0	1.4	68.0	70.8	91.7	97.7	75.0

[a] For mean sensory panel ratings (flavor, juiciness, amount of connective tissue, tenderness and overall palatability): Category I was 6.00 or higher, Category II was 2.99 or lower, Category III was 4.00 to 8.00 and Category IV was 1.00 to 3.99. For mean shear force value: Category I was 3.63 kg or lower, Category II was 6.35 kg or higher, Category III was 4.99 kg or lower and Category IV was 5.00 kg or higher.
[b] Computed by averaging percentages for shear force values for SM, BF and ST muscles.
[c] Computed by averaging percentages for flavor, juiciness, amount of connective tissue, tenderness, overall palatability and three times the percentage for shear force value.

variability: (a) increased for flavor of loin and top round steaks; (b) did not change for juiciness of loin and top round steaks; (c) increased for amount of connective tissue detected in loin and top round steaks; (d) increased for overall palatability of loin and top round steaks; and (f) increased for shear force requirements of loin, top round, bottom round and eye of round steaks. Overall maturity scores were of little value (less than 4.0% C.D.) for predicting overall palatability of loin steaks in the A, B or in the A + B maturity groups but overall maturity

Table 10 — Coefficients of determination ($r^2 \times 100$) for USDA overall maturity score (by percentages within maturity groups) and palatability traits, stratified by type of steak and maturity group

Overall maturity group or groups	Steak[a]	Sensory panel rating					Shear force value
		Flavor	Juiciness	Amount of connective tissue	Tenderness	Overall palatability	
A	LD	0.3ns	5.0**	4.9**	0.4ns	0.0ns	0.6ns
A	SM	12.5**	2.1ns	27.6**	20.4**	17.9**	11.5**
A	BF	–	–	–	–	–	13.4**
A	ST	–	–	–	–	–	10.5**
B	LD	3.1**	0.1ns	3.6**	0.9ns	2.3*	1.2ns
B	SM	1.0ns	0.0ns	0.1ns	1.1ns	2.2ns	1.6ns
B	BF	–	–	–	–	–	0.7ns
B	ST	–	–	–	–	–	3.4ns
C	LD	6.9**	0.8ns	12.7**	6.0**	6.8**	2.8*
C	SM	15.5**	0.2ns	24.6**	17.0**	21.6**	15.8**
C	BF	–	–	–	–	–	4.0ns
C	ST	–	–	–	–	–	6.1*
E	LD	8.0**	0.2ns	0.1ns	0.1ns	3.1*	0.8ns
E	SM	12.5**	0.0ns	0.4ns	0.0ns	3.2ns	3.0ns
E	BF	–	–	–	–	–	1.2ns
E	ST	–	–	–	–	–	1.1ns
A + B	LD	4.0**	2.2**	9.0**	4.1**	3.8**	1.5**
A + B	SM	5.2**	1.0ns	25.8**	20.3**	14.0**	15.1**
A + B	BF	–	–	–	–	–	10.7**
A + B	ST	–	–	–	–	–	12.6**
C + E	LD	32.1**	1.5*	38.7**	22.5**	33.5**	9.2**
C + E	SM	33.6**	4.4**	38.6**	26.6**	42.5**	27.1**
C + E	BF	–	–	–	–	–	21.2**
C + E	ST	–	–	–	–	–	10.4**
A – E	LD	34.5**	5.2**	49.4**	31.8**	37.6**	14.3**
A – E	SM	34.5**	2.9**	59.0**	48.8**	53.4**	41.5**
A – E	BF	–	–	–	–	–	36.4**
A – E	ST	–	–	–	–	–	34.1**

[a] LD = longissimus dorsi; SM = semimembranosus; BF = biceps femoris; ST = semitendinosus.
** $P < 0.01$
* $P < 0.05$
ns $P > 0.05$

scores were of some value (10–18% C.D.) for predicting overall palatability ratings and/or shear force values of top round, bottom round and eye of round steaks in the A and A + B maturity groups. Relative maturity (by groups and percentages within groups) was very important (22–59% C.D.) for determining flavor, amount of connective tissue, tenderness and overall palatability of both loin and top round steaks in the C + E and A – E maturity groups.

Although USDA overall maturity groups A or B, as discrete entities, were meaningful indicators of expected palatability, position within the A maturity group, B maturity group, and within the A + B maturity range was of little or no importance in determining relative palatability of loin steaks and accounted for less than 18% of the observed variability in overall palatability ratings for top round steaks and in shear force values for top round, bottom round and eye of round steaks. A possible reason for the lack of maturity relationship, within the A maturity group, to tenderness may relate to the findings of Boccard et al. (1979) that in muscles that have a low content of collagen, such as the LD and SM, tenderness decreased as animals increased in age from 8 to 16 months but that tenderness then increased as animals increased in age from 16 to 24 months of age. Tatum et al. (1980) reported that among loin steaks from steers, those from A vs. B maturity carcasses did not differ in any of the palatability traits and that those from C maturity carcasses had higher overall palatability ratings than did those from A or B maturity carcasses. Results of the present study confirm that there are substantial differences in palatability when youthful beef is compared to mature beef (e.g., A vs E maturity) but that differences in beef palatability among youthful carcasses (e.g., those in the A maturity group) are of lesser consequence.

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Evaluation of Various Methods for Roasting Frozen Turkeys

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ABSTRACT

Frozen turkeys were roasted to a lower than normal final temperature (71.1°C, 160°F) by one of the following methods: (1) foil tent, 93.3°C (200°F) oven; (2) foil wrap, 93.3°C (200°F) oven; (3) foil tent, 162.8°C (325°F) oven; (4) roasting bag, 176.7°C (350°F) oven; (5) foil wrap, 204.4°C (400°F) oven; or (6) microwave oven. Birds roasted by low temperature roasting methods received highest ratings for most attributes. Basted birds were preferred for all eating quality attributes. Microwave roasted birds reached final temperature fastest and with least energy consumption, but were rated low in appearance and eating qualities, and often had undesirably pink thigh joints. Turkeys inoculated with *Salmonella typhimurium* and *Clostridium perfringens* vegetative cells were essentially sterilized by all roasting methods. These methods can be considered as convenient alternatives to conventional roasting procedures.

INTRODUCTION

MOST TURKEYS for home consumption are purchased in the frozen state. Package directions usually recommend that the turkey be thawed in the refrigerator, a process that may take 2–3 days. Alternatively, the turkey may be thawed in cold water, allowing about 30 min per pound. The inconvenience of thawing is probably one factor limiting home consumption of turkey.

Standard recipes recommend roasting turkey to an internal breast temperature of 85°C (185°F). The pop-up timers used by the industry are also set for 85°C (185°F). However, inquiries are received concerning low temperature roasting, and some recipes recommend roasting turkey at an oven temperature of 93.3–107.2°C (200–225°F) to an internal temperature of 71.1°C (160°F) (Evans, personal communication). One advantage of roasting slowly to a lower final temperature is an increase in juiciness. Hoke et al. (1967) found that yield and juiciness of both light and dark turkey meat decreased as the internal temperature increased from 73.8 to 90.5°C (165 to 195°F), and that above 87.7°C (190°F) the meat was dry and tended to crumble. Similarly, Schmidt and Parrish (1971) have reported that beef was more tender when cooked to lower temperatures, within the 60–80°C (140–176°F) range. Fulton et al. (1967) and Fulton and Davis (1974) have previously demonstrated that frozen birds may be roasted satisfactorily with no effect upon yield or eating quality, compared to thawed birds. Naturally, roasting time was longer for frozen birds. Travnicek and Hooper (1968) reported that palatability scores for flavor intensity and desirability, tenderness and juiciness were similar for frozen turkey breast, whether cooked by roasting or braising.

The microbiological safety of turkey and turkey products cooked by standard procedures has been confirmed (Mercuri et al., 1969; Zottola and Busta, 1971). Wilkinson et al. (1965) confirmed the safety of turkey rolls inoculated internally with *Staphylococcus aureus, Streptococcus faecalis,* or various *Salmonella* species, then roasted to an internal temperature of 71.1°C (160°F) in an oven set at 107°C (225°F). Beloian and Schlosser (1963) concluded that baked foods that reach a temperature of 71.1°C can be considered safe from any *Salmonella* organisms present in the ingredients, specifically spray dried eggs. Vegetative foodborne pathogens, including *Salmonella* and *C. perfringens,* even when present in large numbers (10^7), are reportedly destroyed when held for 1 sec or more at or above 73.9°C (165°F), or by 12 min or more exposure at or above 65.6°C (150°F) (Angelotti et al., 1961; Bryan and Mckinley, 1979). Little or no information is available, however, on the microbiological status of whole turkeys roasted to a lower internal temperature of 71.1°C (160°F). This study was undertaken to provide information on the convenience, sensory attributes, and microbiological status of turkey roasted from the frozen state to an internal temperature of 71.1°C by one of several methods, including microwave, low temperature oven, high temperature oven, and with a roasting bag in a high temperature oven.

PROCEDURES

SIX ROASTING METHODS and three basting methods were tested, for a total of 18 treatment combinations. Three replications of each treatment combination were arranged in an incomplete block design. Nine sets of six frozen turkeys and a thawed (control) turkey were roasted on each of nine occasions for sensory analysis. Within each set of six frozen turkeys, each of the roasting methods was represented once, and each basting method was represented twice. The roasting methods used included; (1) foil tent, 93.3°C (200°F) oven; (2) foil wrap, 93.3°C (200°F) oven; (3) foil tent, 162.8°C (325°F) oven; (4) roasting bag, 176.7°C (350°F) oven; (5) foil wrap, 204.4°C (400°F) oven; (6) microwave oven; and (7) foil tent, 162.8°C (325°F) oven, thawed for 48 hr in a refrigerator (control). The prebasting methods were; (1) unbasted, (2) broth-basted, and (3) oil-basted. The unbasted and broth basted turkeys were obtained from a local turkey processor. The oil basted birds were purchased locally. All birds weighed 11–15 pounds.

For roasting methods requiring a foil tent, a piece of aluminum foil was draped over the breast and down to the roasting pan. Foil wraps were prepared by placing foil under the bird and wrapping the foil tightly together on the top of the breast and ends, leaving a small space over the breast to avoid sticking of the foil to the skin. Nylon film cooking bags (Reynolds, Richmond, VA 23261) were purchased locally. Ovens were not preheated. Giblets and neck were left in the bird throughout roasting.

Immediately before roasting, thermocouples were inserted 3–4 cm into the breast, thigh, and cavity and just under the skin of the breast, using a hammer and 1/8 inch diameter nail to pierce the frozen tissue. Temperatures were recorded every 30 min during roasting, using a multichannel recorder (Leeds and Northrup, Salt Lake City, UT). Foil tents or wraps were removed or unwrapped, respectively, at 30–45 min prior to removal of the turkey from the oven, to allow browning. The birds were removed from the oven when the lowest thermocouple reading was 71.1 ± 2°C (160 ± 4°F). A meat thermometer was used to monitor the temperature increase of the breast and thigh, after removal of the bird from the oven. After roasting, the yield (final weight/initial weight x 100) was determined.

Coded whole birds were displayed for evaluation of degree of browning, uniformity of browning and general appearance. Sliced turkey breast samples were evaluated for flavor, juiciness, tender-

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ness and for an overall rating. The panel consisted of 25 individuals for each of the nine taste sessions. All panelists had previous experience roasting turkeys and were familiar with sensory techniques. All ratings utilized a 7-point scale, with 7 being the most intense or highest score for the particular attribute under consideration. Coded 4 x 5 cm slices were served at room tempeature in individual sensory booths with rinse water available.

Warner-Bratzler shear values were recorded for triplicate samples of breast muscle from each bird, using a 1.6 cm diameter coring knife. Samples were removed so that shears were taken perpendicular to the grain of the muscle. Expressible juice values were obtained on 100g samples of chopped breast muscle, recording the ml of juice expressed during the 5 min application of 1000 psi pressure.

For the microbiology portion of the study, seven unbasted frozen turkeys were purchased locally. After thawing for 48 hr at 2.2°C (35°F), aerobic plate counts were determined on samples of giblets and skin by standard procedures (Messer et al., 1978). Each turkey and the pooled giblets were inoculated by stirring for one minute in a solution containing *Salmonella typhimurium* and *Clostridium perfringens* vegetative cells. The giblets were wrapped in toweling, replaced in the same position in each turkey, and the turkeys were re-frozen. Roasting methods were as previously described. Aerobic plate count, *C. perfringens* plate count, and *Salmonella* isolation procedures were conducted on samples of skin and giblets from each turkey immediately after inoculation and again after roasting. The respective procedures described in the FDA *Bacteriological Analytical Manual* (Messer et al., 1978; Harmon and Duncan, 1978; Andrews et al., 1978) were used with modifications. Skin samples were obtained with a coring knife. Each core had a surface area of 2.0 cm^2. Three cores (two from the breast and one from the thigh) were included in each sample. The coring knife was sterilized between cores by immersion in 70% ethanol, then flaming. Giblet samples were 5g, rather than 25g, due to the limited amount of material available. Appropriate changes in dilutions were also made.

The data were statistically analyzed using the SAS statistical analysis package (SAS Institute, 1979). Analysis of variance and Duncan's multiple range tests were run separately for each sensory attribute and physical measurement.

RESULTS & DISCUSSION

BOTH ROASTING METHOD and basting method resulted in significant variation in the eating quality and appearance ratings of frozen roasted turkeys. In general, the high temperature, fast roasting methods resulted in a greater degree of browning than did the slow, low temperature methods (Fig. 1). The microwave roasted birds received substantially lower scores for uniformity of color than did any other birds. The nonrotary microwave oven used in this study consistently overbrowned the middle-lower portions of the breast and back, even though the birds were manually turned every 5 min. The foil wrapped, 93.3°C oven roasted turkeys were considered less brown but more uniform in color, and were rated significantly higher in general appearance than birds from all other roasting methods. Microwave roasted birds were rated lowest in general appearance.

Frozen turkeys roasted in a low temperature oven were preferred for flavor and tenderness (Fig. 1). Roasting in a bag (176.7°C oven) resulted in significantly lower juiciness ratings. Birds roasted with a foil tent in a 93.3°C oven were significantly preferred for overall quality over the roasting bag, microwave, and foil wrap, 204.4°C oven roasted birds.

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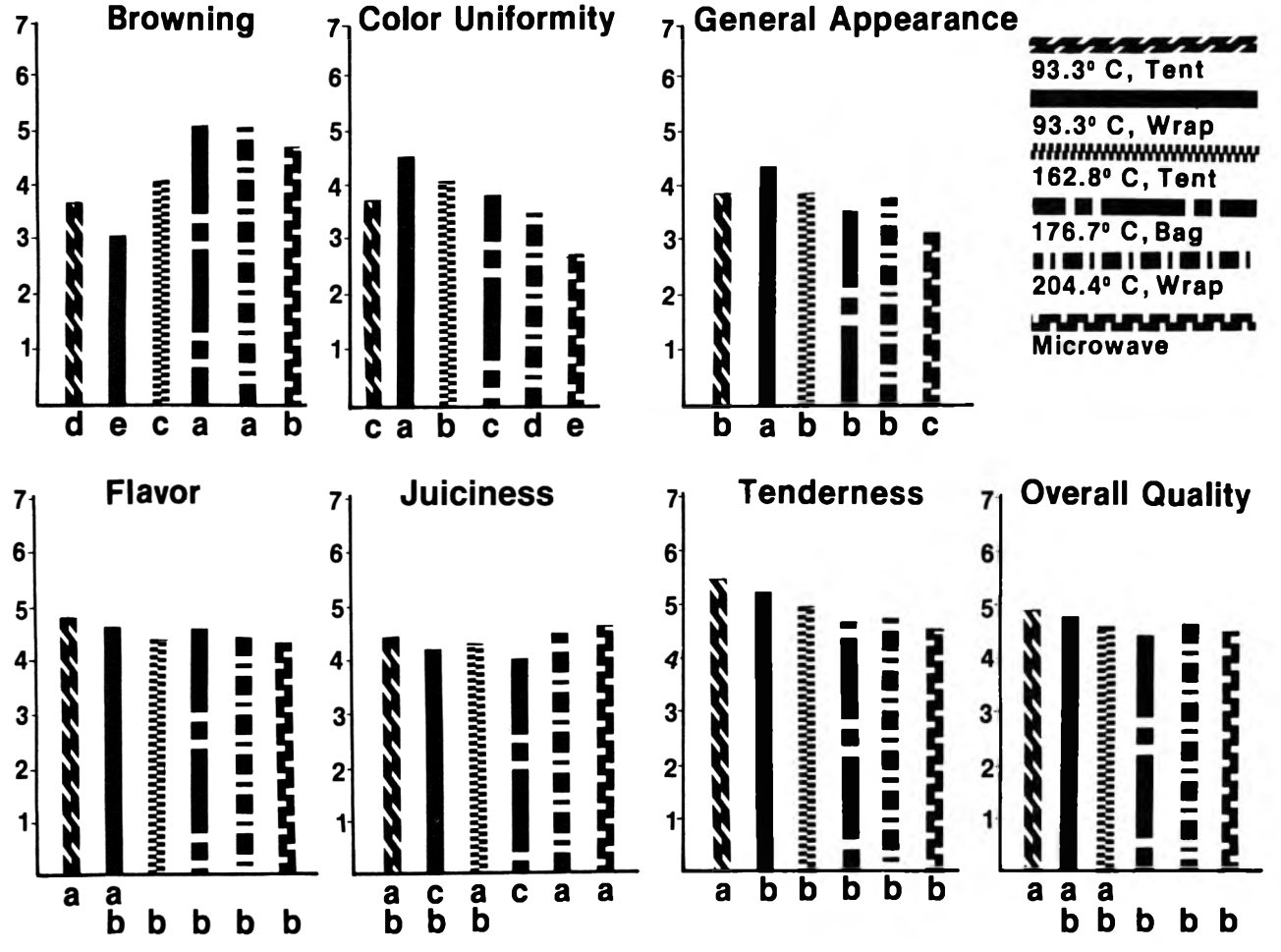

Fig. 1–Mean sensory scores for the roasted turkeys. [Within each attribute, columns with the same letter are not significantly different (α = 0.05, Duncan's multiple range test).]

The foil tent, 176.7°C oven and the foil wrap, 93.3°C oven roasted birds were given intermediate scores for overall eating quality (Fig. 1). The control method of roasting (176.7°C, thawed, foil tent) was not included in these comparisons, since all control birds were unbasted, and thus the lower scores given these birds may have been due to the preference of the panelists for the basted birds. The unbasted, thawed, control birds were compared with the unbasted frozen birds roasted by each of the other methods in a separate analysis of variance. The thawed, control birds were not significantly better than birds roasted from the frozen state on any attribute. This is in agreement with the previous work of Fulton and Davis (1974).

Broth basted and oil basted birds were preferred over unbasted birds for all eating quality attributes (Table 1). Unbasted birds, however, were rated somewhat higher for degree of browning than were basted birds. None of the appearance scores were especially high (Table 1), probably because the birds did not brown to the degree that consumers have learned to expect for birds roasted to 85°C. The oil basted birds were considered the most juicy, followed by the broth basted birds. The unbasted birds received the lowest scores for juiciness. Broth and oil basted turkeys were also considered significantly more tender than unbasted turkeys (Table 1). Oil and broth basted turkeys were rated higher for virtually all eating quality attributes than were unbasted birds. Consumers on a low fat or low sodium diet may consider pre-basting undesirable.

In general, within a given basting method, results of the roasting method comparisons were similar to those previously described (Fig. 1). One exception was noted. Oil basted birds roasted at 93.3°C in a foil tent received significantly lower scores for uniformity of browning and general appearance than did oil basted birds roasted by any other method. The reason for these low browning scores is not readily apparent.

Broth basted birds roasted at 93.3°C with a foil tent received a mean flavor score that was significantly higher than the scores for the 204.4°C roasted and the microwave roasted birds. No significant differences in flavor were observed among roasting methods for either unbasted or oil basted birds. For unbasted turkeys, those roasted at 204.4°C in a foil wrap were rated most juicy. The microwave and control groups received scores that did not differ significantly from that given the 204.4°C roasting method. All other methods received lower juicness ratings. There were no significant differences in juiciness ratings among roasting methods for oil or broth basted birds. Since both oil and broth basted birds received higher juiciness ratings than did unbasted birds, basting must compensate for any effect that roasting method may have on juiciness.

Turkeys roasted at 93.3°C were thought to be most tender (Fig. 1). Similar results were also obtained when the data were analyzed for interaction effects among roasting and basting methods. No significant roasting method-basting method interactions were noted on overall eating quality.

Roasting at 93.3°C with a foil wrap produced birds having a significantly lower expressed juice volume than did either the 162.8°C, foil tent or the microwave method (Table 2). Taste panel scores also indicated that higher temperature, shorter time roasting methods produced juicier breast meat, with the exception of the samples cooked in a roasting bag at 176.6°C, which were evaluated as somewhat dry. Breast meat samples from unbasted birds consistently had more expressed juice than did samples from basted birds, but the difference was not statistically significant.

Warner-Bratzler shear values were significantly lower for birds roasted in a 93.3°C oven (Table 3). Oil basted birds were significantly more tender, with the broth basted and unbasted birds being progressively tougher. Taste panel results corroborated these physical measurements (Table 1). Additional fluid retained in basted birds during roasting may result in less extensive coagulation and toughening. The toughness ratings given microwave and 204.4°C roasted turkeys were probably because the breast was cooked to a somewhat higher temperature than in the low temperature roasting methods, to reach 71.1°C in the cavity. Also, birds roasted by microwave, in a bag, or in a 204.4°C oven tended to exceed the desired endpoint temperature after removal from the oven (Table 4). Bryan and McKinley (1979) previously reported this phenomenon in beef roasts. The higher internal temperature of the breast of turkeys roasted by these three methods probably contributed to the toughness of these samples as measured by both taste panel and Warner-Bratzler shear test.

Table 1—Mean sensory scores for basting methods of frozen turkeys[a]

Basting method	Flavor	Juiciness	Tenderness	Overall	Browning	Uniformity	General appearance
Unbasted	3.61c	3.58c	4.19b	3.78b	4.56a	3.92a	3.87ab
Broth	5.21a	4.62c	5.17a	5.00a	4.09b	3.64b	3.98a
Oil	4.98b	4.88a	5.16a	4.94a	4.17b	3.90a	3.70b

[a] All analysis of variance tests were significant at $\alpha \leq 0.001$. Values in the same column with the same letter are not significantly different (α = 0.05, Duncan's multiple range test).

Table 2-Mean succulometer values[a] *for roasted turkey breast*

	Roasting method[b]							
Basting method	Foil tent 93.3°C	Foil wrap 93.3°C	Foil tent 162.8°C	Bag 176.7°C	Foil wrap 204.4°C	Microwave	Thawed, foil tent 162.8°C	Mean for basting method[d]
Unbasted	7.0[a]	5.2	10.4	9.7	7.6	12.1	8.5	8.7a[c]
Broth	6.0	7.0	6.5	7.1	9.0	9.4		7.5a
Oil	6.1	3.1	11.0	8.4	7.9	11.5		8.0a
Mean for roasting method[d]	6.4bc	5.1c	9.3ab	8.4abc	8.2abc	11.0a	8.5	

[a] Juice expressed (ml) from 100g cooked breast at 1000 psi for 5 min.
[b] All roasting methods used frozen turkeys except for the thawed control.
[c] Calculated for frozen birds only.
[d] Mean values with the same letter are not significantly different (α = 0.05, Duncan's multiple range test).

It was also observed that birds roasted at 93.3°C were more uniform in final temperature throughout the bird than those roasted by other methods (Table 4). For all roasting methods, the skin and thigh thermocouples reached 71.1°C first, followed by either the breast or cavity. The final temperatures of the breast and cavity were nearly equal (within 3°C) for all roasting methods except the control. For the thawed control birds, the average final breast temperature was 74.4°C, while the corresponding value for the cavity was 71.7°C.

Birds roasted in a 93.3°C oven took much longer to reach final temperature than did those from other groups (Table 5). Low temperature roasted birds took about 112 min/pound to reach 71.1°C, while the birds roasted in a bag needed only 22 minutes/pound. The microwave roasted birds required only 9.8 min/pound. Oil basted birds took about 5 min/pound longer to reach final temperature (Table 5).

Average yields for birds roasted in a bag at 176.7°C and by microwave were 72.8% and 72.7%. All other roasting methods resulted in significantly higher yields, ranging from 74.2–74.5%. Overall yield for unbasted birds was 73.2%, compared to 74.0% and 74.5% for broth and oil basted birds, respectively. These differences among basting methods, although small, were all significant at $\alpha \leq 0.05$.

Energy consumption was measured on one turkey of each group plus a thawed, microwave roasted bird, using the same insulated, self-cleaning oven for each measurement (Table 6). As expected, the thawed control bird required less energy than most other methods. The turkey

Table 3—Mean Warner-Bratzler shear values[a] for roasted turkey breast

	Roasting method[b]							
Basting method	Foil tent 93.3°C	Foil wrap 93.3°C	Foil tent 162.8°C	Bag 176.7°C	Foil wrap 204.4°C	Microwave	Thawed, foil tent 162.8°C	Mean for basting method[d]
Unbasted	5.2	6.2	7.1	8.2	7.8	10.5	8.2	7.5a[c]
Broth	5.6	5.6	5.1	8.3	6.8	6.9		6.4b
Oil	4.1	5.3	5.3	5.5	7.4	5.8		5.5c
Mean for roasting method[d]	4.8c	5.7bc	6.0b	7.5a	7.4a	7.5a		

[a] Expressed in pounds to shear a 1.6 cm diameter core.
[b] All roasting methods used frozen turkeys except for the thawed control.
[c] Calculated for frozen birds only.
[d] Mean values with the same letter are not significantly different ($\alpha = 0.05$, Duncan's multiple range test.)

Table 4—Mean final temperatures of skin, breast, thigh, and cavity

	Foil tent 93.3°C	Foil wrap 93.3°C	Foil tent 162.8°C	Bag 176.7°C	Foil wrap 204.4°C	Microwave	Thawed foil tent 162.8°C
Skin	78.0°C	76.7	84.3	88.8	89.0		85.5
Breast	71.2	72.4	73.3	74.0	75.6	75.7	74.5
Thigh	72.3	73.1	78.0	79.3	81.5	78.5	75.0
Cavity	71.3	70.9	74.4	74.4	75.2	74.8	71.7

Table 5—Mean roasting time (min/lb raw turkey) for roasting methods[a]

Basting method	Foil tent 93.3°C	Foil wrap 93.3°C	Foil tent 162.8°C	Bag 176.7°C	Foil wrap 204.4°C	Microwave	Thawed control foil tent 162.8°C	Mean for basting method
Unbasted	112.36	103.93	39.06	20.56	31.06	9.76	27.18	52.78[b]
Broth	112.94	111.85	36.01	22.09	26.12	9.44		53.07
Oil	121.17	122.07	43.15	25.21	29.80			58.60
Mean for roasting method	115.49	112.62	39.41	22.62	28.99	9.80		

[a] All roasting methods used frozen turkeys except for the thawed control
[b] Calculated for frozen birds only

Table 6—Comparison of energy consumption among roasting methods

Roasting method[a]	Foil tent 93.3°C	Foil wrap 93.3°C	Foil tent 162.8°C	Bag 176.7°C	Foil wrap 204.4°C	Microwave	Thawed microwave	Thawed foil tent 162.8°C
Energy[b,c] consumption (Watt hr/lb raw turkey)	468.19	469.62	465.97	318.61	393.72	286.56	191.16	350.88

[a] All roasting methods used frozen turkeys except the thawed controls.
[b] Measured with a Duncan electric portable energy meter, Lafayette, IN 47903.
[c] Calculations per lb based on measurements using 12-lb birds

roasted in a roasting bag required even less energy than did the thawed control, i.e., only 318.64 watt hr/pound for the roasting bag method, compared to 350 watt hr/pound for the control and 469 watt hr/pound for the 93.3°C oven methods (Table 6). Stephens (1977) previously reported a similar low energy consumption using the roasting bag cookery method.

All roasting methods achieved virtually complete sterilization of both giblets and skin of turkeys previously inoculated with viable pathogenic organisms. The pooled giblets had an aerobic plate count of 2.7×10^3 after inoculation. After roasting, the giblets from the frozen bird roasted at 162.8°C with a foil tent had a plate count of less than 10 cells per gram. Further tests confirmed that the colonies were not *Salmonella.* Giblet samples from all other methods showed no growth at all on aerobic plate count agar. Skin samples from turkeys inoculated in a common bath had an aerobic plate count of 1.8×10^2 cells per cm^2 after inoculation, but after roasting no growth occurred. An enrichment procedure was used for the detection of *Salmonella.* Consequently, the procedure could detect the presence of viable *Salmonella* cells, but not their original number. After inoculation with *S. typhimurium,* the organisms were detected in samples of skin and giblets. After roasting, however, *Salmonella* species were absent from skin and giblet samples of all roasting methods. After incoulation, giblet and skin samples contained 11 cells/g and 5 cells/cm^2, respectively, of *C. perfringens.* After roasting, no *C. perfringens* organisms were detected on any of the samples. The birds were removed from the oven when the internal temperature was 71°C. However, the internal temperature of all birds tended to increase after removal from the oven, reaching as high as 75°C for the microwave and high temperature oven roasted birds (Table 4). This temperature overshoot phenomenon probably contributed to the high degree of bacterial destruction observed in this study. However, all methods used in this study produced a microbiologically safe product, when the internal temperature of the bird was 71°C upon removal from the oven.

In contrast, Sundburg and Carlin (1976) found that some *C. perfringens* spores and vegetative cells inoculated on internal surfaces of beef rump roasts survived roasting to an internal temperature of 77°C (170°F) in a 177°C (350°F) oven. They concluded, however, that naturally occurring populations of *C. perfringens* in beef roasts would be much lower than inoculated levels. Thus any organisms surviving in commercially or home prepared roast beef would not likely cause illness if the cooked roasts were refrigerated or eaten immediately. The same would be true for roast turkey.

A number of observations were made regarding roasting, carving, and convenience characteristics of the frozen-roasted birds. One general objection to roasting frozen turkeys is that the bird cannot be stuffed, and the giblets are inaccessible. Perhaps the giblets (in a plastic bag) could be stapled to the wing or in some other fashion made accessible for separate cooking. Alternatively, one could oven-thaw the bird, remove neck and giblets, and return the bird to the oven. Another objection was that all frozen-roasted birds, but especially those roasted in a microwave oven, had excessively pink, underdone appearing thighs, even when thermometer readings verified that the thigh had indeed reached 71.1°C or higher. This was probably because the thigh was not held at elevated temperature long enough to completely denature and coagulate the blood and meat proteins. Up to 4 hr at 68°C is required to destroy the pink bloom characteristic of rare-cooked roast beef (Dymit, 1961). Thus, a final temperature higher than 71.1°C may be preferred by some consumers to decrease the pink appearance of the thigh. A further objection to the microwave method of roasting frozen birds was the poor uniformity of browning and uneven heating of the bird, resulting in overcooked surface areas. A rotary microwave oven may perform more satisfactorily in this regard.

The birds roasted at 93.3°C were highly rated for eating quality. Panelists often commented that breast meat samples from these birds had a desirable light pink, "ham-like" color and texture. The low temperature roasted birds were also easy to prepare, since they could be left in the oven overnight. Low temperature roasting would, however, use the oven for periods often exceeding 24 hr, and were among the most energy consuming procedures evaluated in this study. The actual difference in energy cost between the most and least energy-efficient roasting methods was 2.78 cents per pound or 33.4 cents per 12 pound bird, assuming an energy cost of 10 cents per kilowatt-hour.

Roasting in a bag at 176.7°C or in a 204.4°C oven had the great advantage of speed, and degree of browning was quite satisfactory. Flavor and tenderness scores were lower than average, however, due in part to the deep breast final temperature being closer to 76.7°C than 71.1°C. Despite the previously noted objections, roasting a frozen turkey to a final temperature of 71.1°C by any of the methods used in this study would yield a roasted bird of acceptable appearance and eating quality, and could be considered an alternative to conventional methods.

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Tenderization of Beef: Effect of Enzyme, Enzyme Level, and Cooking Method

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ABSTRACT

The effect of different activity levels of papain and bromelin on shear force values and protein hydrolysis product formation in bovine deep pectoral muscle tissue was studied at two rates of heat penetration. Slow cooking required much lower activity levels than did fast cooking in order to produce the same reduction in shear force for both papain and bromelin. In general, increasd enzyme activity level resulted in increased levels of water-soluble protein hydrolysis products. Regression lines and correlation coefficients for free-amino-bearing compounds and free hydroxyproline vs shear force indicated that progressive enzyme induced loss of physical integrity was accompanied by increased solubilization of the protein hydrolysis products measured.

INTRODUCTION

TENDERNESS OF MEAT is a composite sensation largely related to the physical and biochemical status of two major classes of muscle tissue proteins, the myofibrillar proteins, and the connective tissue protein, collagen. Several researchers have demonstrated that the rate of heat penetration may have differential effects on the physical and biochemical structures and properties of these two groups of muscle tissue proteins (Paul et al., 1973; Penfield and Meyer, 1975; Hearne et al., 1978a, b). Basic conclusions which may be derived from these studies indicate that slower rates of heat penetration resulted in more granulation and coagulation of myofibrillar proteins, less cracks and breaks in fibers, increased solubilization of hydroxyproline, and a trend towards decreased shear force values when compared with faster rates of heat penetration.

Plant enzymes have been utilized by man for hundreds of years in an effort to offset or diminish those factors contributing to decreased bovine muscle tenderness. Among these plant enzymes, papain and bromelin have been at the center of both scientific investigation and practical industrial application. The degradation of the various meat fractions by papain and bromelin has been studied by several workers (Myada and Tappel, 1956; Wang et al., 1957; Kang and Rice, 1970). On the basis of a review of this work it appears that, in regards to the myofibrillar fraction, papain showed stronger proteolytic activity than did bromelin. Bromelin, on the other hand, appeared to exhibit a greater ability to hydrolyze collagen fibers than did papain. It is significant to note, however, that in all cases (Myada and Tappel, 1956; Wang et al., 1957; Kang and Rice, 1970) both enzymes, papain and bromelin, showed some degree of proteolytic action on all of the various protein fractions in bovine muscle tissue.

The present study was undertaken to determine if enzyme-cooking method combinations differentially affect cooked bovine muscle tenderness and the formation of water-soluble protein hydrolysis products.

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MATERIALS & METHODS

Sample preparation

Briskets (deep pectoral muscle) from 3–5 year old cows, Swedish Grade 1M (approximately equivalent to U.S. Commercial), were obtained from a local packing plant. After slaughter, the carcasses were held at +2°C overnight and cut into wholesale cuts the next day. The briskets were aged at +2°C for 4–5 days before freezing (−30°C for 4–6 wk). The frozen briskets were held at room temperature to allow slight surface thawing. Cores (2.5 x 5.0 cm), weighing 20–25g, were excised from the muscle parallel to the muscle fibers, placed in 2.5 cm diameter centrifuge tubes and allowed to thaw completely at 8–10°C.

Enzymes used

The commercial enzyme preparations selected for use in this study were Griffith 77-A containing papain and Griffith 77-C containing bromelin and were obtained from Griffith Laboratories, Ltd., United Kingdom. The ingredient declaration for these commercial preparations was as follows: salt, dextrose proteolytic enzyme, vegetable oil. Specific percentages of each ingredient were unobtainable.

The procedure used for the determination of the relative enzymatic activity of Griffith 77-A and 77-C was a modification of that given in Bergemeyer (1965) using casein as a substrate. The enzyme activities were expressed in the enzyme units, PU^{cas} and BU^{cas}, for papain and bromelin, respectively. Specific modifications of the procedure as incorporated for the purposes of this study included an adjustment in pH of the phosphate buffer from pH 7.6 to pH 6.0. This was necessary in order to simulate the action of papain and bromelin in meat systems. In a similar manner the incubation temperature was adjusted from 35°C (for trypsin) to the optimum temperature of papain (65°C) and bromelin (50°C). Optimum temperatures for these enzymes were chosen in lieu of a common incubation temperature due to the fact that the cooking programs utilized in this project allowed for a considerable amount of time at both 65°C and 50°C.

Cooking programs

The cooking procedures utilized in this project were laboratory simulations of two large-scale cooking programs involving the heating and subsequent cooling of cylindrical 20 cm long x 13 cm diameter muscle pieces. A description of the two large scale cooking methods is as follows: Fast, (1) Cooking water held at a constant temperature of 90°C to an internal meat temperature of 74°C; (2) Cooking water replaced with 10°C cooling water until a final temperature of 20°C is reached; (3) Total cooking time is approximately 4 hr. Slow, (1) Cooking water held constant at 55°C until internal temperature reaches 49°C; (2) Cooking water is then raised to 60°C and held constant for a period of 3 hr; (3) Water temperature is then heated to 90°C to an internal meat temperature of 74°C; (4) Cooking water replaced with 10°C cooling water until a final temperature of 20°C is reached; (5) Total cooking time is approximately 9 hr.

Simulation was accomplished by heating the small (2.5 x 5.0 cm) excised muscle cores in a HETO programmable water bath utilizing computer projected heat penetration rates for 20 cm x 13 cm muscle pieces. Fig. 1 illustrates the simulated cooking programs. Combination of enzyme type and cooking method resulted in four major treatment groups: fast cooked, papain treated; slow cooked, papain treated; fast cooked, bromelin treated; and slow cooked, bromelin treated.

Enzyme injection

One of the early objectives of this project was to evaluate enzyme induced changes in muscle tissue while also maintaining the

physical integrity of the tissue over shear value ranges common to all four major treatment groups. The enzyme activity levels shown in Table 1 were selected, on the basis of preliminary trials, in an effort to maintain shear force values (1.25 cm core) above 1.0 kg for all four enzyme-cooking method combinations.

The excised muscle cores were weighed and multiple stitch injected with the enzyme solutions given in Table 1 at a level of 10% of green weight. Control samples were injected at the 10% level with distilled water. The equipment used for the multiple stitch injection consisted of a hypodermic syringe equipped with a needle that had several openings spaced along its length so as to simulate large scale brine injection systems. Following injection, the cores were held overnight (13–16 hr) at 8–10°C. Five ml of distilled water were then added to each cooking tube and the cores were cooked in a HETO programmable water bath according to heat penetration curves which simulated the cooking programs previously outlined for 20 cm x 13 cm muscle pieces. Individual cooking trials were performed at a given heating rate (either fast or slow) including both papain-treated and bromelin-treated muscle tissue samples with varying enzyme activity levels (Table 1).

Warner-Bratzler shear (Bratzler, 1932)

The cooked muscle cores were separated from the cooking water and excess drippings were removed. The samples were then cored with a 1.25 cm Warner-Bratzler coring tool parallel to the muscle fibers. Each sample was sheared two times perpendicular to the muscle fibers and the average recorded as the kg of force needed to shear a 1.25 cm cooked muscle sample. It should be noted that the number of shear measurements per sample was limited by the small sample size (2.5 X 5.0 cm). This limitation was somewhat compensated for by replicating the experiment five times.

Preparation of cooking water

The cooking drip was transferred to 15 ml centrifuge tubes and centrifuged for 15 min at 3,600 rpm. The supernatant was decanted, the ml of cooking water recorded and the sample retained for analysis of free amino groups by ninhydrin and for free hydroxyproline.

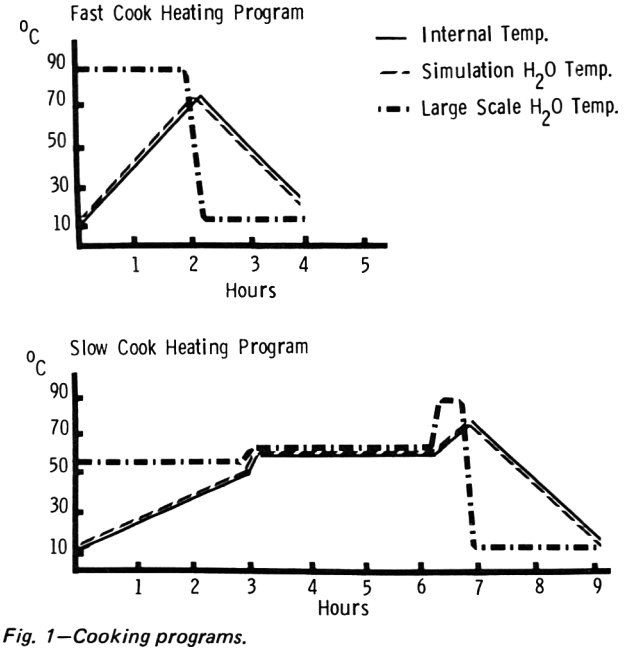

Fig. 1—Cooking programs.

Ninhydrin analysis

Proteases, such as papain and bromelin, break down proteins and their degradation products, polypeptides and peptides, by hydrolyzing the -CO-NH-peptide linkages to give either peptides or amino acids (Underkofler, 1975). The ninhydrin reaction is based on the formation of blue color by reaction of ninhydrin and compounds having free NH_2 groups including amino acids, peptides, primary amines, and ammonia. The amino acid leucine is used to determine the standard curve as it gives a high level of color development in relation to other amino acids.

One ml of cooking water was diluted to 50 ml with distilled water and analyzed for compounds having free NH_2 groups as described by Colowick and Kaplan (1957). Results are expressed as mg of leucine equivalent solubilized per gram of raw sample weight.

Assay for free hydroxyproline

A modification of the methods described by Blumenkranz and Asboe-Hansen (1975) and Kivirikko et al. (1967) was used to determine the amount of hydroxyproline solubilized in the cooking water during heating of the cores. One ml of cooking water was placed in 125 x 15 mm Teflon-lined culture tubes with screw caps. One and a half ml of borate-alanine buffer (Blumenkranz and Asboe-Hansen, 1975) were added to each tube. Approximately 2g of solid KCl was added to saturation and the tubes allowed to stand at room temperature for 20 min with occasional mixing. Oxidation was accomplished by adding exactly 0.6 ml of 0.2M Chloramine T in methyl cellosolve. Tubes were allowed to stand at room temperature for 20 min with occasional mixing. Two ml of 3.6M sodium thiosulfate were added to each tube and mixed. Exactly 5 ml of toluene were added and the caps tightened. Samples were heated in boiling water for 30 min and then cooled by running tap water. Samples were shaken for 5 min and centrifuged at 2,000 x *g* for 5 min in order to separate organic and aqueous phase. Exactly 2.5 ml of the organic phase were rapidly mixed with 1.0 ml of Ehrlich reagent (Kivirikko et al., 1967). After 30 min standing at room temperature the absorbance at 560 nm was read. Standard curve was determined by using 1–10 μg/ml solutions of L-hydroxyproline. The results were expressed as μg of free hydroxyproline solubilized per gram of raw sample weight. Free hydroxyproline, as referred to in this paper, is defined as that hydroxyproline which is solubilized in the cooking water during heating of enzyme treated muscle cores. No correction was made for the initial hydroxyproline or collagen content of individual, uncooked muscle pieces.

Statistical analysis

All analytical procedures were performed in duplicate and the experimental design was replicated five times. Statistical analysis for the effect of enzyme levels was accomplished using the Least Squares and Maximum Likelihood General Purpose Program of Harvey (1968). Linear regression, standard error of estimate for the regression line, and correlation of protein hydrolysis products vs shear force (Table 3, fig. 3–6) were determined according to Dixon and Massey (1969) and were used to evaluate the effects of cooking method and enzyme type.

RESULTS & DISCUSSION

Effect of cooking method on control samples

The effect of cooking method on control samples (no enzyme; injected with distilled water at 10% of green

Table 1—Activity concentrations of injected enzyme solutions

Enzyme	Cooking program	Activity levels used[a]							
Papain (77-A)	Fast	1.00	1.43	4.00	5.70	8.00	11.40	12.00	22.80
Papain (77-A)	Slow	0.05	0.20	0.60	1.00	1.20			
Bromelin (77-C)	Fast	3.64	5.00	14.60	15.00	25.00	25.60	35.00	36.50
Bromelin (77-C)	Slow	0.50	1.46	2.50	3.65	7.50	10.95	12.50	29.20

[a] Papain Activity Levels = Papain Units $^{casein}/_{1}$ ($PU^{cas}/_{1}$)
Bromelin Activity Levels = Bromelin Units $^{casein}/_{1}$ ($BU^{cas}/_{1}$)

weight) was explored in an effort to establish time-temperature relationships exclusive of enzyme treatments. Comparisons of the effect of cooking method on control samples are given in Table 2. Cooking method did not have a significant ($p < 0.05$) effect on either shear force value (kg; 1.25 cm core) or cooking water free hydroxyproline of the control samples. The faster rate of heat penetration did result in significantly ($p < 0.05$) higher levels of free-amino-bearing compounds in the cooking water of control samples. Intuitively, it may seem logical to expect the slower rate of heating to produce such an effect. Hearne et al. (1978a, b) found that a faster rate of heating resulted in increased disintegration and fragmentation of myofibrillar proteins while lower rates showed a greater degree of myofibrillar coagulation with considerably less disintegration and fragmentation. The increased disintegration and fragmentation which appears to occur at the faster rates of heating would seem to indicate a breaking apart of the myofibrillar proteins, possibly resulting in the formation of water-soluble protein components which would readily react with the ninhydrin reagent. On the other hand, the increased degree of coagulation noted in slower rates of heating would indicate a trend towards the formation of an insoluble protein network (Hamm and Deatherage, 1960; Cheng and Parrish, 1979). This would therefore limit the amount of water-soluble components having the ability to react with the ninhydrin reagent. These two effects, taken together, would seem to provide a viable explanation for the higher levels of free-amino-bearing compounds detected at the fast rate of heating.

Effect of enzyme, enzyme level and cooking method on W-B shear force (kg; 1.25 cm core)

It was the intent of this project to evaluate enzyme induced biochemical changes in cooked bovine muscle tissue while at the same time maintaining a predetermined degree of physical integrity common to all four major enzyme-cooking method treatments. Therefore, enzyme levels (activity basis) were deliberately chosen so as to provide significant changes in shear force values within the predetermined range.

As expected, increasing levels (activity basis) of enzyme resulted in significant ($p < 0.05$) decreases in shear force values for papain-fast, papain-slow, bromelin-fast, and bromelin-slow enzyme-cooking method treatments (Fig. 2). It is also apparent from Fig. 2 that, for both papain and bromelin, slow cooking required much lower activity levels than did fast cooking in order to produce the same decreased degree of physical resistance to shear. This relationship was a reflection of the basic catalytic properties of virtually all enzymes and is based on time-temperature interaction, with extended periods of time at optimum temperatures accounting for increased substrate hydrolysis (Underkofler, 1975). It therefore follows that less enzyme would be required at the slow rate of heating in order to produce levels of hydrolysis (as measured by loss of physical integrity) equal to that produced by higher levels of enzyme when meat was heated at a faster rate.

It can futher be seen from Fig. 2 that under the conditions of this experiment, papain appeared to be more effective in producing significant decreases in shear force than did bromelin, since less papain (activity basis) was required to produce the same reduced degree of physical resistance to shear. This relationship appeared to hold true for both fast- and slow-cooking methods. Again, this relationship can be related to the basic factors affecting all enzymes including substrate specificity, pH, time and temperature (Underkofler, 1975).

Effect of enzyme, enzyme level and cooking method on free amino bearing compounds in cooking water

The use of the ninhydrin procedure for the detection of protein hydrolysis products was limited by its lack of specificity due to its reaction with any compound having free NH_2 groups (Colowick and Kaplan, 1957). Inferences as to specific origins of detected ninhydrin positive products must be made with care in that hydrolysis of all three major muscle protein fractions (myofibrillar, sarcoplasmic and stroma) result in formation of compounds capable of reacting with the ninhydrin reagent. However, it should be noted that of the three major muscle protein fractions, approximately 89% is composed of myofibrillar and sarcoplasmic proteins (Scopes, 1970). It would therefore seem reasonable to expect that, under the conditions of this experiment, observations drawn from detection of ninhydrin products may be closely related to hydrolysis of these two muscle fractions. This statement would not hold true if the design had included an enzyme specific for collagen.

Regression lines for free-amino-bearing compounds in the cooking water (CWFAC) vs muscle core shear force are presented in Fig. 3 and 4. It is apparent from these regression lines that, for all four major enzyme-cooking method treatments, as free-amino-bearing compounds in the cooking water increased, a corresponding decrease in muscle core shear value was experienced. It was established earlier in this paper that increasing enzyme activity level resulted in decreased shear force values, thus it seems reasonable to propose that progressive enzyme induced losses of physical integrity, as measured by shear force, are accompanied by increased amounts of solubilized protein hydrolysis products in the cooking water.

It should be further noted that thermal degradation of the injected enzymes themselves might have been responsible

Table 2—Effect of cooking method on least squares means for texture and amount of solubilized protein components in cooked bovine muscle tissue (control samples[a])

	Cooking method	
	Fast[b]	Slow[c]
Shear (kg; 1.25 cm core)[n.s.]	5.97	5.59
CWFAC[d] ($\frac{\text{mg leucine eq.}}{\text{g sample}}$)*	2.08	1.66
CWFHP[e] (μg/g sample) n.s.	0.91	1.01

[a] Injected with distilled water at a level of 10% of green weight
[b] 90°C constant (water bath)
[c] 55°C to internal temperature of 49°C, held at 60°C for 3 hr, heated again at 90°C (water bath).
[d] CWFAC = Cooking water free-amino-bearing compounds
[e] CWFHP = Cooking water free hydroxyproline
n.s. = Non significant ($p < 0.05$)
* Significant ($p < 0.05$)

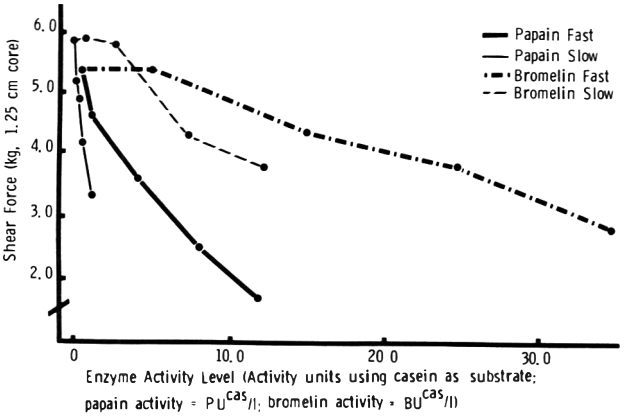

Fig. 2—Effect of enzyme, enzyme level and cooking method on shear force.

for some of the observed increase in CWFAC associated with increasing enzyme concentrations. However, the quantity of enzyme actually injected into individual tissue samples would appear to be sufficiently small so as to limit any significant contribution to CWFAC. Further cooking trials involving solely the enzymes themselves may be indicated in order to determine their specific contribution to CWFAC.

Correlation coefficients (Table 3) between CWFAC and shear force were highly significant ($p < 0.01$) for papain-fast cook and papain-slow cook and were significant ($p < 0.05$) for bromelin-fast cook and bromelin-slow cook. It was noted earlier that detection of ninhydrin products could possibly be related to hydrolysis of the myofibrillar and sarcoplasmic proteins. Kang and Rice (1970) observed that papain was more effective on both the myofibrillar and sarcoplasmic fraction than was breomelin. The simple correlations presented in Table 3 support the results of Kang and Rice (1970) indicating the greater relationship of papain in both cooking methods to loss of physical integrity as measured by shear force.

Table 3—Simple correlation coefficients (r), regression line slope (b) and standard error of estimate for the regression line ($s_{Y \cdot X}$) for amount of solubilized protein components vs shear force (Fig. 3–6)

	Cooking method					
	Fast[a]			Slow[b]		
Enzyme	r	b	$s_{Y \cdot X}$	r	b	$s_{Y \cdot X}$
CWFAC[c]						
Papain	−0.50**	−0.11	0.17	−0.60**	−0.17	0.33
Bromelin	−0.41*	−0.15	0.41	−0.30*	−0.11	0.49
CWFHP[d]						
Papain	−0.34*	−0.05	0.42	−0.47**	−0.15	0.39
Bromelin	−0.21	−0.04	0.32	−0.38*	−0.14	0.56

[a] 90°C constant (water bath)
[b] 55°C to internal temperature of 49°C, held at 60°C for 3 hr, heated again at 90°C (water bath)
[c] CWFAC = Cooking water free-amino-bearing compounds
[d] CWFHP = Cooking water free hydroxyproline
* Significant at $p<0.05$
** Highly significant at $p<0.01$

The regression lines for CWFAC vs shear force presented in Fig. 3 and 4 indicate that papain-slow cook produced higher overall levels of CWFAC than did bromelin-slow cook. This relationship would appear to be consistent with the notion that papain was more active on myofibrillar and sarcoplasmic fractions of muscle tissue than was bromelin.

On the other hand, the regression lines of CWFAC vs shear force presented in Fig. 3 and 4 for the fast cook method indicate that for this cooking program, bromelin produced higher overall levels of CWFAC than did papain. This apparent inconsistency may be resolved by considering the fact that the ninhydrin procedure has an inherent lack of specificity and as such can provide only a quantitative and not a qualitative picture of the protein hydrolysis products which have been formed. This is significant to note, since the specific nature of all enzymatic reactions would suggest that the hydrolysis products formed by action of bromelin and papain on muscle tissue would most likely differ in their specific biochemical nature (Underkofler, 1975). Furthermore it would seem that the specific nature of the hydrolysis products formed may be more important in determining the extent of the loss of physical integrity in enzyme treated muscle tissue than the total amount of hydrolysis products formed.

The regression lines presented in Fig. 3 and 4 also indicated that for a given degree of physical integrity (as measured by shear force) the four major treatment groups each resulted in the formation of different levels of cooking water free-amino-bearing compounds (CWFAC). This may further support the notion that the specific hydrolysis products formed as a result of enzyme-cooking method interaction may be of more significance than the absolute quantitative amount of such products. Additional research may be indicated to determine the specific nature of free-amino-bearing hydrolysis products formed by the action of papain and bromelin on muscle tissue.

In regards to cooking method, regression lines for CWFAC vs shear force (Fig. 3 and 4), indicated that fast cooking, independent of type of enzyme, resulted in higher

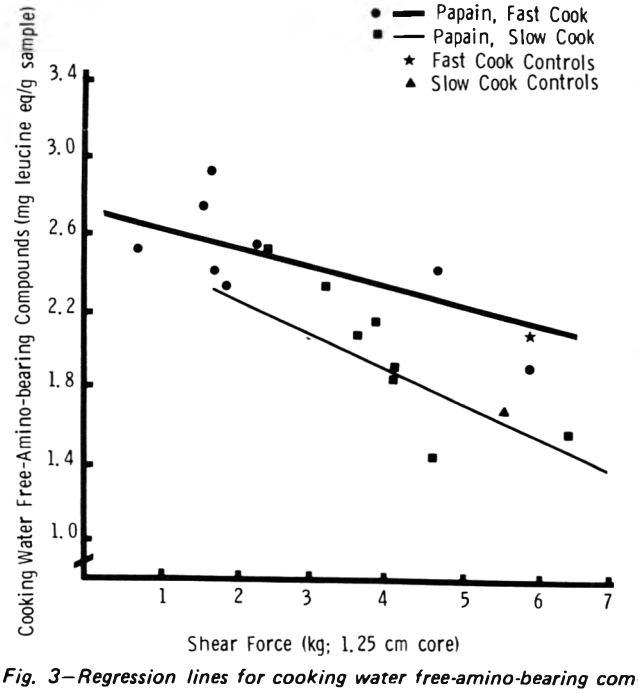

Fig. 3—Regression lines for cooking water free-amino-bearing compounds vs Warner-Bratzler shear force in papain-treated deep pectoral muscle.

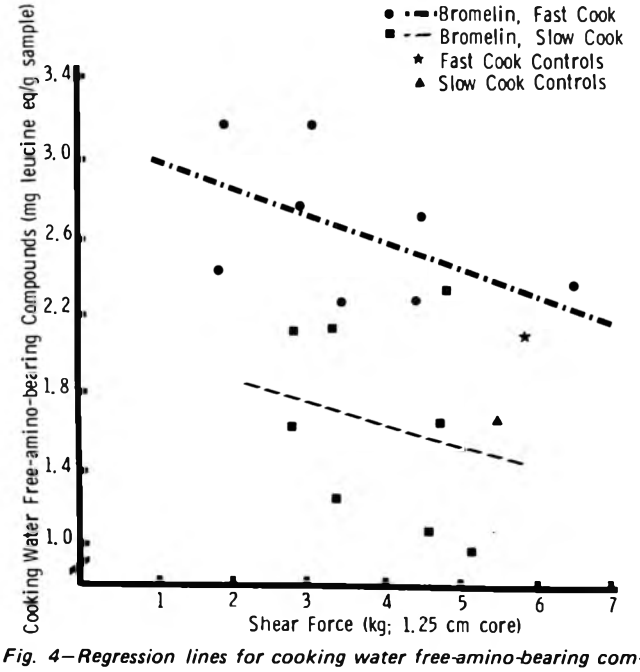

Fig. 4—Regression lines for cooking water free-amino-bearing compounds vs Warner-Bratzler shear force in bromelin-treated deep pectoral muscle.

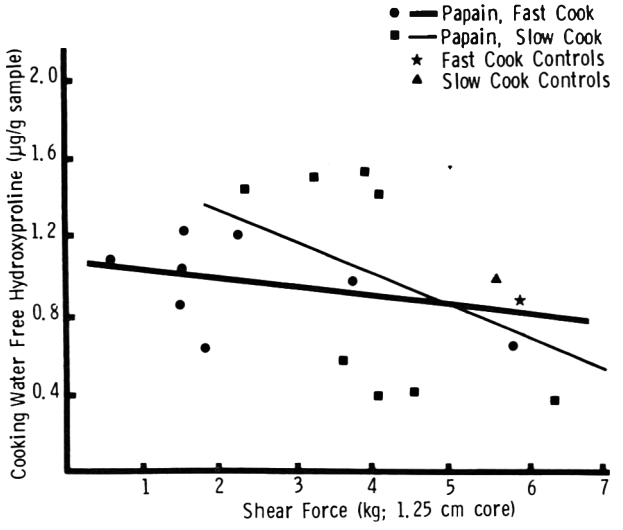

Fig. 5—Regression lines for cooking water free hydroxyproline vs Warner-Bratzler shear in papain-treated deep pectoral muscle.

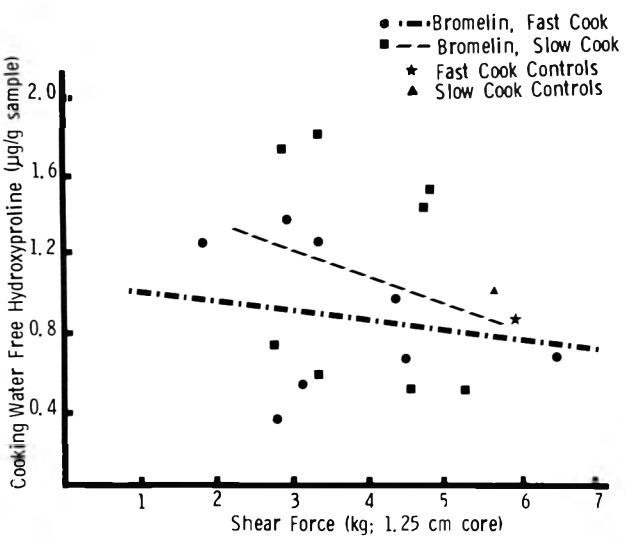

Fig. 6—Regression lines for cooking water free hydroxyproline vs Warner-Bratzler shear in bromelin-treated deep pectoral muscle.

levels of these products at a given shear force value, than did slow cooking. This was consistent with results obtained from control (no enzyme) samples in which fast cooking produced significantly ($p < 0.05$) higher levels of CWFAC than did slow cooking. It would therefore seem apparent that cooking method has the greatest influence on the overall level of these compounds solubilized in cooking water while increased enzyme activity level did account for increased formation of CWFAC within cooking treatment group and within enzyme type.

Effect of enzyme, enzyme level and cooking method on cooking water free hydroxyproline

Regression lines showing the relationship between cooking water-free hydroxyproline (CWFHP) and shear force are presented in Fig. 5 and 6. Correlation coefficients and slopes for CWFHP vs shear force are presented in Table 3. The regression lines for CWFHP vs shear force seem to indicate, for all four major enzyme-cooking method treatments that as the amount of free hydroxyproline in the cooking water increased the shear force decreased. This observation was supported by the correlation coefficients of CWFHP vs shear force which were highly significant ($p < 0.05$) for fast cooked, papain treated muscle tissue and slow cooked, bromelin treated muscle tissue. Although the correlation between CWFHP and shear force for fast cooked, bromelin treated samples was not significant, the regression line for the same enzyme cooking method treatment indicated a general trend towards increased CWFHP with decreased shear force.

The slopes of the regression line (Table 3) for CWFHP vs shear force were greater for slow vs fast cooking for both papain and bromelin. This observation seems to indicate that hydrolysis of collagenous tissue, as measured by increases in CWFHP, in papain and bromelin-treated samples responded more readily to increased enzyme activity level for the slow, as opposed to the fast, cooking method. This conclusion is further supported by the correlation coefficients presented in Table 3 as slow cooking resulted in correlations of higher magnitude, as compared to fast cooking, for both papain and bromelin. The differential effects of slow vs fast cooking on the regression lines and correlation coefficients for CWFHP vs shear force were a reflection of the fact that the slow cooking method provided for a considerably longer period of time at optimum incubation temperatures (50°C for bromelin, 65°C for papain) than did the fast cooking method.

It would seem logical to expect that the apparent differential effect of slow vs fast heating on the level of free hydroxyproline solubilized in the cooking water would also result in differential effects on shear values. However, it was evident from the regression lines presented in Fig. 5 and 6 that this was not the case since a given shear force value was associated with varying levels of cooking water free hydroxyproline (CWFHP). This ovservation was consistent with earlier work by Penfield and Meyer (1975) dealing with the effect of rate of heat penetration on tenderness and collagen of beef semitendinosus. In that study, slow and fast rates of heat penetration resulted in a significant difference in solubilized hydroxyproline but not in shear values. It has been suggested by other workers (Cross et al., 1973; Paul et al., 1973; Penfield and Meyer, 1975) that the effects of increased collagen solubilization were overshadowed by other factors important in the determination of shear force and that tenderness of heated bovine muscle tissue was more closely related to muscle fiber properties than to connective tissue components.

From these studies it would appear that, in terms of large scale, practical application of enzyme tenderization of cooked bovine muscle tissue, the single most important consideration is the individualized tailoring of enzyme levels to complement enzyme type and cooking method. This must be so, as the catalytic activity of all enzymes is intimately associated with the environment in which they must function. Further studies involving such areas as residual enzyme activity, influence of muscle tissue source and various enzyme combinations may be beneficial in defining specific enzyme systems with a wide range of practical applications.

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Significance of Lactobacilli and Film Permeability in the Spoilage of Vacuum-Packaged Beef

A. F. EGAN and B. J. SHAY

ABSTRACT

The spoilage of vacuum-packaged fresh beef during storage at 5°C was studied using analytical taste panels. In the absence of contaminating micro-organisms, meat spoiled due to the development of an "off" flavor described as "liver-like." This occurred even when the meat was packaged in bags made of film of very low oxygen permeability, but the rate of spoilage increased as the film permeability increased. Thus, vacuum-packaged beef has a limited shelf life even in the absence of a significant population of contaminating micro-organisms. Pure cultures of lactic acid bacteria increased the rate of spoilage, which was then due largely to the development of flavor defects described as sour, acid and bitter. Depending upon the strain of bacteria chosen, off flavor became significant 13–28 days after the population reached $10^8/cm^2$.

INTRODUCTION

WHEN FRESH BEEF is vacuum-packaged in plastic films of low gas permeability the shelf life is greatly increased (D'Alessandria and Pagliaro, 1975; Dainty et al., 1979; Newton and Rigg, 1979). This increase is due to the replacement of the normal aerobic spoilage flora by one comprised mainly of lactic acid bacteria (Dainty et al., 1979). Examination of the literature indicates that this group of organisms is considered of less significance in spoilage than others (Gill and Newton, 1978; Newton and Rigg, 1979), but there are few quantitative data to support this.

When vacuum-packaged meat is opened after prolonged storage, odours variously described as cheesy, sour or stale are noticed. This raises the question as to whether the shelf life could be further extended by the elimination of the lactic acid bacteria. This in turn requires an evaluation of the ultimate shelf life of vacuum-packaged beef, i.e. the shelf life of sterile vacuum-packaged beef.

We have been using a trained analytical panel to evaluate the changes produced by the presence of pure cultures of bacteria on packaged meats. Experiments of this type have shown that *Brochothrix thermosphacta* caused spoilage of vacuum-packaged sliced luncheon meats at 5°C much more rapidly than did lactobacilli (Egan et al., 1980). In further studies fresh beef was inoculated with this organism, vacuum-packaged in a plastic film of relatively high gas permeability and stored at 5°C. Spoilage was rapid and was due to the development of an "off" aroma and flavor, which were significant by the time the population reached 10^8 cells/cm^2 (Egan and Grau, 1981).

In the present study we have examined the spoilage of vacuum-packaged beef stored at 5°C in the absence of a significant population of bacteria. The effects of packaging film permeability and the presence of lactic acid bacteria on the rate of spoilage have been studied.

EXPERIMENTAL

MOST PROCEDURES have been described in detail previously (Campbell et al., 1979; Egan et al., 1980).

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Bacteria

Strains of psychrotrophic lactic acid bacteria were selected from a collection of isolates obtained from vacuum-packaged fresh beef which had been stored for 9 wk at 0°C. Strain L7 is a heterofermentative coccus and has been tentatively identified as *Leuconostoc mesenteroides*. Strains L70 and P40 are homofermentative rods which could not be classified down to a species level. They are thus atypical streptobacteria. Whereas P40 is unable to deaminate arginine, L70 deaminates this compound in the presence of a low (0.05% w/v) but not of a high concentration of glucose. This latter strain closely resembles *Lactobacillus plantarum*. All strains grow readily in pure culture on vacuum-packaged beef at temperatures down to 0°C.

Growth of bacteria

Liquid medium for the growth of lactic acid bacteria contained 0.2% w/v glucose, 0.3% w/v yeast extract, 0.1% v/v Tween 80 and 0.005% w/v $MnSO_4 \cdot 4H_2O$ in half-strength medium 56 (Monod et al., 1951). Solutions of glucose and manganese sulphate were sterilized separately and added to the bulk of the medium which had been sterilized by autoclaving. Inocula were grown at 5°C under anaerobic conditions. Cells in the exponential phase of growth (ca 5.0×10^8 cells per ml) were harvested by centrifugation at $3000 \times g$ for 10 min, suspended in distilled water to give ca 1.0×10^6 cells per ml and placed in a sterile glass chromatogram sprayer.

Meat samples

Beef biceps femoris muscles of pH 5.4–5.8 were selected on the basis of surface pH (Campbell et al., 1979). The muscles were trimmed with a sterile knife, hung in a sterile cabinet and flamed for ca 15 min using a Bunsen burner. This resulted in a cooked layer of at least 0.5 cm depth. Using aseptic techniques this layer was removed and the underlying muscle cut into slices ca 1.25 cm thick (100–200g). Slices of meat were inoculated by spraying both sides with the suspension of cells so that an initial viable count of ca 10^4 per cm^2 was produced. After vacuum-packaging (Egan et al., 1980) samples were stored at 5°C and 90% RH in the dark. Samples to be used as frozen controls were stored at −20°C.

Taste panel assessment of stored samples

Each experiment consisted of at least three treatments. Inoculated samples were compared to uninoculated or frozen controls. To reduce possible variability due to differences in test material, three separate beef muscles were used in each experiment. Each time point thus comprised three separate tasting sessions. At a session, packs from the same muscle were compared and each muscle was assessed at every time point. After sampling for microbiological analysis, meat was frozen and stored at −20°C. Frozen samples were thawed at 0–1°C and then minced. After mincing, beef was mixed with an equal weight of water and brought to the boil in a small stainless steel saucepan fitted with a lid. After simmering gently for 20 min the warm mince was presented to the taste panel for evaluation. Fourteen tasters, experienced in meat flavor assessment, scored a sample from each treatment for "meat" and off aromas and flavors and for overall acceptability. Aroma and flavor intensities were rated on 9-point structured scales (0-none, 2-slight, 4-moderate, 6-strong, 8-very strong) and overall acceptability was rated using a 9-point hedonic scale (0-very poor, 2-poor, 4-moderate, 6-good, 8-very good). Sample packs were selected at random for tasting at each time point. The order of sample assessment was randomised amongst panelists to cancel out any sequential effects.

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Microbiological analysis

Using a sterile stainless-steel cork borer a sample (area 5 cm^2 x ca 3–4 mm deep) was excised from the surface of each piece of meat. At each time point, sub-samples from each of the three packs from each treatment being tasted were pooled to produce a 15 cm^2 sample. Samples were placed in a blender bowl which contained 90 ml of 0.1% w/v peptone water, blended at 12,000 rpm for 30 sec and appropriate dilutions made in peptone water. All samples were plated for enumeration on tryptone soya agar (Oxoid CM131) supplemented with 0.2% w/v yeast extract and 0.2% w/v glucose. Samples inoculated with lactic acid bacteria were plated also on the medium of de Man et al. (1960). Duplicate plates were incubated in an atmosphere of nitrogen containing 10% (v/v) of carbon dioxide for 4–5 days at 25°C and also for 21 days at 5°C.

Packaging films

Four different films, varying in gas permeability were used. The film of lowest permeability was a multi-layered material which had a very low oxygen permeability (<1 $cm^3/m^2/24$ hr/atm measured at 25°C and 75% RH). It consisted of a 12 μm thick layer of aluminum foil held between two 12 μm layers of polyester and bonded to a 50 μm internal layer of polypropylene copolymer. The other low permeability film consisted of polyvinylalcohol (thickness 50 μm) coated on both sides with polyvinylidene chloride and laminated to 50 μm thickness ethylene-vinylacetate copolymer (oxygen permeability, ca 1 $cm^3/m^2/24$ hr/atm measured at 25°C and 75% RH).

The third film used had an oxygen permeability of ca 25 $cm^3/m^2/24$ hr/atm at 25°C and 75% RH and consisted of a layer of polyvinylidene chloride coated on both sides with ethylene-vinylacetate copolymer. Bags made of this film are widely used in the Australian chilled meat export trade (Barrier Bag, W.R. Grace & Co.). The oxygen permeabilities of these three films do not vary with changes in relative humidity. The highest permeability film consisted of nitrocellulose coated cellophane laminated to polyethylene. It had an oxygen permeability of ca 1000 $cm^3/m^2/24$ hr/atm at 25°C and 90% RH.

RESULTS

THE pH VALUES of the meat samples chosen were in the range of 5.45–5.65. Whereas the pH of uninoculated samples remained unchanged through the period of storage, the pH values of the samples inoculated with lactic acid bacteria fell slightly and were in the range of 5.3–5.45 after 24–35 days storage.

Uninoculated samples used as controls were initially contaminated with low numbers of microorganisms, but the count usually declined during storage. On no occasion did it reach $10^3/cm^3$ and on most samples it was less than 50/cm^2 (the limit of detection). For uninoculated samples, counts obtained by incubating plates at 5°C agreed well with those for the corresponding plates incubated at 25°C.

In the first experiment the rate of spoilage at 5°C of meat inoculated with a pure culture of the homofermentative lactobacillus (strain P40) was determined. Inoculated meat samples were packaged in bags made of plastic films with oxygen permeabilities of either < 1 or ca 1000 $cm^3/m^2/24$ hr/atm, chosen since these films were the least and most permeable, respectively, of those used. Control samples consisted of uninoculated meat packaged in bags made of the film of lowest permeability.

Lactobacillus P40 grew readily on all inoculated samples. From an initial count of ca 3 x 10^4 the population reached $10^8/cm^2$ after 11 days storage and remained at 1–2 x $10^8/cm^2$ for the duration of the experiment. At each sampling point the counts obtained using the selective agar agreed well with those of the complete nonselective medium, and the counts obtained at 5°C agreed with those at 25°C. Taste panel evaluation showed that meat inoculated with P40 developed an off aroma and off flavor which increased with storage time (Fig. 1). These defects of the cooked minced samples were much more intense for the meat packaged in the film of highest permeability. For such samples the "off" aroma became significantly different from that of the uninoculated controls after 18 days storage ($P < 0.1\%$) and "off" flavor became significant after 24 days ($P < 0.1\%$). Acceptability declined with time, became significantly different after 18 days storage ($P < 5\%$) and was rated poor to very poor after 24 days ($P < 0.1\%$). For samples inoculated with P40 and packaged in film of the lowest permeability, all three parameters became significantly different from those of the control samples after 24 days storage (aroma, $P < 5\%$; flavor, $P < 1\%$; acceptability, $P < 0.1\%$). Further the uninoculated control samples appeared to be slowly but steadily developing both an "off" aroma and flavor.

In the second experiment the effect of packaging tilm permeability on the storage life of uninoculated meat was examined. Samples were packaged in films of three different oxygen permeabilities (ca 1, 25 and 1000 $cm^3/m^2/24$ hr/atm) and stored at 5°C. Similar samples packaged in the

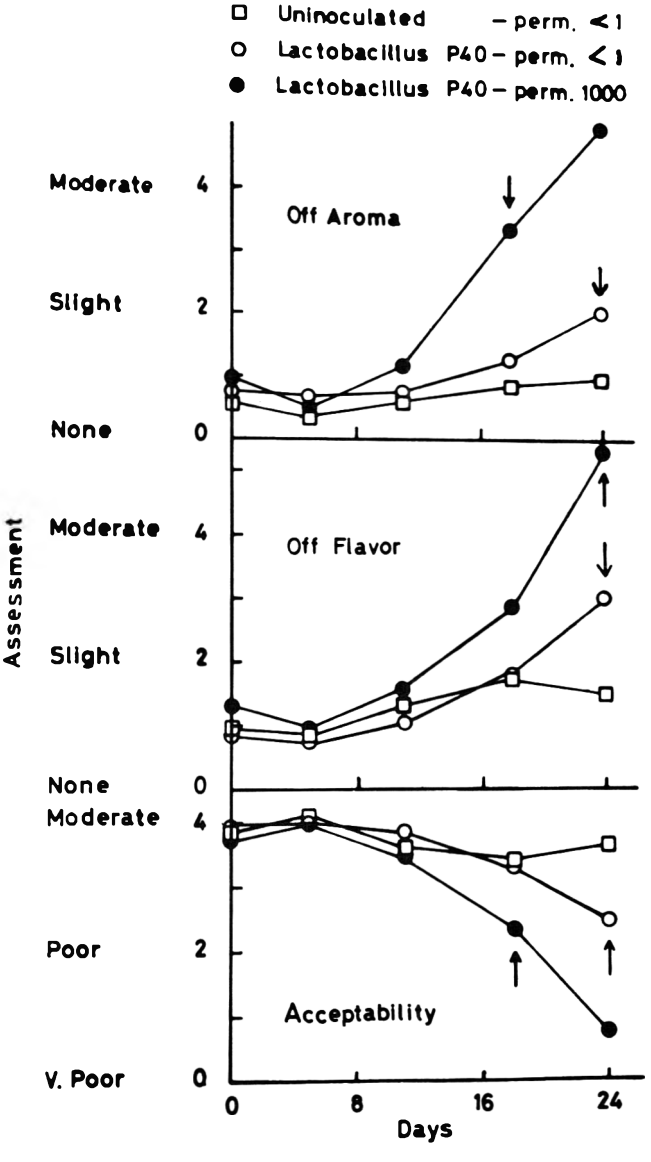

Fig. 1—Taste panel assessments of vacuum-packaged fresh beef (pH 5.45–5.65) stored at 5°C. Samples inoculated with strain P40, a homofermentative lactobacillus, and packaged in bags with oxygen permeabiliteis of either <1 or ca 1000 $cm^3/m^2/24$ hr/atm, were compared to uninoculated control samples stored in bags of the lower permeability. Arrows indicate the times at which samples first became statistically significantly different from the controls. The population of P40 reached $10^8/cm^2$ at 11 days.

film of permeability 1 cm^3 /m^2 /24 hr/atm, but frozen and stored at −20°C, were used as controls.

When compared to the frozen controls, all samples stored at 5°C developed aroma and flavor defects (Fig. 2) which resulted in a reduction in acceptability. These defects developed more rapidly as film permeability increased. In the case of meat packaged in the film of permeability 1000 cm^2 of O_2/m^2/24 hr/atm, off flavor developed very rapidly and it was significantly different from that of the control samples after 14 days storage ($P < 0.1\%$). Acceptability also became significantly reduced at this time ($P < 0.1\%$) but off aroma did not reach a significant level until 27 days storage ($P < 0.1\%$).

The defects developed much more slowly for samples packaged in the other two films. For meat stored in bags with an oxygen permeability 25 cm^3/m^2/24 hr/atm the aroma and flavor defects did not become significant until 27 days ($P < 0.1\%$ and $< 5\%$, respectively) whereas acceptability was significantly reduced at 21 days ($P < 1\%$). For samples in bags of oxygen permeability ca 1 cm^3/m^2/24 hr/atm the "off" aroma and flavor which developed did not become significantly different from the aroma and flavor of the frozen control samples throughout the duration of the experiment (27 days). Acceptability was significantly reduced at 21 days ($P < 5\%$) but was again non significant at 27 days.

This experiment also allows a comparison of the rate of spoilage of uninoculated meat stored in bags of permeability 25 cm^3/m^2/24 hr/atm with that of meat stored in bags of permeability 1 cm^3/m^3/24 hr/atm. After 27 days storage no significant differences had developed in off flavor and acceptability but samples stored in bags of the higher permeability had developed an off aroma which was significantly worse than the aroma of samples in bags of the lower permeability ($P < 0.1\%$).

In the final experiment the rates of spoilage produced by *Lactobacillus* L70 and *Leuconostoc* L7 were evaluated. Again uninoculated meat was used as the control samples. All samples were packaged in bags made of plastic film with an oxygen permeability ca 25 cm^3/m^2/24 hr/atm.

Both strains of bacteria grew readily on the meat (Fig. 3). Strain L70 increased from an initial count of $2 \times 10^4/cm^2$ and reached a population in excess of 10^8 /cm^2 after 7 days storage at 5°C. Strain L7, the leuconostoc, was at a lower

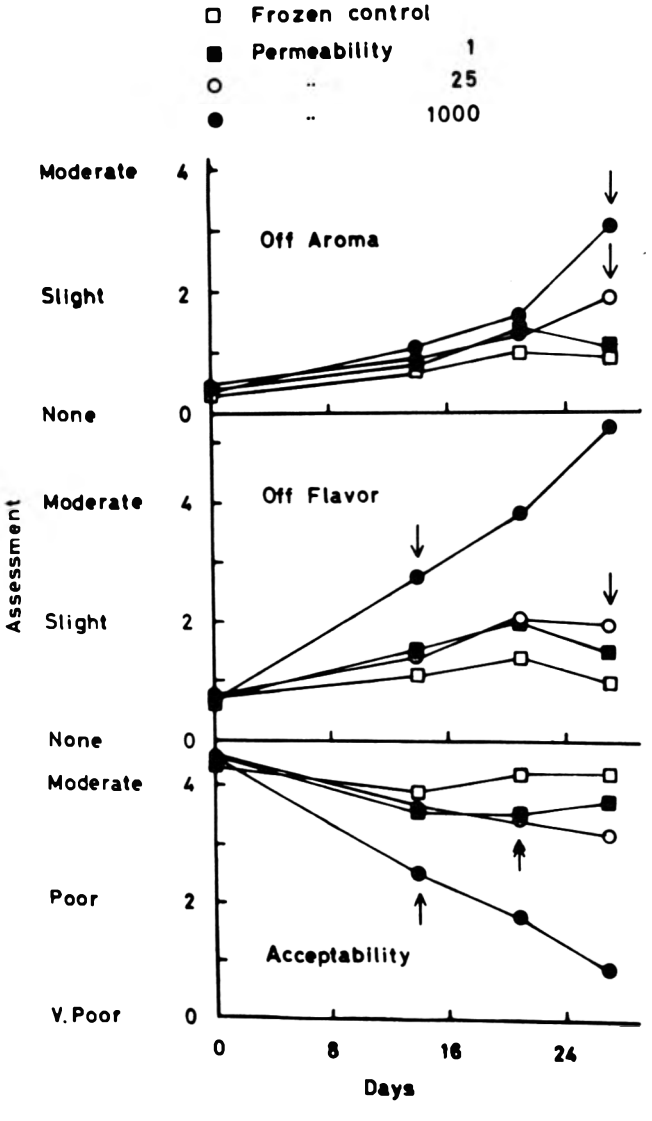

Fig. 2—Taste panel assessments of uninoculated "sterile" vacuum-packaged beef stored at 5°C. Samples stored in bags with oxygen permeabilities of either <1, 25 or ca 1000 cm^3/m^2/24 hr/atm were compared to frozen controls. Arrows indicate times at which samples first became significantly different from the controls.

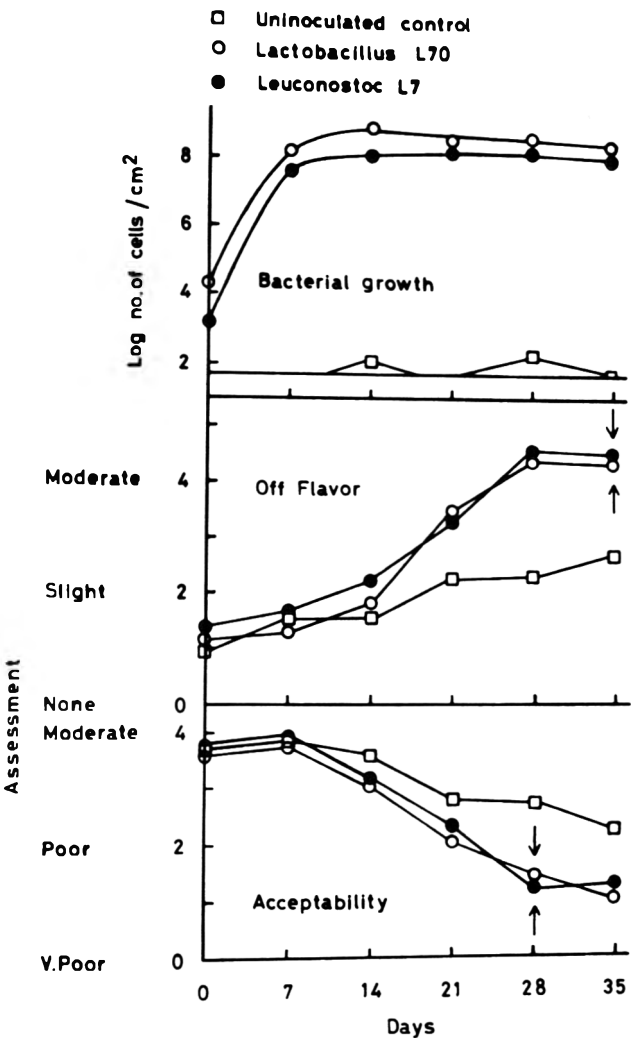

Fig. 3—Growth of lactic acid bacteria on vacuum-packaged fresh beef and taste panel assessments of resulting changes in flavor and acceptability. Samples inoculated with pure cultures of Leuconostoc L7 or Lactobacillus L70 were compared to uninoculated control samples. All meat was packaged in a film with an oxygen permeability of 25 cm^3/m^2/24 hr/atm. Arrows indicate the times at which samples first became significantly different from the controls.

population level throughout the experiment (as determined by viable count). However this organism forms chains of cells and as the cultures of the two strains were at the same turbidity at the inoculation, we believe they were both present on the meat at approximately the same mass per unit area. With both inoculated sets of samples a slight off aroma slowly developed. This became significant for the leuconostoc (strain L7) after 35 days storage ($P < 5\%$) but did not become significant for the homofermentative lactobacillus. Flavor defects gradually developed (Fig. 3) and these became significant with both strains after 35 days ($P < 1\%$ in each case). Acceptability declined slowly and became significantly lower than that of the uninoculated controls after 28 days ($P < 5\%$ in each case).

As noted in the first experiment, the quality of the uninoculated samples gradually declined with time (Fig. 3). The taste panel noted a slight but steady increase in "off" flavor and a corresponding reduction in acceptability.

Throughout the experiments browning of meat occurred with samples stored in bags made of film with an oxygen permeability of ca 1000 $cm^3/m^2/24$ hr/atm, but not with samples stored in bags made of the films of lower permeability. This was the only visual defect noted. No slime was observed on samples inoculated with the leuconostoc. Stored samples gradually developed a "confinement" odour often noted with vacuum-packaged beef. This dissipated rapidly after opening the packs.

The off flavor which developed in the cooked mince prepared from samples was consistently described by tastepanellists as "liver-like." The defects which arose with samples inoculated with lactic acid bacteria were described as sour, acid, bitter and also "liver-like."

DISCUSSION

THE MICROBIAL STATUS of the muscle tissue of the carcasses of freshly-slaughtered animals has been discussed in detail recently (Gill, 1979). It appears generally accepted that the muscle tissue of healthy animals is sterile and that sterile muscle samples can be readily obtained by a variety of methods such as the one used in the present study. We were unable to detect either psychrotrophic or mesophilic bacteria on most of the uninoculated samples used in the present experiments. In those cases where organisms were detected no growth occurred during storage. We are unaware of any report describing the growth of obligate anaerobes on vacuum-packaged beef of low or normal pH at 0–5°C. Whilst we cannot exclude the possibility that very small populations of strict anaerobes were present on some samples, the population level would have been unlikely to have contributed in a significant manner to the organoleptic changes reported in this study.

The experiments show that vacuum-packaged fresh beef spoils at 5°C in the absence of a significant population of contaminating micro-organisms. Whilst the rate of spoilage is dependent upon the oxygen permeability of the packaging film, it occurred even in bags made of film with an oxygen permeability of less than 1 $cm^3/m^2/24$ hr/atm. To our knowledge this is the first study to demonstrate a limited shelf life for fresh beef stored "sterile" under conditions of very low oxygen tension.

Even though lean meat was used throughout the experiments, the dependence of spoilage rate upon film permeability suggests that oxidative rancidity was one factor in spoilage. However, the fact that spoilage occurs even in films of very low permeabilities means that other mechanisms must also be involved. Spoilage appears to be mainly due to the development of a flavor rather than an aroma defect. This off flavor, described as "liver-like," became significant for meat stored in bags made of film of oxygen permeability 25 $cm^3/m^2/24$ hr/atm after 27 days storage. For samples stored in bags made of film of permeability 1 $cm^3/m^2/24$ hr/atm the "off" flavor which developed had not become significantly different from the flavor of the frozen control samples after 27 days. However, the results strongly suggest that sterile lean meat slowly deteriorates even when stored in bags made of very impermeable film, i.e. vacuum-packaged fresh beef has an "ultimate" shelf life and eventually spoils, even in the absence of a contaminating microbial flora.

In practical terms there were only slight differences in the rates of spoilage of sterile meat stored in bags made of films with oxygen permeabilities of 1 and 25 $ml/m^2/24$ hr/atmosphere (Fig. 2) suggesting that the use of bags of very low permeability would not necessarily lead to significantly improved results in commercial use. Further studies are needed to clarify this point.

The presence of pure cultures of selected strains of lactic acid bacteria increased the rate of spoilage of vacuum-packaged beef (Fig. 1 and 3). This is in agreement with the results of Smith et al. (1980) who showed that inoculation of naturally contaminated beef steaks with lactobacilli prior to vacuum-packaging increased the rate of spoilage. In our experiments, the defect which developed was once again one of flavor rather than of aroma. When packaged in bags made of films with oxygen permeabilities of up to 25 $ml/m^2/24$ hr/atm, meat contaminated with pure cultures of the three strains of bacteria remained acceptable for a considerable period of time after the bacterial populations had reached $10^8/cm^2$. Depending upon the strain used, off flavor became significantly different 13–28 days, and acceptability 13–21 days after the count had reached $10^8/cm^2$. These times are only estimates as the organisms, particularly the leuconostoc, tend to form chains making an exact determination of viable count difficult. For meat packaged in bags made of film of the highest permeability, and carrying a pure culture of the homofermentative lactobacillus, strain P40, significant aroma and flavor defects developed 7 and 13 days respectively after the population reached $10^8/cm^2$ (Fig. 1). This result can be compared with our previously reported results for meat stored under similar conditions and inoculated with a pure culture of *Brochothrix thermosphacta*. In this case, both aroma and flavor defects were already significant at the time the population reached $10^8/cm^2$ (Egan and Grau, 1981). Thus our present results extend our earlier observations (obtained using vacuum-packaged sliced luncheon meats) that lactic acid bacteria are less significant in the spoilage of meat than is *B. thermosphacta* (Egan et al., 1980). It needs to be emphasised that this comparison has, to date, only been made for meats stored in bags made of film of relatively high permeability.

It should also be noted that whilst the available evidence indicates that lactic acid bacteria may be less significant in spoilage than the other types of organisms commonly found on packaged meat, exceptions do occur. For example, we have recently shown that a pure culture of *Lactobacillus* L13 causes spoilage of vacuum-packaged beef due to the development of a strong off aroma. This organism produces hydrogen sulphide from cysteine and this property appears to be plasmid-mediated (Shay and Egan, 1981).

There are various estimates of the shelf life of vacuum-packaged primal cuts of beef stored at 0°C in bags made from low permeability and contaminated with a natural mixed flora consisting predominantly of lactic acid bacteria. These estimates range from 8–15 wk (Dainty et al., 1979; Newton and Rigg, 1979). Our present results suggest a shelf life of about 4–5 wk at 5°C. We believe this result should extrapolate to about 10–12 wk for large cuts stored at 0°C when allowance is made for the following; (1) reduction of temperature from 5°C to 0°C; (2) the higher surface to volume ratio of the meat slices used in our experiments;

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Safety and Sensory Evaluation of Canned Rice

R. N. SHARP, A. A. KATTAN, C. Q. SHARP, and J. A. COLLINS

ABSTRACT

Rice, thermally canned as an acid food, was studied to determine if the process would provide a product which was safe and acceptable. Heat penetration studies showed that heat transfer was initially by convection and then by conduction as the rice imbibed sufficient water to restrict the convection currents. Although heat penetration studies indicated that a temperature of 180°F (82°C) was reached in 8 min and inoculated packs processed for 20 minutes in actively boiling water did not support bacterial growth, 30 minutes processing time was required to attain optimum quality. Canned rice, rice with textured vegetable protein (TVP®), Spanish rice, and Spanish rice with TVP were rated acceptable in general appearance, texture and taste. Experimental Spanish rice was rated superior to one and equal to two commercial Spanish rice products in general appearance and flavor.

INTRODUCTION

SPORE-FORMING BACTERIA are common contaminants of cereal grains and their products. Most do not pose any threat to public health, but food poisoning types such as *Clostridium perfringens* and *Bacillus cereus* may be found. *B. cereus* has been reported in a wide variety of dried foods including rice (Blakey and Priest, 1980). Boiled and fried rice were found to be excellent media for the growth of *B. cereus* (Beuchat et al., 1980; Ueda et al., 1980), and neither spore nor the toxin was destroyed during the reheating process (Gilbert et al., 1974). Spira and Silverman (1979) reported *B. cereus* growth was retarded at pH 5.5 and inhibited at pH 5.0, with the production of toxin being severely retarded at pH 5.5. The temperature ranges of growth are similar for *C. botulinum* (50–122°F) and *B. cereus* (50–113°F) (Riemann, 1965). Nebesky et al. (1950) found thermal inactivation time curves of enzymes of certain acid foods followed the pattern of thermal death time curves of bacteria, and the procedure has evolved as a measure of adequate process time. However, enzyme activity in parboiled rice is essentially nonexistent (Luh and Mickus, 1980). Thermal retardation of bacterial growth can be assessed by measuring the vacuum of cans inoculated with a gas producing, spore-forming organism. *B. polymyxa* is a spore-former capable of growing and producing gas in the pH range 4.5–6.8 (Gordon et al., 1973) and has outgrowth limits similar to those reported for *C. botulinum* (Ito et al., 1976).

The documented occurrence of *B. cereus* related food poisoning in cooked rice (Gilbert and Parry, 1977; Gilbert et al., 1974), corresponding growth conditions for *C. botulinum* and *B. cereus* (Riemann, 1965) and the lack of heat tolerance of the rice kernel (Sharp et al., 1981) necessitate the investigation of survivability of selected organisms in canned rice. This study was conducted to assess the adequacy of the thermal process of acidified canned rice products and to determine sensory quality of selected rice products canned as acid foods.

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MATERIALS & METHODS

Acidification

Nonhydrated parboiled rice was weighed into 211 x 400 R-enameled cans at 40, 45, 50, 55, 60, 65, or 70g levels. Acidification was accomplished by adding 4.0, 4.5, 5.0, 5.5, or 6.0 ml of 10% (W/V) citric acid monohydrate to each can prior to filling with hot (194°F) water. All cans were processed in actively boiling water at atmospheric pressure for 45 min. The pH of each treatment was recorded the following day.

Heat penetration

Ecklund nonprojecting connector type copper-constantan thermocouples (O.F. Ecklund, Cape Coral, FL) were placed at either the geometric center or 1/3 the can height from the bottom of 211 x 400 and 303 x 406 metal cans. Product temperatures were recorded for 60 min at a frequency of: 1-min intervals for 20 min; 2-min intervals for 20 min; and 5-min intervals for 20 min. A thermocouple was placed in the retort to monitor the temperature of the boiling water. Temperature profiles were obtained in triplicate for each thermocouple position of each can size. Sixty and 90g of rice, respectively, were placed into 211 x 400 and 303 x 406 size cans that were previously fitted with thermocuoples. The cans were filled with 194°F brine containing 0.16% citric acid monohydrate and 0.5% table salt, closed and submerged in actively boiling water at atmospheric pressure.

Inoculated packs

Ingredients shown in Table 1 were placed in either 211 x 400 or 303 x 406 cans to which approximately 10,000 spores each of *B. cereus* and *B. polymyxa* (National Food Processors Association, Berkeley, CA) were added. The cans were processed as shown in Table 1. Eight cans of each size were cooled and stored for 3 wk at

Table 1—Procedures for canning rice products

Ingredients	Canned rice	Canned rice with TVP[a]
Parboiled milled rice[b] (g)	60.0	55.0
Sugar (g)	3.5	4.5
Dehydrated onion[c] (g)	1.0	1.0
Poultry seasoning[d] (g)	0.2	0.2
Vegetable oil[5] (ml)	2.5	2.5
Textured vegetable protein[a] (g)	0.0	10.0
Brine (ml)	275.0[f]	275.0[g]

The indicated amounts of ingredients were placed into 211 x 400 R-enamel cans (for 303 x 406 cans increase all values by a factor of 1.5). The cans were filled with 194°F brine, leaving approximately 5 mm headspace, sealed immediately, and processed in actively boiling water at atmospheric pressure for 45 minutes. The cans were cooled in cold water.

[a] Textured vegetable protein (TVP) — Archer Daniels Midland Co., Decatur, IL
[b] Parboiled rice — Riceland Foods, Stuttgart, AR
[c] Dehydrated onion — Basic Vegetable Products, Inc., Vacaville, CA
[d] Poultry seasoning — McCormick and Co., Inc., Baltimore, MD
[e] Vegetable oil — Mazola Pure Corn Oil, Best Foods, A Division of CPC International, Inc., Englewood Cliffs, NJ
[f] Brine — 300 ml water, 1 chicken bouillon cube (Wyler's Chicken Bouillon, Wyler Foods, Borden, Inc., Chicago, IL), 0.5% salt, 0.16% citric acid monohydrate.
[g] Brine — 300 ml water, 1 chicken bouillon cube (Wyler's Chicken Bouillon, Wyler Foods, Borden, Inc., Chicago, IL), 0.5% salt, 0.32% citric acid monohydrate.

73, 104, or 122°F. After storage, vacuum and pH were recorded for each can. Tubes containing thioglycollate medium (Difco, 1953) were inoculated with free liquid from the product and incubated at 99°F for 24 hr. Gram strained smears from the thioglycollate medium tubes were observed microscopically.

Sensory evaluation

Experimental products were processed under the conditions described by Sharp et al. (1981) according to the procedures shown in Tables 1 and 2. Sugar: acid ratios were determined by preliminary sensory evaluations.

Nine untrained panelists composed of food science students and faculty evaluated canned rice, rice with TVP (Archer Daniels Midland Co.), Spanish rice and Spanish rice with TVP for appearance, texture and taste. A reference sample was prepared by cooking parboiled rice in water as recommended by the rice supplier. Three commercially canned Spanish rice products from local supermarkets, and experimental Spanish rice were evaluated for appearance and taste. Entire can contents of each treatment were thoroughly mixed prior to sampling. Each panelist was allowed to take a sample from each treatment. The different types of products were evaluated separately from the Spanish rice products. Duplicate samples were evaluated on separate days. Samples were presented to the panel in random order at room temperature in an open area, fluorescent lighted laboratory. Panelists were instructed to rate each sample on its own merits on a 5-point scale (5 = excellent and 3 = acceptable) using the cooked parboiled sample as a reference. The reference sample was not rated by the panel. Ratings were analyzed statistically by analysis of variance (Steel and Torrie, 1960).

Process time vs texture and safety

Rice was canned according to Table 1 except the processing time varied from 20 to 50 min in 5 min increments. These products were presented the following day to a sensory panel as described above for texture evaluation. Fifty 211 x 400 size cans were inoculated as described above and processed in 10 can lots for 20, 25, 30, and 35 min in actively boiling water. One 10 can lot was not acidified and was processed for 35 min. All inoculated cans were stored at 100°F for 3 wk.

RESULTS & DISCUSSION

Acidification

Rice processed at atmospheric pressure must be acidified to pH 4.6 or less to prevent outgrowth of *C. botulinum* spores. Cans that received less than 55g initial rice weight contained excessive water while rice products with more than 60g initial rice weight were matted and hard to remove from the can. Fig. 1 shows the final product pH resulting from the initial rice weight and the acidification level. The superimposed box enclosed the area between 55 and 60g initial rice weight and between pH 4.3 and 4.5. The optimum acidification level would then be 4.5–5.0 ml of 10% (W/V) citric acid monohydrate per 211 x 400 size can. The citric acid monohydrate may be added directly to the hot water. Comparable acidification may be accomplished by substituting 0.16% or 0.20% (W/V) citric acid monohydrate for the canning water.

Table 2—Procedures for canning Spanish rice products

Ingredients	Spanish rice	Spanish rice with TVP[a]
Parboiled milled rice[b] (g)	45.0	40.0
Sugar (g)	2.8	3.5
Dehydrated green peppers[c] (g)	1.0	1.6
Dehydrated red peppers[c] (g)	1.3	1.3
Dehydrated onion[d] (g)	0.5	0.5
Paprika[e] (g)	0.3	0.3
Vegetable oil[f] (ml)	2.5	2.5
Textured vegetable protein[a] (g)	0.0	10.0
Tomato juice[g]	275.0	275.0

The indicated amounts of ingredients were placed into 211 x 400 R-enamel cans (for 303 x 406 cans increase all values by a factor of 1.5). The cans were filled with 194°F tomato juice, leaving approximately 5 mm headspace, sealed immediately, and processed in actively boiling water at atmospheric pressure for 45 minutes. The cans were cooled in cold water.

[a] Textured vegetable protein (TVP) — Archer Daniels Midland Co., Decatur, IL
[b] Parboiled rice — Riceland Foods, Stuttgart, AR
[c] Dehydrated green and red peppers — California Vegetable Concentrates, Modesto, CA
[d] Dehydrated onion — Basic Vegetable Products, Inc., Vacaville, CA
[e] Paprika — McCormick and Co., Inc., Baltimore, MD
[f] Vegetable oil — Mazola Pure Corn Oil, Best Foods, A Division of CPC International, Inc., Englewood Cliffs, NJ
[g] Tomato juice — 1 part tomato paste (30—32% solids) (H.J. Heinz Co., Pittsburg, PA), 6 parts water, 1.5% salt. Juice was adjusted with citric acid monohydrate to pH 3.8 for Spanish rice, and to pH 3.5 for Spanish rice with TVP.

Heat penetration

The rates of heat penetration into selected size cans processed in actively boiling water were monitored. The come up time of the retort was negligible, since the temperature of the boiling water did not decrease when the cans were submerged. The coldest spot in the can (one-third the can height from the bottom) indicated that heat pentration was by convection. A heating curve was derived by plotting product temperature (°F) vs time on semilog scale (Fig. 2). The broken curve indicated that heat penetration was initially by convection and changed to conduction after approximately 8 min of processing time.

The minimum process for acid products was described by Ball (1938) as that which brings the temperature of every particle of the product to 180°F. Rice must imbibe water to reach a temperature of 180°F at the center of the kernel. The break in the heating curves (Fig. 2) indicated that rice had imbibed considerable water after 8 min of heating. The convection currents inside the can at this time were restricted and heat transfer changed from convection to conduction. The minimum processing time as described by Ball (1938) was, therefore, estimated to be approximately 8 min. However, a process of 45 min to attain optimum quality was earlier established (Sharp et al., 1981).

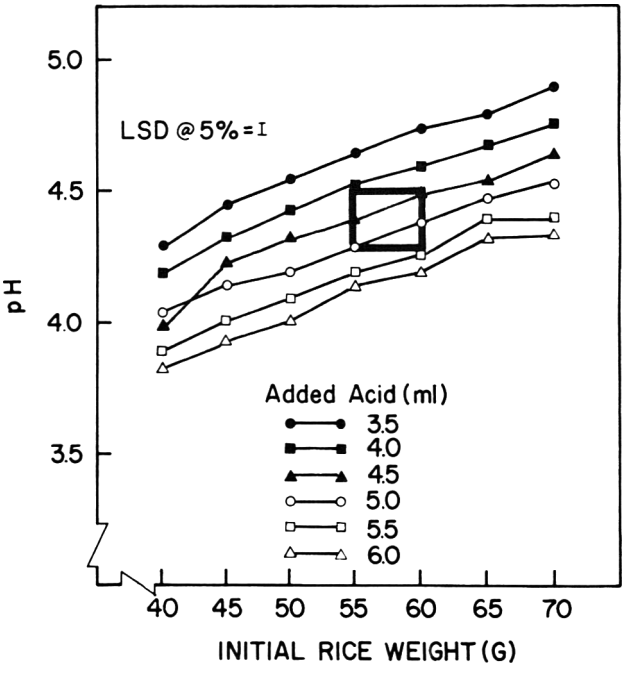

Fig. 1—Effects of initial rice weight and level of acidity on final product pH.

Inoculated packs

The heat process in preservation of acid foods is designed to kill the microorganisms that can grow and spoil the product, and not necessarily to kill spore-forming organisms such as *C. botulinum* that will not grow below pH 4.6 (Odlaug and Pflug, 1977). Therefore, many adequately preserved acid foods may contain viable bacterial spores. The bacterial suspension used to inoculate packs in this study contained both spores and vegetative cells of *B. cereus* and *B. polymyxa* as verified by direct microscopic observation. Swelling did not occur in any of the cans and good vacuum was maintained (Table 3), indicating that no vegetative cells of *B. polymyxa* survived the thermal process. This does not confirm the absence of spores, only their inability to produce gas within the storage period. Direct microscopic observation of gram stained smears from the incubated thioglycollate medium indicated the presence of gram positive rods (both *B. cereus* and *B. polymyxa* are gram positive rods). Spores were prevented from germinating by the low pH attained by acidification (Table 3).

Sensory evaluation

The sensory panel evaluated canned rice, rice with textured vegetable protein (TVP) (Spanish rice and Spanish rice with TVP using cooked rice as a reference. Texture was good for all products (Table 4). Product types did not differ significantly in acceptability of appearance and texture. The product receiving the lowest rating for taste, though still acceptable, was rice with TVP. Rice with TVP tended to be rated lower in taste than did rice without TVP, but the reverse was found in the Spanish rice product. The reason for this tendency was unclear, but possibly was due to interactive effects of other ingredients.

Experimental canned Spanish rice was evaluated alongside commercial canned Spanish rice and received good ratings in both general appearance and flavor (Table 5). The experimental Spanish rice was significantly more acceptable than one and not significantly different from two of the commercial products in general appearance and taste. Objective color values were not tested statistically; however, numerically the experimental product was darker (lower "L" value) and contained less yellow (lower "b" value) than any of the commercial products, while two commercial products contained more red (higher "a" value) than did the experimental product. The "a" and "b" color values became more meaningful when expressed as a ratio and compared to the general appearance ratings. The Spanish rice product given the highest general appearance rating had the highest numerical a/b ratio indicating that the product containing more red and less yellow was more acceptable to this panel.

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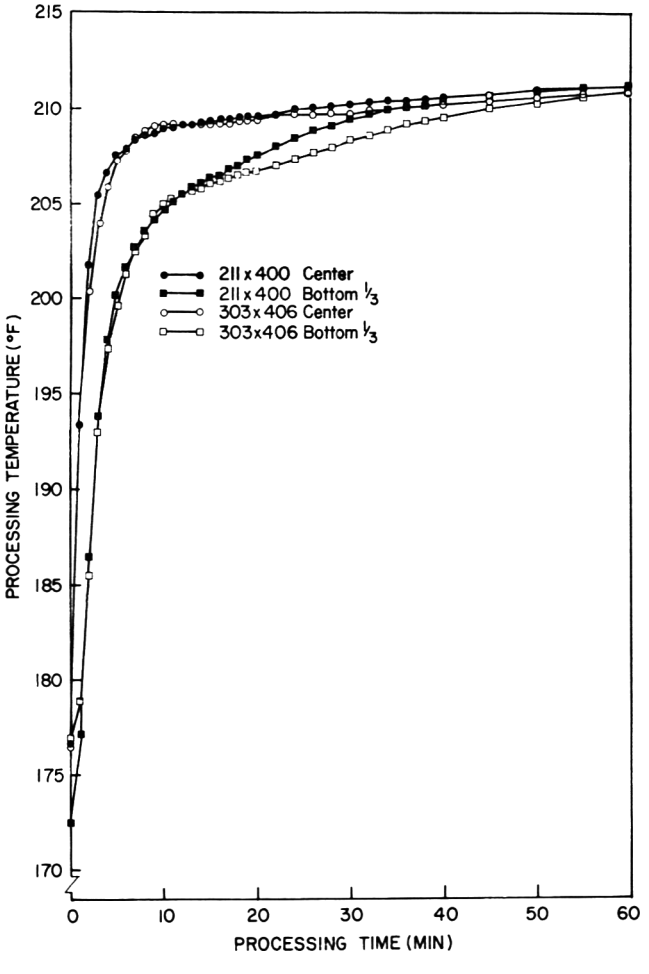

Fig. 2—Rate of heat penetration into rice in two can sizes with thermocouples at two locations processed in actively boiling water.

Table 3—Percent swollen cans, vacuum and final product pH of canned rice stored at three temperatures for 3 wk

Can size	Storage temp (°F)	Swollen cans (%)	Vacuum[a]	Final product pH[b]
211 x 400	73	0	14	4.5
	104	0	14	4.4
	122	0	13	4.4
303 x 406	73	0	10	4.4
	104	0	11	4.4
	122	0	10	4.4

[a] Average inches of Hg of 8 cans
[b] Average pH of 8 cans

Table 4—Sensory evaluation[a] of experimental canned rice using cooked parboiled rice as a reference

Product type	Appearance[b]	Texture[b]	Taste[b]
Canned rice	4.2	4.1	3.5
Canned rice with TVP[c]	3.9	4.2	3.0
Canned Spanish rice	4.3	4.6	4.1
Canned Spanish rice with TVP[c]	4.0	4.3	4.8
LSD @ 5%	NS	NS	0.7

[a] Nine panel members
[b] Rating scale 5 to 1: Excellent = 5.0, acceptable = 3.0.
[c] Textured vegetable protein (Archer Daniels Midland Co., Decatur, IL)

Table 5—Objective and subjective[a] evaluations of experimental canned Spanish rice with locally purchased commercial canned Spanish rice

Product	CDM[b] L	a	b	a/b	Appearance[c]	Taste[c]
Commercial # 1	54.5	23.9	30.2	0.79	4.5	4.3
Commercial # 2	56.1	20.8	30.8	0.68	3.8	3.9
Commercial # 3	56.9	15.3	29.9	0.51	2.1	2.5
Experimental	52.4	16.8	24.8	0.68	4.1	4.3
LSD @ 5%					0.7	0.7

[a] Nine panel members
[b] Gardner Color Difference Meter values
[c] Rating scale 5 to 1: Excellent = 5.0, acceptable = 3.0

Process time vs texture and safety

Samples processed for varying times were presented to panelists to determine the process time required to yield the most acceptable texture. Six panelists preferred the texture of the rice processed for 30 min while one panelist each preferred that of the 20, 25, and 35 min process.

All inoculated cans containing nonacidified rice exhibited can swelling while all cans containing acidified rice retained good vacuum (no less than 10 in mercury column).

These data indicate that approximately 30 min of processing time in actively boiling water was required for preferred texture of canned rice, even though the inoculated acidified packs did not support bacterial growth when processed for only 20 min.

SUMMARY

THESE DATA indicate that the process described by Sharp et al. (1981) produced an adequately processed canned rice product. Processing in actively boiling water for 20 min was adequate to destroy the vegetative cells in an inoculated pack while a pH of 4.4 prevented spores from germinating. The potential of the processing method was demonstrated by the development of four products that were rated acceptable.

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We thank K.A. Ito for supplying the bacterial cultures. Use of a trade name does not imply endorsement of guarantee of the product or exclusion of other products of similar nature.

SPOILAGE OF VACUUM-PACKAGED BEEF. . . From page 1122

and (3) the higher initial populations of bacteria in our experiments (ca 10^4 cells/cm^2 compared to an initial average count of 10–100/cm^2 for lactic acid bacteria on naturally contaminated meat).

These experiments show that fresh beef stored at 5°C in plastic films of very low permeability spoils slowly, even in the absence of a contaminating microbial flora. The rate of spoilage is increased by the presence of lactic acid bacteria. We have commenced further studies designed to estimate the ultimate shelf life of vacuum-packaged beef stored at 0°C and to determine the practical significance of lactic acid bacteria in the spoilage of vacuum-packaged beef.

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A Pilot-Plant Study of Continuous Ultrasonic Extraction of Soybean Protein

K. J. MOULTON and L. C. WANG

ABSTRACT

Methods were studied to extract soybean proteins from commercial defatted flakes with ultrasonic energy, continuously and efficiently, in a pilot plant. Studies also included batchwise sonication of aqueous soybean slurries in a tank. Parameters investigated included soybean-to-solvent ratios of 1:10, 1:20, and 1:30; solvents of tap water and 0.1N NaOH; and extraction with and without sonication. Protein yield and energy requirements for extraction were measured to correlate the operating condition with the extent of protein extraction. For a residence time of 0.7 sec in the continuous sonication process, the extracted protein yield was greatest at 1:30 soy-to-alkaline solvent ratio. Energy input to produce protein isolate is the least at 1:10 soy-to-alkaline solvent. Although the continuous sonication procedure is workable, the commercial operation remains to be tested.

INTRODUCTION

ISOLATED SOY PROTEIN (ISP) generally has been produced commercially by batch operations since its conception over 45 years ago. A yield of 30% protein extracted from soy flakes (60% on a total protein basis) is common for a commercial batch process (Mustakas and Sohns, 1976; Smith, 1958), but yield of protein is generally higher in laboratory extractions. Many investigators have studied and reported on factors that affect the laboratory peptization of soybean proteins in batch processes. Beckel et al. (1946) reported the effect in the pilot plant of varying the ratio of distilled water-to-soy flakes and temperature on extracting soy proteins. More protein was extracted at 1:30 ratio at 30°C than at 1:10 ratio. Type of solvent, variety of beans, age of the meal, particle size, time of extraction, pH, salt concentration, stirring action, and conditions for preparing the defatted flakes have been investigated as variables and the results reported by Circle (1950) and Circle and Smith (1972). Smith and Circle (1939) concluded that, to prepare ISP with desirable functional properties, the extracting solvent exerts a greater influence on proteins than does the precipitating agent. They favored water as the solvent because the precipitate settled faster, was not hydrated as much, and was lighter in color than when alkali was the solvent. Smith (1958) and Circle with Johnson (1958) reviewed the technology for producing protein isolates. Circle et al. (1959) reported an improvement in the isolation process by dispersing the protein from partially heat-denatured meal in a hot aqueous system. Mustakas and Sohns (1976) surveyed and described commercial batch methods for extracting soy proteins. Other information describing commercial or pilot-scale operations, either batch or continuous, is incomplete or sketchy. Information in the literature regarding the extraction energy requirements of a stirred batch tank process is lacking also.

Wang (1975, 1977, 1978) applied ultrasonic energy to disperse soy proteins in distilled water or aqueous alkali and compared the efficiency to a process using conventional extraction methods. His results were promising and suggested additional work to investigate the feasibility of ultrasonication as a method to peptize soy protein. Childs and Forte (1976) combined the effects of ultrasonic and enzymatic extraction of protein in distilled water or alkali from heat-treated cottonseed meal with a degree of success.

In the present work, a continuous ultrasonic extraction and a batchwise ultrasonic extraction were used to study the degree of processing variables for peptization of soy protein. These extractions were evaluated and compared based on the yield of protein in the ISP product. Electrical energy required to peptize proteins in both continuous and batch sonic processes was measured.

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MATERIALS & METHODS

Protein source material

Defatted and low-temperature desolventized soybean flakes, purchased from the Archer Daniels Midland Company, were used for all experiments. Analytical results for a representative sample of these flakes are listed in Table 1. AOCS (1979) methods were used to measure total protein, protein dispersibility index (PDI), nitrogen solubility index (NSI), ash, crude fat, crude fiber, and urease activity. Moistures of defatted flakes were determined by Rapid Moisture Tester (C.W. Brabender Instruments, Inc., South Hackensack, NJ).

Solvents

Tap water with similar analysis to commercial treated water (Johnson, 1978) was used for the tests. The tap water analyzed as follows: iron, 0.01 ppm; manganese, trace; hardness, 300 ppm; alkalinity, 190 ppm; and pH, 7.5. Aqueous alkali (0.1N NaOH) and soy flakes were used to give a slurry with a pH of 8.

Equipment

Fig. 1 illustrates the arrangement of equipment for the continuous sonic extraction, separation, and isolation of soluble protein from soy flakes. To collect data for comparison of the batch process with the continuous process, the slurry mixing equipment and continuous cell were replaced by a tank sonifier.

Continuous sonication process

In the continuous process, ultrasonic energy was applied to the soybean flake/solvent slurry by a Branson Sonic Power Company Model J 32A power supply, supplying up to 550 watts at 20 kHz to the converter. A booster horn to increase by 50% the longitudinal mechanical vibrations of the probe was connected to the converter. The probe extended into a continuous high-pressure processing cell.

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Table 1—Analysis of defatted soy flakes

Total protein	50.2%
Protein dispersibility index, PDI	80%
Nitrogen solubility index, NSI	74%
Moisture	8.0%
Ash	6.2%
Crude fat	0.8%
Crude fiber	3.6%
Urease activity, pH increase	2.1

A wattmeter connected to the power supply measured the power consumed during sonication.

Soy flakes, metered by a variable speed feeder (A) and Eriez Hi-Vi vibrator (B), were combined with solvent, delivered through a peristaltic pump (C) into a small vessel (D) equipped with a solely rotating stirrer. The resulting slurry was then pumped by a Moyno pump (E) through the ultrasonic processing cell (F) to discharge the slurry on a 61 cm x 122 cm vibrating 100-mesh screen (G). A portion of the filtrate from the filter screen was pumped to a Sharples Super Centrifuge (30,000 x *g*) (H) to recover the peptized protein in the supernatant. The separated nonpeptized residues retained both on the filter screen and from the Sharples centrifuge were combined as spent flakes. Protein in the supernatant was precipitated batchwise at pH 4.5 with 1N HCl while agitating in vessel (I) and then separated from the whey in an International centrifuge (2,000 x *g*) (J). The concentrated precipitate, dried in a freeze dryer (K), was recovered as isolated soy protein (ISP).

Twelve combinations (3 x 2 x 2) of flake (as-is)-to-solvent ratio (1:10, 1:20, and 1:30), with and without ultrasonic energy, and type of solvent (*tap* awater or dilute alkali) were tested in two replicates. (Passing the slurry through the processing cells without ultrasonic energy applied gave control data.) The 24 runs were conducted in a random order. Residence time in the effective zone (2.4 cc) of the sonication cell was calculated to be 0.72 sec at a fluid flow rate of 200 cc per min for all tests. This rate was chosen because it was the slowest reliable rate of the pump. Slurry was processed at a rate of 12 to 15 kg per hr depending on the soy flakes-to-solvent ratio. No extrernal heat was supplied to the slurry other than the heat resulting from cavitation in the sonifier, which raised the temperature from ambient to about 40°C.

To measure reproducibility of the continuous sonication process, samples of the slurry, spent flakes and filtrate from the filter, curd and supernatant from the Sharples centrifuge, and freeze-dried ISP product were withdrawn from the process and analyzed. Slurry samples from the sonifier and filtrate samples from the filter were also centrifuged in a laboratory centrifuge at 10,000 x *g* for 10 min, separated, weighed, and analyzed for total solids and protein. Samples of spent flakes from the filter and curd from the Sharples centrifuge were air dried, then vacuum dried and weighed to determine total solids and analyzed for protein. The Sharples supernatant was weighed and analyzed for total solids and protein.

Batch sonication process

In the batch sonication process, 50g (dry basis) defatted soy flakes, slurried with 500g tap water or alkali, were sonicated in a 29½ x 15¼ x 14 cm stainless steel sonic cleaning bath, Model 90C1 (Ultrasonic Industries, Inc.), with an output capacity of 800 watts. Slurries of 1:10 ratio were sonicated for 2.5, 5, and 60 min. Samples were withdrawn from the batch sonifier, treated and analyzed in the same manner as those from the continuous cell. It takes 2.5 minutes to process 500 cc slurry (capacity of the batch sonifier) through the continuous ultrasonic cell.

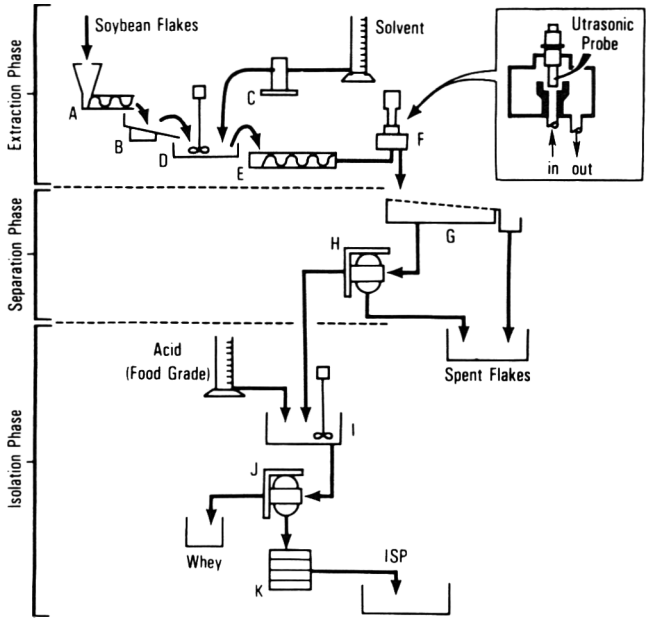

Fig. 1—Pilot-plant production of isolated soy protein (ISP) from defatted, low-temperature desolventized soybean flakes.

RESULTS & DISCUSSION

THE ANALYTICAL VALUES for soluble protein samples from the sonfier, the filter, the centrifuge and the freeze dryer were assessed statistically to identify significant effects. Only analysis of filtrate samples from the filter indicated significant interactions between (1) flake/solvent ratio on solvent and (2) with and without energy of the sonifier. The Least Significant Difference (LSD) for the filtrate samples was 3.4, with a standard deviation of 5%. Analyses of all other samples indicated no significant interactions. Specifically for the ISP samples, the LSD was 5.2 and the standard deviation was 10%. In the continuous system, when the sonifier was operating, the recovery of dispersed protein increased progressively as the flake/solvent ratio changed from 1:10 to 1:30. Without the sonifier, there was less dispersed protein recovered at a 1:10 ratio and no change in the amount of protein recovered at soy/solvent ratios of 1:20 and 1:30. When the pH of the slurry was adjusted with alkali to pH 8, more protein was recovered both with and without use of the sonifier; however, the improvements from sonication were smaller in alkaline systems.

Protein yield

The effects of varying the flake/solvent ratio, type sol-

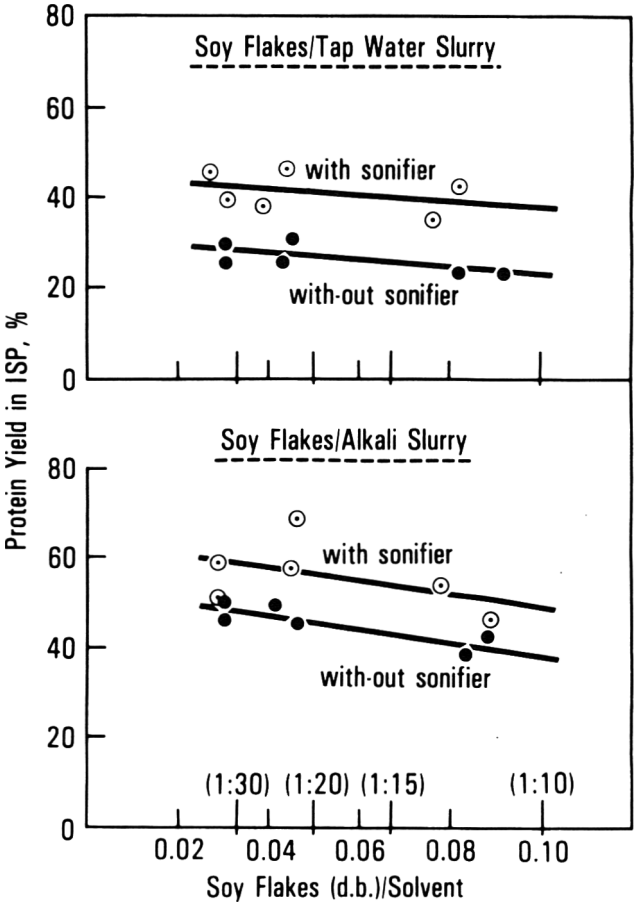

Fig. 2—Effect of sonifier, type of solvent, and flake/solvent ratio on protein yield in ISP for continuous sonication.

vent, and sonication energy on the yield of protein extracted (measured in ISP) are shown in Fig. 2 for the continuous sonication process. The yield of protein was calculated from the measured protein in the recovered ISP relative to the total protein content of the starting flakes. The balance of the protein was left in the filter cake and whey.

Maximum protein yield was observed when the flake/solvent ratio was 1:30, dilute alkali was the solvent, and the sonifier supplied the energy to extract protein. The relationship between protein yield and flake/solvent ratio is linear for both solvent and sonifier action variables. There were no significant differences in the rate of decrease in protein recovery per unit flake/solvent ratio between the four solvent-sonifier combinations. The mean decrease in protein was 10.8 units per 0.1 unit flake/solvent ratio.

Yields of recovered protein (in ISP) for the batch sonifier process are compared in Table 2 with those from the continuous sonifier process. The continuous process extracted 54% more protein from a 1:10 aqueous slurry than the batch process. Processing times were equivalent (2.5 min) for each process. Furthermore, to recover the same amount of protein from a slurry, the batch process required almost 60 min (nearly 24 times that required for the continuous process). For alkali, the continuous process extracted 23% more protein than the batch process.

Sonication energy requirements

In the continuous sonifier process, the energy required to extract 1g of protein from soy flakes was 20 to 30% less for alkali than for *tap* water. At a soy/solvent ratio of 1:10, less than 1 watt per g extracted protein was required regardless of solvent (Fig. 3). For a sonication period of 0.72 sec, 4.5–4.8 watts were expended. Sonifier efficiency generally improved with the greater load of a thicker slurry. Nearly 60% of the available protein was extracted continuously with less than 3 watts per g protein (flake-to-alkali ratio of 1:30).

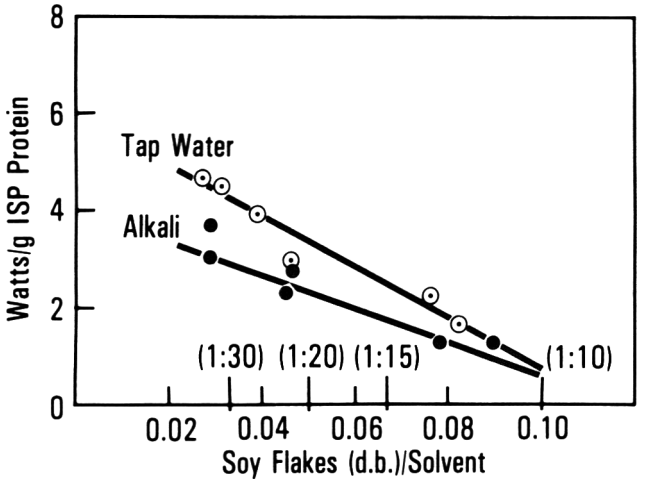

Fig. 3—Power required to extract soy protein from defatted, low-temperature desolventized soybean flakes in tap water or alkali slurries at various flake/solvent ratios for continuous sonication.

The continuous process required ca 70% less energy than the batch process to extract the same amount of protein from a 1:10 slurry (aqueous or alkali) in 2.5 min (Table 2). The batch process, operating for 60 min, required 250 times more energy to extract the same amount of protein as the continuous process in 2.5 min. Thus, a batch tank-type sonifier required more energy than a continuous in-line sonifier to extract an equivalent amount of protein.

CONCLUSION

DEFATTED, LOW-TEMPERATURE desolventized soybean protein was peptized in less than 1 sec in the ultrasonic continuous processing cell. It would require many minutes in a batch-stirred tank or a batch-sonifier tank to obtain the same effect. ISP yields in the continuous pilot plant under the conditions of this study were about equivalent to a batch commercial process (60% of total protein recovery requiring 40–50 minutes extraction time). The flake/solvent slurry flowed in a continuous stream through the sonifier cell without separation, undue foaming, or biodegrading. Ultrasonic probes are available to peptize soy protein at almost any commercial throughput. Although a 1:30 ratio flake-to-alkali slurry resulted in the greatest yield of protein extracted, the sonication energy required to extract protein was lowest when thicker slurries were used. Therefore, a compromising flake/solvent ratio between 1:10 and 1:20 would appear more practical for commercial production of ISP. Further work is needed to investigate the contribution of other variables such as temperature, pressure, sonifier cavitation, energy, and residence time to improve commercial yields.

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Table 2—Continuous vs batch sonication of soy flakes

Process[a]	Sonication time, min	Tap water: % of total flake protein recovered in ISP	Tap water: Power, watts per g protein	Tap water: Final temp, °C	Alkali: % of total flake protein recovered in ISP	Alkali: Power, watts per g protein	Alkali: Final temp, °C
Continuous (0.7 sec residence time)	2.5	37	1.64	26	49	1.29	36
Batch	2.5	24	1.24	27	44	0.68	26
Batch	5	29	2.01	32	44	1.34	24
Batch	60	46	15.4	49	53	13.44	43

[a] Soy flake/solvent ratio of 1:10 (d.b.)

Improved Aseptically Filled Sweet Potato Purees

D. A. SMITH, T. A. McCASKEY, H. HARRIS, and K. S. RYMAL

ABSTRACT

A high quality sweet potato puree with better color stability than frozen purees was produced by flash sterilization followed by aseptic packaging. Starch conversion time was shortened by slicing the sweet potatoes and accurately controlling the temperature during conversion. Processing of inoculated purees for 13 sec at 138°C followed by aseptic filling was adequate for commercial stability. The effect of several processing variables on puree and sweet potato souffle quality was examined. Improved quality and storage stability of sweet potato purees were accomplished by flash sterilization at very high temperatures followed by aseptic packaging.

INTRODUCTION

SWEET POTATOES are a good source of dietary carbohydrates, provitamin A, ascorbic acid, dietary fiber, and provide moderate amounts of protein. However, because of the length of the growing season and the requirement of special storage and handling conditions, marketing of fresh sweet potatoes is limited. Production of a shelf stable pureed product could lengthen the marketing season for this crop and provide a high quality product for distribution to other areas. Frozen sweet potato puree is well accepted in products such as pies and souffles (Turner and Danner, 1957; Marshall and Danner, 1959).

The technology for producing purees has been investigated by several researchers. The steam kettle process developed by Harris (1955; Harris and Barber, 1958) produces excellent quality purees. With this process, peeled whole sweet potatoes are cooked in a steam jacketed kettle with either syrup or a small quantity of water as medium for beginning the cook. Heat penetration is optimum for starch hydrolysis (Harris, 1949) and concentration of sweet potato solids is controlled by regulating the rate of evaporation and the length of the cooking period. The puree can be marketed in the frozen state or is suitable for drum drying. The process was modified by Hoover (1967) for a semi-continuous operation. Ice et al. (1980) studied the effects of pH, enzymes, and storage time on the rheology of sweet potato puree.

The process time required by conventional pressure retort processing in large containers for a safe, shelf stable pack of sweet potato puree would result in a product with low market quality. Therefore, only special packs of pureed sweet potatoes in small cans are now processed commercially. Processes have been described for aseptic processing recent years (Brody, 1972; Duffy, 1973), but sweet potatoes and other crops which normally exhibit pseudoplastic flow (Muller, 1973), increase in both apparent viscosity and yield stress when heated sufficiently to form gels of pectin and starch. This gives the puree the apparent flow characteristics of a solid and retards heat transfer which can result in fouling of a heat exchanger and underprocessing.

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This study was undertaken to produce a shelf stable sweet potato puree by methodologies adaptable to continuous line production using high-temperature, short-time sterilization, and aseptic packaging. The aim of the process was to produce a product equal to or superior to frozen puree and accomplish a net energy savings over a product maintained in frozen storage.

EXPERIMENTAL

Sweet potatoes

Roots of the 'Red Jewel' cultivar which had been cured for 8 days at 29.5°C and 95% relative humidity were used in this study. After curing, all roots were held in storage at 13°C and 95% relative humidity until processed.

Processing treatments and evaluations

The roots were peeled by high pressure steam with cold water injection as described by Smith et al. (1980). A series of purees were produced utilizing different processing methodologies to find the optimum process for in-line production of an aseptically filled shelf stable product. The processing variables consisted of:

Process 1. Slicing the peeled sweet potatoes into 1.25 cm thick slices and holding in a jacketed kettle at 75°C for 15 min with 1 kg of water for every 5 kg of potatoes, to achieve optimal saccharization. This was followed by pulping through a screen with 1.5 mm diameter openings to remove the fiber.

Process 2. Slicing and holding at 75°C as above for 15 min followed by grinding through an Alpine Kolloplex mill to produce a puree which passes through a 40-mesh screen.

Process 3. Slicing, grinding through the Alpine Kolloplex mill followed by a 75°C holding period for 15 min.

Process 4. A control consisting of the Harris kettle method (1955).

All of the above processes were carried out in an atmosphere of steam to minimize oxidative browning and the products were flash heated to 132°C and stored at −23°C for further analysis. The flash heating was accomplished in a specially designed heat exchanger which allows uniform heating and accurate temperature control without fouling. With this patented, steam injection process (Harris, 1981) puree was pumped into a primary mixing chamber and combined with steam thus accomplishing heating. The heated puree was further mixed in a connecting mixing chamber before being pumped through the holding tube to the filler. A holding time of 13 sec was used for all samples followed by flashing to atmoshpere in the filling operation. The temperature did not vary by more than ±1.0°C from the desired temperature with respect to time and position within the holding tube. All cans were sterilized in steam (10 psig) then filled directly from the holding tube and sealed in an environment of atmospheric steam.

To eliminate any possible danger of inadequate heating of particulate material from the kolloplex processed samples and to insure uniformity of conditions with purees being tested for the effect of processing temperature on organoleptic quality all samples undergoing organoleptic testing were packaged in polyethylene containers and stored at −23°C for further analyses.

A second test was designed whereby samples were prepared as in Process 1 and were flash heated to 99°, 110°, 121°, 132°, or 138°C to determine the effect of processing temperature on organoleptic quality. A similar sample was homogenized at 250 kg/cm^2 in a Gaulin 31M laboratory homogenizer before flash heating to 132°C. These samples were frozen for later analysis of the effect of heat treatment on the organoleptic character of sweet potato souffles. Replicates processed at 138°C were aseptically filled in plain 401 x

411 cans and stored at 27°C for 4 months to determine if the quality differed from similar samples held in frozen storage.

A third test was designed whereby purees processed as in the second test were subjected to a laboratory scale microbiological test. The puree was inoculated with spores of *Bacillus subtilis*, to a population of 1 spore/g; *Bacillus stearothermophilus*, (1 spore/g); and *Clostridium sporogenes* (3,900 spores/g). After inoculation samples were flash heated and aseptically filled directly into sterile 401 x 411 cans and sealed in an atmosphere of steam. Each thermal process consisted of 50 cans. Cans were incubated at 25°C and 55°C and were examined at 24-hr intervals for swelling. After storage at these temperatures for 2, 4, and 8 wk, aliquots of puree were plated by diluting 50g of puree with 450 ml of sterile phosphate buffer. Five 20-ml aliquots of the diluted puree were dispensed into 14 x 150 mm screw-capped tubes and heat shocked at 80°C for 10 min. After the five tubes were heat shocked, three tubes were randomly selected and plated on brain heart infusion (BHI) agar. Agar plates prepared from Tube 1 were incubated aerobically at 37°C, plates from Tube 2 were incubated aerobically at 55°C, and plates from Tube 3 were incubated anaerobically at 32°C. All agar plates were incubated for 48 hr. The two tubes not plated were divided into two groups; one was incubated at 37°C for 48 hr and the second was incubated at 55°C for 48 hr. The two tubes of puree were cultured on BHI agar to determine whether sporeforming bacteria survived the puree thermal process but were not detected in the original plating process. Puree from the tube incubated at 37°C was plated in duplicate on BHI agar and incubated aerobically and anaerobically at 32°C for 48 hr. The puree incubated at 55°C was cultured aerobically at 55°C for 48 hr. All samples of puree were analyzed for viable sporeforming bacteria by further diluting 1:10 dilutions of puree 1:1 with sterile phosphate buffer, and plating 10 replicates of 2 ml each of the final 1:20 dilution on BHI agar. Ten replicates of 2 ml each of the final dilution represent 1g of puree.

Evaluation of purees

After 8 months of storage, the puree quality was examined. The purees were evaluated for color using a Hunter Color Difference Meter standardized to a yellow standard at L = 79.1, a = 3.1, b = 21.9. Total solids were determined by drying to a consistent weight in a vacuum oven at 65°C and 737 mm of vacuum. Soluble solids were read on a hand held refractometer.

Sensory evaluation

Souffles were prepared from the various purees using a recipe developed by Harris and Barber (1958). These souffles were ranked using the multiple-comparison ranking method (Kramer and Twigg, 1966) taste panel design with five trained panelists. These panelists were asked to evaluate the product for flavor, texture and color using a 10-point scale with descriptive terms for the numerical values (Fig. 1).

The souffles were tested in a sensory evaluation laboratory with individually partitioned booths and overhead lighting. Red lighting was used to mask the color when the samples were judged for flavor. Four, five-digit randomly coded samples were presented at each mid-morning and mid-afternoon session. The souffles were served warm (46°C) and water was provided for rinsing. Analyses of variance was used to identify significant differences.

RESULTS & DISCUSSION

THE INOCULATED TEST PACK of puree indicated that a commercially sterile sweet potato puree can be processed by this method (Table 1). No evidence of spoilage in cans stored at 25°C or 55°C was indicated in purees processed at 132°C or 138°C with a 13 sec holding time followed by aseptic closing. Plate counts of viable microorganisms (Table 2) confirmed the adequacy of the process at 138°C for 13 sec. No organisms were found after aerobic incubation at 37°C, 55°C, or after anaerobic incubation at 32°C.

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SCORE SHEETS FOR SWEET POTATO PUREES

Judges:

Please evaluate the samples for their qualities as sweet potato souffles. Do not base your scores on a personal like or dislike for the product in general. Please do not communicate with anyone while scoring the sample.

Use numerical scores from the following score card. Enter your score under the sample numbers in the scoring chart below. Please accompany all scores with comments when requested. If not requested, comments are optional.

Score Card

Range	Class	Score
Unacceptable	Very poor	1 or 2
	poor	3 or 4
Barely acceptable	Fair	5 or 6
Acceptable	Good	7 or 8
Highly acceptable	Unusually good	9 or 10

Scoring Chart

Qualities to be tested		Sample		
Color	Score			
	Comment			
Texture	Score			
	Comment			
Flavor	Score			
	Comment			

Name_____

Date_____

Fig. 1—Sensory evaluation score sheet.

Table 1—Effect of the processing temperature for 13 sec with aseptic filling on the microbiological stability of sweet potato purees as measured by the swelling of 401 x 411 cans when stored at 25°C[a]

Processing (°C) temp	Number (percent) of swollen cans at N days of storage										Total % swollen
	day 1	2	3	4	5	6	7	8	9	10	
99	25(100%)										100
110		23(92%)	2(8%)								100
121						4(16%)	19(76%)	2(8%)			100
127										2(8%)	8
132											0
138											0

[a] Puree processed at 132°C and 138°C and held at 55°C showed no evidence of spoilage.

Processing operations designed to speed the conversion of starch to sugar in the preparation of puree were successful as long as these operations were conducted in an atmosphere of steam to prevent browning. Good conversion was accomplished by slicing the roots into 1.25 cm thick sections and holding at 75°C for 15 min rather than the 45 min required for the whole roots in the Harris kettle method. This process yielded soluble solids levels in excess of 18% (Table 3), while the Harris kettle cook method (control) produced a puree higher in soluble solids due to concentration during the longer 45-min cook.

Varying the processing operations (Table 3) had little effect on the starch conversion except when the sweet potatoes were processed in a colloid mill prior to cooking. The lower sugar level appears to be the result of excessive cell breakage and more rapid heating of the freed liquids which may have inactivated much of the α amylase. Total solids (Table 3) were higher in purees made by colloid grinding of the sweet potatoes than in those which had been pulped. This was due to the retention of the sweet potato fiber in the finished puree. This type of processing may merit consideration because of the inclusion of dietary fiber. Processing temperature had no effect on either soluble solids or total solids (18.5% and 20.6% respectively).

Table 2—Viable microbial counts for flash sterilized, aseptically filled sweet potato purees after 2, 4, and 8 wk of storage at 25°C

Processing (°C) temp	—Puree stored at 25°C— Microbial count after incubation at 37°C (aerobic)	55°C (aerobic)	32°C (anaerobic)
		2 Weeks storage	
127	5/g[a]	1/g	1/g
132	1/g	1/g	<1/g
138	<1/g[b]	<1/g	<1/g
		4 Weeks storage	
127	1/g	1/g	1/g
132	1/g	<1/g	<1/g
138	<1/g	<1/g	<1/g
		8 Weeks storage	
127	2/g	<1/g	<1/g
132	<1/g	<1/g	<1/g
138	<1/g	<1/g	<1/g

[a] Total number of colonies on 10 plates each with 0.1g puree. Number of organisms is average of 10 samples.
[b] Puree which contained no viable organisms per gram (<1/g) after storage in hermetically sealed 401 X 411 cans was diluted 1:10 with phosphate buffer and incubated at 37°C and at 55°C for 48-hr and again plated on BHI agar. No viable microorganisms were detected in ten 0.1g subsamples.

After storage, the aseptically packaged puree had better color retention than any of the frozen samples (Table 3). Incorporation of fiber into the puree by grinding the sweet potatoes after cooking resulted in slightly lower color value than the fast cooked and pulped purees or the control. The color was still acceptable and nearly indistinguishable from the pulped purees by visual examination. Comminuting prior to cooking resulted in a dark-colored puree which was unacceptable. The temperture of final processing had no effect on the color values of the purees.

When the various purees were incorporated into souffles and rated by taste panel, all samples except those made from roots ground before cooking, where the color was rated as unacceptable, were rated as acceptable or better (Table 4). The final processing temperature of the sweet potato purees had no effect on the color, texture or flavor of the souffles. Even though preliminary testing had indicated better flavor acceptance for purees which had been heated to 132°C or above, such ranking was not carried over into the corresponding souffles.

The quality of souffles as measured by color, flavor, and texture by taste panel was found to vary with the processing variable used in the constituent puree (Table 4). Acceptable to highly acceptable colored souffles were produced from purees processed by pulping the sweet potatoes which had been sliced and held at 75°C for 15 min before flash heating to 138°C. Similar ratings were received for souffles where the processing variable for the puree had been pulping followed by homogenation, and colloid grinding following cooking. The souffles produced from purees originating from the Harris kettle cook method were also rated as highly acceptable in color.

Souffles made with homogenized pulped puree were rated as barely acceptable to acceptable in texture while souffles which incorporated fiber by grinding the sweet potatoes in the puree through a colloid mill either before or after cooking were judged to have acceptable texture. The puree which was produced by pulping the sliced, steamed

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Table 4—Effect of the puree processing variables on the quality of souffles by flashing to 138°C after slicing and cooking at 75°C for 15 min[a]

Processing variable	Color	Texture	Flavor
Pulped and frozen	8.7^{b}	8.5^{bc}	8.7^{b}
Pulped, homogenized, frozen	8.6^{b}	6.6^{d}	7.5^{c}
Comminuted after cooking, frozen	8.0^{b}	7.9^{c}	8.6^{b}
Comminuted before cooking frozen	3.6^{c}	7.9^{c}	8.9^{b}
Harris kettle cook, frozen	8.8^{b}	8.8^{b}	8.7^{b}

[a] Ten-point scale with ten being excellent and six being the borderline of acceptability.
[bcd] Values with the same superscripts do not differ significantly at $p < 0.05$.

Table 3—Effect of processing variable on the soluble solids, total solids and color of sweet potato puree processed by slicing and holding at 75°C for 15 min prior to flash heating to 138°C for 13 sec, and following 4 mo storage

Processing variable	Soluble solids (%)	Total solids (%)	L[a]	a[b]	b[c]
Pulped and asceptically filled	18.3^{e}	20.0^{f}	55.4^{d}	24.5^{d}	36.4^{d}
Pulped and frozen	18.5^{e}	20.5^{f}	54.0^{e}	23.5^{e}	30.8^{e}
Pulped, homogenized, frozen	18.1^{e}	20.6^{f}	54.3^{e}	23.8^{e}	31.2^{e}
Comminuted after cooking, frozen	18.0^{e}	23.0^{e}	49.9^{f}	22.8^{f}	29.8^{f}
Comminuted before cooking, frozen	15.9^{f}	22.6^{e}	37.5^{g}	14.0^{g}	21.7^{g}
Harris kettle cook, frozen (control)	22.4^{d}	26.7^{d}	53.8^{e}	23.5^{e}	30.3^{ef}

[a] L = total light transmittance when standardized to a yellow plaque where L = 79.1, a = −3.1, and b = 21.9
[b] a = redness, where a = −3.1
[c] b = yellowness, where b = 21.9
[defg] Values with the same superscripts do not differ significantly at $p < 0.05$.

Sweet Potato as an Ingredient of Yeast-Raised Doughnuts

J. L. COLLINS and N. A. ABDUL AZIZ

ABSTRACT

Jewel sweet potatoes (SP) were prepared as flour and puree of baked and steam-cooked roots. SP replaced 0, 7, 14, and 21% of the wheat flour in a recipe for yeast-raised doughnuts. Composition of doughnuts was apparently affected by type and amount of SP. Caloric content ranged from 6.32–7.03 Kcal/g dry material. Texture and specific volume of doughnuts were affected by SP. Crumb color of doughnuts with SP was yellow-orange. Six quality attributes tested by a panel were affected by SP, but for the most part, overall quality of the doughnuts was not lowered.

INTRODUCTION

SWEET POTATOES (SP) are an important crop in many tropical and subtropical areas of the world and in certain areas of the United States (Cooley, 1948). The highest consumption of SP in the United States is in the areas of production (Mathia, 1975). Members of rural families consume more SP than members of urban and rural nonfarm families. In 1919 per capita consumption for the United States was 13.3 kg, but since then, consumption has decreased steadily such that consumption in 1971 was 2.2 kg per capita (USDA, 1967, 1980). In the more recent years there has been a small increase in consumption. The declining overall rate of SP consumption has been attributed to the fact that many people consider SP to be a low status food, being eaten primarily by economically deprived people. Another reason for the declining consumption is the misconception that SP are high in calories (Fitzgerald, 1976).

SP are an excellent source of many food nutrients (Technical Committee S-101, 1980). A 113.4g (4 oz) serving of canned SP, Centennial and Jewel cultivars, should provide 60 and 71%, respectively, of the recommended daily allowance (RDA) of provitamin A for males 23–50 yr old and 19 and 21%, respectively, of the RDA for vitamin C for males of the same age group. A computation of data presented by Adams (1975) indicates that vitamin C retention in raw SP flesh was high when the flesh was processed: approximately 72% in baked SP; 87% in SP which had boiled in the skin and mashed with the skin removed; and 70% in dehydrated SP. Junek and Sistrunk (1978) stated that a 150g baked SP should contribute approximately one-half of the RDA of vitamin C and six times the provitamin A for adult males.

Presently, SP are available primarily as fresh roots, too often of low quality, and canned small, whole roots. Both forms require additional preparation before being eaten (Edmond and Ammerman, 1971). To overcome these problems new products should be offered to meet the needs of the consumer. One immediate approach is to incorporate SP into different products already accepted by the consumer. An example is the doughnut–a popular food item.

The purpose of this study was to prepare yeast-raised doughnuts with SP as an ingredient and to evaluate certain quality factors including chemical composition, physical and sensory attributes, and caloric content.

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MATERIALS & METHODS

Source of sweet potatoes

The Jewel cultivar of SP was grown on the Plant Science Farm, Knoxville, for use in this study. After harvest, the roots were cured, then stored at 13°C for 1 month prior to being used. Jewel roots have orange colored flesh and are classified as a "moist" type.

Preparation of sweet potatoes

SP roots were prepared to yield flour, puree from baked roots, and puree from steam-cooked flesh. Roots used for flour were washed in water and peeled in a boiling solution of 10% lye for 5 min. The roots were removed from the lye solution and washed with water to remove hydrolyzed tissue and lye and placed into 0.5% citric acid solution to neutralize the residual lye. The roots were cut into 0.6 cm slices which were heated in an atmospheric steam cooker for 6 min. The slices were dried in a forced-air dehydrator at 48°C until they were sufficiently dry to be ground into a flour. The dried pieces were converted to flour by grinding first in a small hammer mill followed by pulverizing in colloid mill. The material which passed through a 100-mesh sieve was retained for use. The flour was placed in glass containers over desiccant and stored in the dark at ambient room temperature until used.

Puree was prepared from roots which had been washed and baked at 191°C for 1.5 hr. After baking, the roots were allowed to cool to room temperature, peeled, and pureed for 6 min with a Hobart Food Chopper. The puree was filled into plastic bags and stored at −17°C until used.

Puree was prepared also from steam-cooked flesh. Roots which had been peeled and sliced similarly as roots used for flour were cooked in an atmospheric steam cooker for 20 min. The cooked slices were cooled, pureed, and stored by procedures similar to those used to prepare puree of baked SP.

Preparation of dougnut dough without sweet potato

The following ingredients were used in the recipe for preparing yeast-raised doughnuts (Sultan, 1976):

wheat flour, all-purpose	425.4g
cane sugar	56.8g
salt	7.1g
nonfat milk solids	21.3g
margarine	42.6g
water	180.0g
whole egg	46.4g
vanilla extract	2.0g
mace	1.8g
active dry yeast (suspended in water)	28.4g
sodium stearoyl-2-lactylate (SSL)	3.8g

SSL was not listed as an ingredient by Sultan (1976) but was added to overcome the adverse effect on texture volume anticipated in doughs which were prepared with SP as an ingredient. The procedures listed by Sultan (1976) were followed to prepare the doughnuts.

Preparation of doughnut dough with sweet potato

Doughnuts were prepared according to the recipe presented above, but a portion of the flour was replaced with an equivalent portion of SP ingredient, and water was adjusted to give desired dough consistency. One part wheat flour (approximately 12% moisture) was replaced by one part solids, and SP solids of each of the three types were added at 0, 7, 14, and 21% levels (Table 1).

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Consistency of doughs decreased as the percentage of SP was increased and the amount of water was held at a constant level. To correct this problem, the recipe was altered by reducing the amount of added water so that the consistency would become similar to that of dough without SP. To determine the amount of water that should be added to each recipe the following procedure was used. Three levels of water were used to prepare each recipe. The levels of water were selected so that at least one of the doughs had a consistency greater than the consistency of dough without SP, and at least one was of a consistency less than that of dough without SP. Two replications were prepared. Three samples were taken from the dough of each replication; five measurements of consistency (procedure presented below) were made on each sample. Thus, 30 measurements were made for the dough of each SP type-level combination. By linear regression analysis, calculating the amount of water needed to prepare a dough with SP so that it possessed a consistency similar to that of dough without SP was possible (Little and Hills, 1972).

Procedure for measuring consistency of dough

Doughs were prepared as described previously, but only one-half recipes were used. Three 70-g portions were taken from the dough of each recipe. Each portion was wrapped in aluminum foil to retain moisture, then rolled to 1.5 cm thickness with a rolling pin just before the measurement was made. The foil was removed before the measurement was made.

An Instron Food Testing Machine (model 1132) equipped with a 5-kg capacity load cell and probe of 1 cm diameter were used for making the measurements. The crosshead and chart were operated at a speed of 10 cm per min. Measurement consisted of allowing the probe to penetrate 6 mm into the dough. The peak force (kg) required to penetrate the dough was recorded as the measurement of consistency. Five measurements were made on each of the three portions of dough. Two replications were tested.

Preparation of dough for doughnuts

A full recipe was used to prepare dough for making doughnuts. The amounts of water used to prepare doughs with SP of a common consistency are presented in Table 1. The dough was placed into a greased bowl and proofed at 38–40°C for 1 hr at 98% relative humidity. The proofed dough was rolled to a 1.2 cm thickness and cut with a doughnut cutter of 6.7 cm diameter. The cut pieces were placed in a greased bowl, held under a cloth for 10 min, then proofed at 27°C for 30 min.

Method of frying

The cut pieces of dough were fried in soybean oil at 191°C using a small commercial-type fryer. The doughnuts were heated on each side 50 sec. Four doughnuts were fried at a time just prior to being used for analysis. Frying order for doughnuts of a given recipe was determined by use of a random numbers table.

Table 1—Amount of sweet potato, wheat flour and water used in recipes for yeast-raised doughnuts

Percentage[a] SP solids	Ingredients[a,b] altered in recipes	Type of sweet potato		
		Flour	Baked	Steam-cooked
0	SP, g	–	–	–
(Control)	WF, g	425	425	425
	Water, ml	180	180	180
7	SP, g	32	80	114
	WF, g	396	396	396
	Water, ml	181	115	113
14	SP, g	63	159	228
	WF, g	366	366	366
	Water, ml	166	51	74
21	SP, g	95	239	342
	WF, g	336	336	336
	Water, ml	159	6	36

[a] SP = sweet potato; WF = wheat flour
[b] Amount of SP ingredient is equivalent (dry weight basis) to the percentage WF replaced: SP flour had 5.9% water; baked SP, 62.6% water; steam-cooked SP, 73.9% water.

Analysis of sweet potato and doughnut material

Samples of raw SP and fried doughnuts were analyzed for moisture, crude fat, crude protein (N = 6.25), crude fiber and ash (AOAC, 1975), and the amount of carbohydrate was calculated by difference. Cooked SP was analyzed for moisture content according to the AOAC (1975) method. For each measurement three analyses were made per sample from each of two replications.

Samples of doughnuts (0.7g) were used to measure the gross energy value with a Parr Oxygen Bomb Carlorimeter (AOAC, 1975). Three samples from each of 2 replications per recipe were taken for analysis.

Color measurement of doughnuts

Color measurements were made on the crust and crumb of doughnuts using the Hunter Colorimeter (model D25D2M). L, "a", and "b" values were recorded. The instrument was standardized against a white tile (Hunter C2-136). To measure the crust color, the crust with the underlying crumb of approximately 4–5 cm thickness was "peeled" from the remaining crumb. The crust side of the material was placed face down on the optical glass bottom of a Hunter cuvette and weighted to compress the sample against the glass. The bottom of the cuvette was covered completely. The crumb color was measured by covering the bottom of the cuvette with the remaining crumb and compressing it as described for the crust. One measurement of the crust and crumb was made on each of three doughnuts of a given recipe. Two replications of each recipe were prepared and measured.

Firmness measurement of doughnuts

Firmness of doughnuts was determined with the Instron Machine equipped with a 50-kg load cell. The crosshead and chart were operated at speeds of 10 and 25 cm per min, respectively. The measuring device consisted of two facing pieces of metal each being circular, flat surfaced, and 15 cm in diameter.

Measurement was made by compressing a doughnut 20% of its thickness between the two pieces of metal. The maximum force required to compress the samples was taken as the measurement for firmness. One measurement was made on each of three doughnuts of a given recipe. Two replications were prepared and measured.

Volume measurement of doughnuts

Four doughnuts were selected at random from each recipe and used collectively to determine the specific volume in a cake volumeter (Cathcart and Cole, 1938). Specific volume (cc/g) per doughnut was calculated by dividing the total weight into the total volume displaced.

Sensory evaluation of doughnuts

The Quantitative Descriptive Analysis (QDA) method was used to evaluate certain organoleptic attributes of the doughnuts (Stone et al., 1974). The QDA method consisted of a continuous 15 cm line anchored by terms at one end (left) to indicate a weak sensation and terms at the opposite end to indicate a strong sensation of the characteristics being evaluated (Table 2). Ten panelists, consisting of faculty members and graduate students, were trained in the QDA procedure. During the training period the panelists evaluated the doughnuts for 19 factors (categories: aroma, taste, texture, color) considered appropriate by four individuals familiar with doughnut quality. Doughnuts used in this part of the study had 0, 5, 10, 15, and 20% SP flour as replacement for equivalent amounts of wheat flour.

Testing was conducted in a laboratory designed for sensory evaluation. Samples were presented to the panelists in a clear plastic wrap and illuminated with cool white fluorescent light. Five training sessions were held. Testing was conducted between 2:30 and 4:30 p.m.

After each training session the scores were tabulated, and the results were discussed at the next session in a group of all the panelists. When the training sessions were concluded, the data were analyzed to determine which factors were redundant. As a result, six factors were deleted.

At this point the panel evaluated another set of doughnut samples. The doughnuts contained 0, 7, 14, and 21% SP flour,

puree from baked roots, or puree from steam-cooked SP. The list of 13 attributes for which the samples were tested is presented in Table 2. For flavor evaluation, panelists were instructed to lift the plastic wrap from the cup containing the sample, sniff the aroma, and then taste the sample before marking the score sheet. Five textural qualities were determined (Larmond, 1976). Elasticity (degree and quickness of recovery to original shape) was evaluated first by partially compressing the doughnut between the thumb and fingers. Next, the panelists bit through the doughnut to determine tenderness (force required to bit through the sample). This was followed by chewing the material to ascertain stickiness (degree to which the material sticks to the teeth and palate after 5–6 chews) and gumminess (degree to which the mass holds together after 5–6 chews). For appearance and color evaluations an uncut doughnut and a one-half section of a cut doughnut were placed on a white plate, covered with clear plastic wrap, and illuminated with white fluorescent light.

Statistical analysis of data

Data for the different tests were analyzed by analysis of variance as factorials of a complete block: proximate analysis (3 x 4, type x level of SP); volume, color, and texture (3 x 4 x 2, type x level of SP x replication); and panel data (3 x 4 x 2 x 10, type x level of SP x replication x panelist). Significance among means of the proximate analysis was determined by Duncan's Multiple Range Test. Means for proximate analysis and caloric energy are presented. Data for hardness, volume, color, and panel evaluations are presented graphically; polynomials of the highest applicable order were used to fit the curves (Little and Hills, 1972).

RESULTS & DISCUSSION

CONSISTENCY of the doughnut dough decreased as progressive increases in the amount of SP material were added to the recipe. This decrease in consistency occurred when the amount of water was held at a constant level. By reducing the amount of added water, doughs could be prepared with increased consistencies. The three levels of water used to prepare doughs of a given recipe (wheat flour-SP ingredient combination) were suitable for determining the amount of added water required to produce a dough with consistency similar to that of dough without SP. Correlation coefficients (r) for the three levels of water and the corresponding consistency values within individual recipes were −0.9800 or higher, with one exception. For the recipe with 7% SP flour, r = −0.9273. The mean consistency measurement four dough without SP was 163g force.

The moisture content of each SP ingredient was determined in order to calculate the amount of SP material required to replace an equivalent amount of wheat flour in the recipes. The SP flour had 5.9 ± 0.0% moisture; puree from baked roots, 62.6 ± 0.2% moisture; and puree from steam-cooked SP, 73.9 ± 0.1% moisture. Table 1 presents the amounts of SP, wheat flour, and water used in the recipes.

The raw SP flesh contained the following components (dry weight basis): crude protein, 4.5 ± 0.1%; crude fat, 1.1 ± 0.1%; ash, 3.4 ± 0.0%; crude fiber, 2.4 ± 0.0%; and carbohydrate, 88.6%. The moisture content was 66.1 ± 0.3%. A more extensive analysis of SP is available (Technical Committee S-101, 1980).

The proximate compositional values for the fried doughnuts are presented in Table 3. Type and level of SP ingredient and the type x level interaction showed an apparent effect on the composition of doughnuts for all the recipes. However, the data and the notations for significance presented pertain only to the interaction effect.

Differences existed in the moisture content among the doughnuts of the different treatments (Table 3). Overall,

Table 2—Reference standards used by sensory panel for evaluating organoleptic attributes of yeast-raised doughnuts containing sweet potato[a]

Factor evaluated	Ends of evaluation scale	
	Left-weak sensation	Right-strong sensation
Flavor		
Sweet	2% sucrose solution	5% sucrose solution
Salty	0.05% NaCl solution	0.2% NaCl solution
Bready	Uncooked canned biscuit dough	Canned biscuit baked 8 min at 246°C
Oily	White potato, 0.3 cm slice, fried 3 min in soy oil, blotted	White potato, 0.3 cm slice, fried 5 min in soy oil, not blotted
Spicy	0.024% mace in water	0.05% mace in water
Sweet Potato	Sweet potato, boiled 30 min, mashed. 150g mixed with 100g dehydrated white potato and 300 ml water	Sweet potato, boiled 30 min, mashed
Texture		
Elasticity	Uncooked canned biscuit dough rolled in wheat flour	Household sponge
Firmness	Fresh commercial unglazed doughnut	Stale commercial unglazed doughnut
Adhesiveness	Sugarless chewing gum	Taffy candy
Gumminess	Overbaked canned biscuit, baked 246°C for 15 min	Canned biscuit baked 246°C for 6 min
Appearance, Color		
Surface appearance	Smooth sensation from experimental doughnut fried 191°C for 40 seconds	Rough sensation from overproofed experimental doughnut fried 191°C for 60 seconds
Crust color	Light brown sugar	Mixture light brown sugar (2 parts) and dark cocoa (1 part)
Crumb color	Muncell color chip, 5Y 9/2, for light cream color	Muncell color chip, 7.5YR 7/10, for yellow-orange color

[a] Scale used by sensory panel to evaluate samples — a score of 0 represented the weakest sensation, while a score of 15 represented the strongest sensation.

the moisture content of the doughnuts exhibited a direct relationship with the amount of moisture in the doughs. Moisture content of the dough was calculated (but not reported) by summing the amounts of added water and component moisture of the SP material used in each recipe. For doughs containing SP flour and baked SP, the moisture content decreased as the amount of SP was increased. However, the amount of moisture increased in doughs as the level of steam-cooked SP was increased.

The percentage of fat (Table 3) in the doughnuts showed a positive relationship to the amount of moisture in the dough. This condition is consistent with the positive moisture-fat uptake relationship in certain other deep fried foods, eg, potato chips. A first impression resulting from observation of the data may indicate that the type and level of SP influenced the amount of fat absorption, and indeed the amount of fat does increase as the level of steam-cooked SP was raised. Doughnuts with 21% SP flour also exhibited a higher fat content than doughnuts with lower levels of SP flour. However, it seems correct to assume that the level of SP probably had little to do with the amount of fat uptake. No explanation can be given to account for the small, but significant, difference in fat content among the control samples.

Table 3—Proximate analysis of doughnuts containing sweet potato[a,b]

Level of sweet potato	Sweet potato ingredient		
	Flour	Baked	Steam-cooked
%		Moisture, %	
0	23.4^{hi}	23.6^{gh}	23.9^{gh}
7	24.4^{ef}	23.0^{i}	24.7^{de}
14	24.0^{fg}	23.0^{i}	25.1^{d}
21	22.9^{ij}	22.5^{j}	27.1^{c}
		Crude fat, %	
0	23.6^{i}	24.2^{ghi}	24.4^{fgh}
7	23.7^{i}	24.6^{fg}	24.8^{f}
14	23.3^{ij}	23.0^{j}	27.6^{d}
21	26.2^{e}	23.9^{hi}	29.1^{c}
		Crude protein, %	
0	15.3^{cd}	15.4^{cd}	15.8^{c}
7	14.8^{de}	14.0^{f}	15.5^{cd}
14	13.8^{f}	14.1^{ef}	13.7^{f}
21	12.7^{g}	12.8^{g}	13.8^{f}
		Ash, %	
0	2.87^{e-h}	2.81^{fgh}	2.78^{gh}
7	2.95^{efg}	2.72^{h}	2.87^{e-h}
14	3.19^{cd}	3.03^{de}	3.04^{de}
21	3.01^{e}	2.98^{ef}	3.28^{c}
		Carbohydrate, %	
0	58.3	57.6	57.0
7	58.5	58.7	56.8
14	59.7	59.9	55.7
21	58.1	60.3	53.8

[a] Mean of six measurements; all components except moisture on dry basis. Values for dry components may not equal 100 due to rounding.
[b] Means for ash not followed by the same letter are different at $P < 0.05$; means for each of the other components not followed by the same letter are different at $P < 0.01$.

Addition of each type of SP caused the level of protein to decrease as the level of SP was increased. This was caused by the relatively low percentage of protein in the SP material. The wheat flour had approximately 12% protein (Watt and Merrill, 1963) while the raw SP flesh had 4.5% protein (dry basis). The milk solids, whole egg and yeast contributed to the protein content. The relatively high amount of protein in these ingredients was responsible for the fairly high protein content (mean 14.3%) of the doughnuts. According to Watt and Merrill (1963), yeast-raised doughnuts contained 8.8% protein (dry basis).

Several of the ingredients including NaCl, milk solids, SP, and yeast contributed collectively to the ash content (mean 3.0%) of the doughnuts. Raw SP had a higher percentage of ash (3.4%) than the wheat flour (0.43%; Watt and Merrill, 1963). The carbohydrate content ranged from approximately 54 to 60%.

Being a fried food item, the doughnuts had relatively high caloric values (Table 4). On the dry basis, caloric value decreased for doughnuts containing SP flour and baked SP, but increased in doughnuts containing steam-cooked SP. It is doubtful that SP per se influenced the caloric level. Most likely, caloric content was an indirect result of the effect of the moisture content on the fat uptake (previously discussed). For example, caloric level of doughnuts containing steam-cooked SP was closely related to fat content of the doughnuts ($r^2 = 0.8949$). These samples had an oily surface, a condition not observed in doughnuts containing the two other types of SP.

When eaten, doughnuts contribute significantly to the caloric intake of the consumer. One hundred grams of the doughnuts (as is basis) contained a mean 505 Kcal, being calculated from the mean caloric value of 6.64 Kcal/g dry material and the mean solids content of 76.0%. Accordingly a 42g doughnut contained a mean 212 Kcal.

Addition of SP affected hardness of the doughnuts (Fig. 1). Both SP flour and baked SP at the two highest levels tended to increase hardness. All levels of steam-cooked SP produced doughnuts that were softer than doughnuts without SP. Consequently, the amount of force required to compress the samples with steam-cooked SP was less than one-half that required to compress the samples without SP or samples with SP flour and baked SP.

Specific volume (Fig. 2) of the doughnuts was not affected by incorporating SP flour or baked SP into the recipes. All levels of steam-cooked SP, however, produced

Table 4—Caloric values of yeast-raised doughnuts containing sweet potato[a]

Level of sweet potato	Sweet potato ingredient					
	Flour		Baked		Steam-cooked	
	g Dry matter	Doughnut, 42g	g Dry matter	Doughnut, 42g	g Dry matter	Doughnut, 42g
%	Kcal[b]					
0	6.86 ± 0.17	221	6.72 ± 0.12	216	6.61 ± 0.04	211
7	6.66 ± 0.03	212	6.67 ± 0.18	216	6.68 ± 0.17	211
14	6.50 ± 0.13	208	6.35 ± 0.27	205	6.78 ± 0.44	213
21	6.50 ± 0.05	211	6.32 ± 0.60	206	7.03 ± 0.10	215

[a] Mean of six measurements ± one standard deviation
[b] Calculation of caloric content was made for a 42g doughnut using the % solids content determined by subtracting the % moisture from 100% in Table 3; % solids x Kcal/g = Kcal/42g doughnut.

an increase in specific volume over the control. Descriptively, these doughnuts were larger and softer (Fig. 1) than doughnuts containing the other types of SP or no SP.

The presence of SP had no effect on crust color of the doughnuts. The range in Hunter color values were as follows: L, 35.0–39.5; "a", 6.5–11.9; and "b", 19.0–22.1. The color as it appeared to the eye was similar to that of commercially prepared doughnuts.

Color of the crumb was affected by the presence of SP. The Hunter values were affected by the presence of SP ($P < 0.01$), and the L and "a" values were affected by the interaction of SP type and level ($P < 0.01$). Fig. 3, 4, and 5 present data for Hunter L, "a" and "b", respectively. Luminosity (Hunter L) of the crumb was decreased by puree of baked and steam-cooked SP (Fig. 3). The greatest decrease was 6.9, from 69.8 (0% steam-cooked puree) to 62.9 (21% steam-cooked puree). Luminosity was essentially unchanged by increased levels of SP flour. Hunter "a" values ranged from just below zero to slightly above zero (Fig. 4). Therefore, a realistic interpretation of these data is that the crumb was neither green (–"a") nor red ("a") but neutral. Yellowness of the crumb was increased ($P < 0.01$) as the level of all types of SP was raised to 14%, above which no change was found (Fig. 5). From an appearance standpoint, the crumb of doughnuts with 0% SP was a cream color while the crumb with SP was yellow-orange color.

Of the 13 attributes evaluated (Table 2) by the sensory panel, only six (Fig. 6 to 11) were affected by the presence of SP in the doughnuts. Each of the attributes, with one exception, was affected by type and level of SP and by the interaction of the two variables. Crumb color was affected by the interaction only.

Sweetness of the doughnuts was increased as the level of SP flour and baked SP was raised (Fig. 6). Steam-cooked SP, however, caused no change in sweetness. Baked SP, as might have been expected, had the greatest effect on sweetness since baking allowed the amylase enzymes to convert some of the starch to sugar. The heat treatment used to prepare the other SP materials caused a rapid rise in temperature and inactivated the enzymes before hydrolysis of starch could occur. Actually, the relative sweetness of the doughnuts was very low since the highest mean score was 3.9 on the scale of 0–15 (footnote, Table 2).

The addition of SP imparted a SP flavor to the doughnuts (Fig. 7). The flavor was increased as the level of SP was increased up to 14%. Above this level, except for baked SP, no further increase in flavor was detected. Overall, SP flavor was weak since the highest mean score was 3.9.

Elasticity (or springiness) of the doughnuts was not affected appreciably by SP flour and steam-cooked SP (Fig. 8). Baked SP at 21% caused a great reduction in elasticity. Samples from all other treatments were scored as fairly elastic since the scores ranged from 8.3–10.

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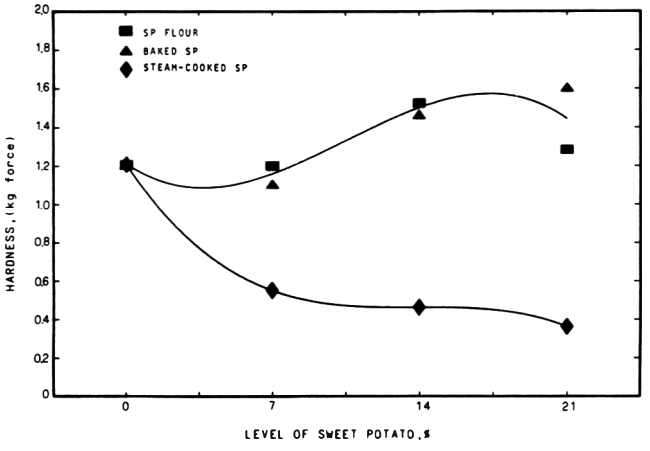

Fig. 1–Force required to compress yeast-raised doughnuts containing sweet potato (SP).

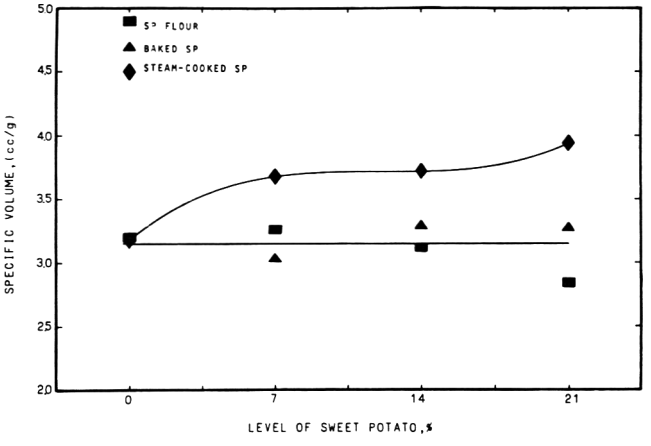

Fig. 2–Specific volume of yeast-raised doughnuts containing sweet potato (SP).

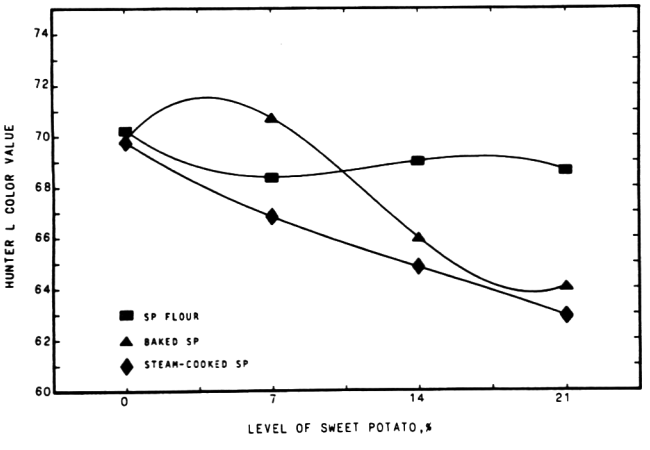

Fig. 3–Hunter L values (luminosity) of the crumb of yeast-raised doughnuts containing sweet potato (SP).

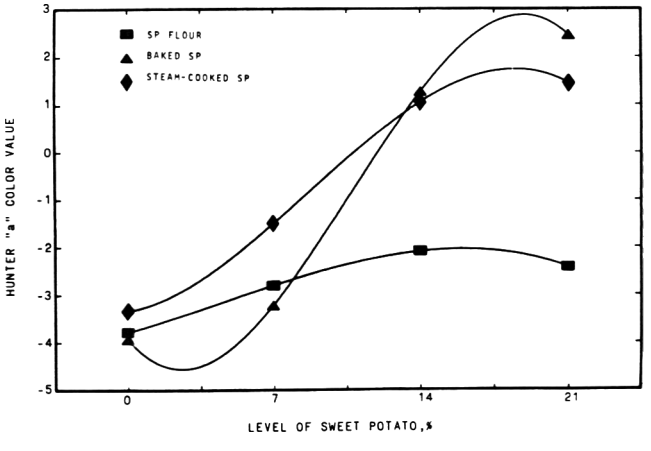

Fig. 4–Hunter "a" values (greenness-redness) of the crumb of yeast-raised doughnuts containing sweet potato (SP).

Puree from baked and steam-cooked SP caused the doughnuts to be more tender (softer) than the doughnuts with SP flour or without SP (Fig. 9). Doughnuts with 14 and 21% steam-cooked SP were very soft. Fig. 1 indicates that doughnuts with steam-cooked SP required the least amount of force to compress the sample. SP flour caused no effect on tenderness except at the 21% level; a slight softening was detected.

The presence of SP affected adhesiveness of the doughnuts (Fig. 10). However, no trends are evident with progressive increases in the amount of SP, but the scores for samples with 14 and 21% SP flour and 21% baked SP are

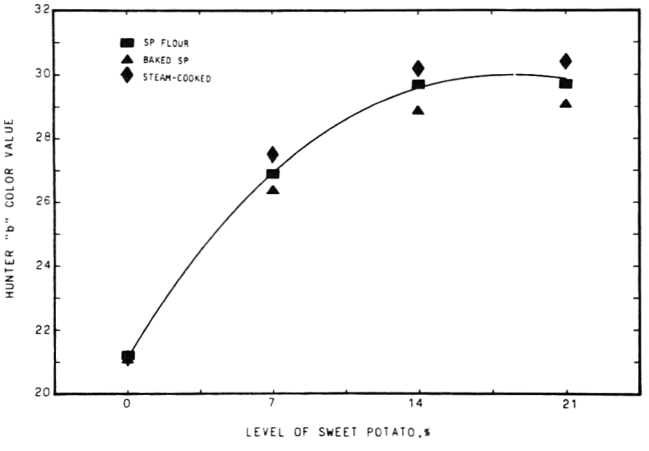

Fig. 5—Hunter "b" values (yellowness) of the crumb of yeast-raised doughnuts containing sweet potato (SP).

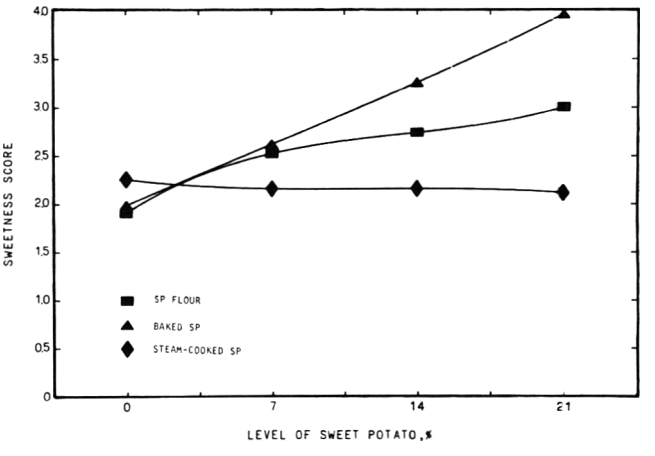

Fig. 6—Panel scores for sweetness of yeast-raised doughnuts containing sweet potato (SP). Scale is in Table 2 footnote.

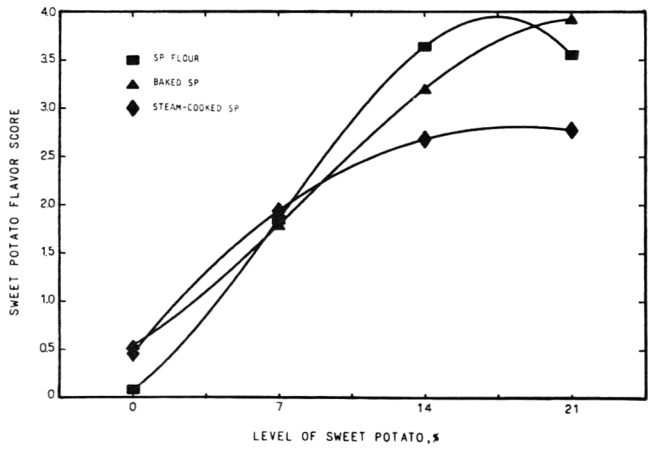

Fig. 7—Panel scores for sweet potato flavor of yeast-raised doughnuts containing sweet potato (SP). Scale is in Table 2 footnote.

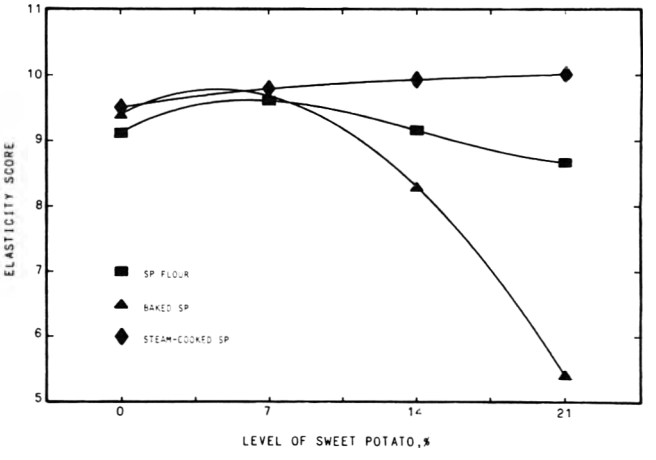

Fig. 8—Panel scores for elasticity of yeast-raised doughnuts containing sweet potato (SP). Scale is in Table 2 footnote.

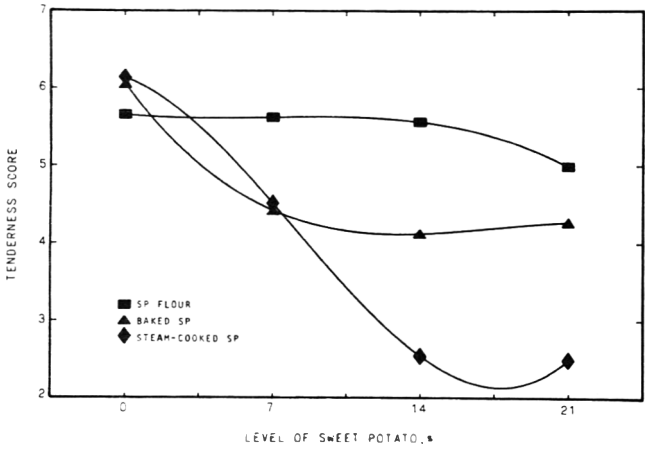

Fig. 9—Panel scores for tenderness of yeast-raised doughnuts containing sweet potato (SP). Scale is in Table 2 footnote.

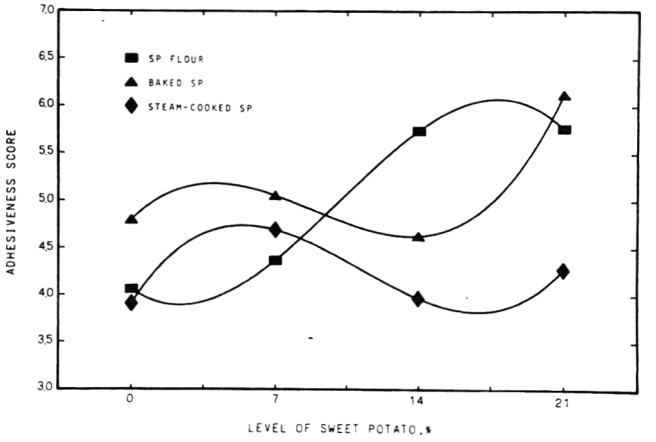

Fig. 10—Panel scores for adhesiveness of yeast-raised doughnuts containing sweet potato (SP). Scale is in Table 2 footnote.

definitely higher than the other scores. With a range of scores from 3.9–6.1 the degree of adhesiveness of doughnuts was low.

Analysis of scores for crumb color did not indicate a difference in the effect of the three types of SP (Fig. 11). However, since the type x level of SP interaction was significant ($P < 0.05$), the data are represented by two curves rather than by one mean curve. Steam-cooked SP at the 14% level caused the crumb to exhibit a more yellow-orange color than that exhibited by doughnuts with the other types of SP. Just why this value did not follow the trend exhibited by the other treatments is not known. With the one exception, each increment increase in the amount of SP in the doughnuts produced a more intense color.

In conclusion, SP as flour and puree can be used as an ingredient of yeast-raised doughnuts. Measurements and analyses of samples which contained SP up to 21% showed that certain attributes and components were altered by the addition of SP. From a quality standpoint baked SP puree was probably the most desirable form; doughnuts did not undergo any adverse changes and, in fact, some of the changes may be desirable. From the standpoint of composition, amount of fat and caloric content, steam-cooked SP would seem to be the least desirable form to use. Comparatively, doughs with steam-cooked SP required a greater amount of moisture to develop a consistency comparable to that of the control of dough. The higher level of moisture resulted in greater uptake of fat when the doughnuts were fried. Concomitant to the increase in fat uptake was an increase in caloric content. Generally, use of SP flour and baked SP resulted in a calorie reduction in the doughnuts. SP flour might be most desirable from the production standpoint since it is a dry material and could be handled and stored more easily and inexpensively than the other forms.

Why, then, consider the addition of SP to doughnuts? First, cooked SP provides a significant amount of provitamin A as well as important amounts of vitamin C, calcium, iron, and energy. Loss of vitamin C should be minimal as a result of deep frying the doughnuts since the frying time was less than 2 min (50 sec on each side). Provitamin A (carotene) is less heat sensitive than vitamin C and should undergo no important changes. The yellow-orange color imparted to the crumb should be an attractive feature to many potential consumers.

While preference tests were not conducted, it is our opinion that SP-containing doughnuts would have been scored at least as high, if not higher, than the control doughnuts. This opinion is based on the results of the difference tests and on the fact that all extra samples were quickly consumed by the panel members.

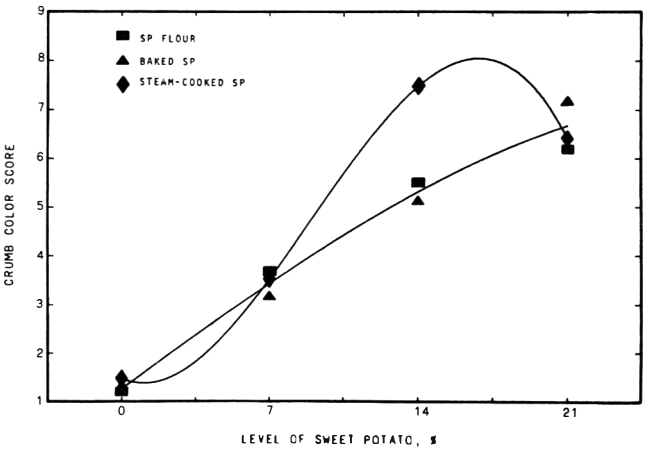

Fig. 11—Panel scores for crumb color of yeast-raised doughnuts containing sweet potato (SP). Scale is in Table 2 footnote.

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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.

Preliminary Evaluations of a New Type of Kishk Made From Whey

AHMED M. HAMAD and M. L. FIELDS

ABSTRACT

An acceptable whey-based kishk was prepared by mixing fermented whey with parboiled wheat at a ratio of 3:1 (v/w). Amino acid analyses for both total and free amino acids indicated that both kishks had adequate amino acid balances for a nutritious food. The riboflavin content of the whey-kishk was 0.14 mg/100g whereas it was only 0.08 mg/100g in the yogurt-kishk. The niacin contents were 3.17 mg and 3.36 mg/100g of yogurt-based kishk and whey-based kishk, respectively.

INTRODUCTION

FERMENTED FOODS play an important role in the diets of many people in Asia, the Near East and parts of Africa (Van Veen and Steinkrause, 1970). Kishk, an extremely popular food in many parts of the middle East, is made by mixing parboiled wheat with yogurt. Kishk is included in the diets of young children and young adults as well as elderly people. Kishk has different names: Kishk in Syria, Palestine, Jordon, Lebanon, and Egypt; Tarhana in Turkey and Kushuk in Iraq (Van Veen and Graham, 1969). Methods of preparation differ from one place to another, but yogurt is almost always one component of kishk. In general, two to three parts of yogurt are mixed with one part of cereal, and the product is dried in the sun or shade. The protein content varies from 15–30% and the moisture content between 10–14 (El-Sadek et al., 1958; Frankul and Pelletot, 1959). The final product is not hygroscopic and can be stored in cloth bags or open jars for 2–3 yr without any detrimental changes. The low moisture content and the low pH are a safeguard against the growth of pathogenic microorganisms. Other ingredients, may be added to the cereal-milk combination. For example, turnips are added in Iraq (Alnouri et al., 1979) as well as tomato paste, red peppers and chopped onions (Van Veen and Graham, 1969).

Manufacturing kishk from yogurt during times when milk supplies are in surplus is a means of preserving milk. This would be especially helpful in tropical and subtropical regions.

For many decades, disposal of whey, a cheese by-product, has been a serious environmental pollution problem. Accordingly, efforts should be made to find new ways and means to utilize this proteinaceous and carbohydrate waste as food for man. The amount of fluid whey produced in the U.S.A. in 1970 was 22 billion pounds, about a third of which was used in food or as a supplement in animal feed (Gillies, 1974). Liquid whey contains about 7% solids of which 4.9% is lactose, 0.9% protein and 0.5% ash.

This research was an investigation of the modification of the conventional method of manufacturing kishk by replacing the yogurt with whey. This modification has two major goals: (1) to produce a food product with an inexpensive but balanced protein content, and (2) to find a new ou for whey.

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MATERIALS & METHODS

White soft cheese and whey production

Fresh whole milk used in this study was obtained from a mix herd (Holstein and Jersey) at the farm of the Ministry of Agricultu Experiment Station-Hofuf, Saudi Arabia. Milk was heated to 65° for 5 min then cooled to 37°C. For setting the milk, rennet extra was ued at the rate specified by the manufacturer (Hansen's Labor tories, Denmark). After about 1 hr, the curd was cut into small cub about 0.5 cm on each side to help expel the whey. Then the cu was transferred to a cheesecloth and the whey was collected an kept refrigerated until used.

Yogurt and fermented whey

Fresh whole milk was heated at 85°C for about 30 min, coole to 45°C and inoculated with a mixed starter culture of *Lactobacillu bulgaricus* and *Streptococcus thermophilus* at the rate of 2%. Afte thorough mixing, the milk was incubated at 45°C for about 6 h and was placed in the refrigerator until used.

Procedure for preparing fermented whey was the same metho used for preparing yogurt.

Kishk preparation

Yogurt-based kishk was produced by the conventional methoc (Table 1). Coarse grained parboiled wheat bulgur was purchasec locally. The bulgur was sifted and extraneous materials were re moved. Then, it was mixed with yogurt at a ratio of 1:2 (250g bul gur to 500g yogurt w/v). The porridge-like mix was spread evenly in metal trays (40 x 20 cm) and covered with protective cheese cloth. The mix was sun-dried until it reached about the same mois ture content of the original bulgur (8–9%). The temperature during the drying period ranged from 40–45°C.

Whey-kishk was prepared in the same way as the yogurt kishk. Preparation steps are listed in Fig. 1. The consistency of the mix was thinner than the yogurt-bulgur mix due to the high water content of whey. Following drying, the samples were ground in a Wiley Mill and passed through 1-mm screen. Samples were replicated three times and kept at room temperature until used.

Chemical analysis

pH of the yogurt, fermented whey and final product (kishk) was determined with a Beckman Automatic pH meter.

Moisture and nitrogen

Moisture and nitrogen content of kishk samples were analyzed as described in AOAC (1975). Moisture content was measured by drying the samples at 130°C for 1 hr in a drying oven. Nitrogen content was determined by using the microkjeldahl method.

Vitamin analysis

Riboflavin and niacin contents were determined for yogurt-kishk and whey-kishk by microbiological methods. The test organisms were *Lactobacillus fermenti* ATCC 9338 and *Lactobacillus plantarum* ATCC 8014 for riboflavin and niacin. The procedure followed for preparation of test materials for riboflavin and niacin was according to the Association of Vitamin Chemists (1966) while the preparation of stock cultures, working standard solutions, inoculum and assay tubes were those of Difco (1977).

Amino acids assay

Amino acid profiles of the kishks were analyzed by the method of Benson and Patterson (1971). Samples were hydrolyzed with 6N HCl for 22 hr at 110°C. Prior to hydrolysis, the samples were oxidized by performic acid for the determination of cystine as cystic acid. The amino acid analyses were performed by automated, cation-exchange chromatography (Benson and Patterson, 1971). Free amino acids were extracted by shaking 500 mg of the sample in 15 ml of 3% sulfosalicylic acid for 30 min.

RESULTS & DISCUSSION

DATA IN TABLE 2 indicate that the pH and percent moisture of the yogurt and whey kishks were similar before and after mixing. The crude protein, however, was higher for yogurt-based kishks than for whey-based kishks. A further examination of the kishks with column chromatography showed that the yogurt-based kishk had a higher total amino acid content (14.62%) than the whey-based kishk (11.7%) (Table 3). The mean percent of free amino acids for yogurt-based and whey-based kishk were 0.87 and 1.02, respectively. The percentage of threonine, valine, leucine and phenylalanine were higher in the whey-based kishk than in the yogurt-based kishk.

From the percentageof free amino acids obtained in these analyses, it appears that the lactic starter culture did not hydrolyze either the milk or the whey proteins. Part of the free amino acids was probably utilized by the lactic culture during their growth.

The percentage of total crude protein in yogurt-based kishk (14.53%) was higher than in whey-based kishk (12.02%). This difference was expected since the yogurt-based kishk contained casein.

The riboflavin content of the whey-based kishk was 1.75 times higher than that of 0.08 mg/100g for the yogurt-based kishk (Table 4). There are no values reported in the literature with respect to the riboflavin content of whey-based kishk. Watt and Merrill (1963) reported that the riboflavin of yogurt made from whole milk was 0.16 mg/100g and that of dry bulgur wheat was 0.10–0.14 mg/100g. Although riboflavin is more stable in acid than in alkaline environments, some destruction apparently occurred during sun drying. A riboflavin content of 0.08 mg/100g was found for yogurt. Presumably the riboflavin content in whey-based kishk also would have decreased due to the sunlight.

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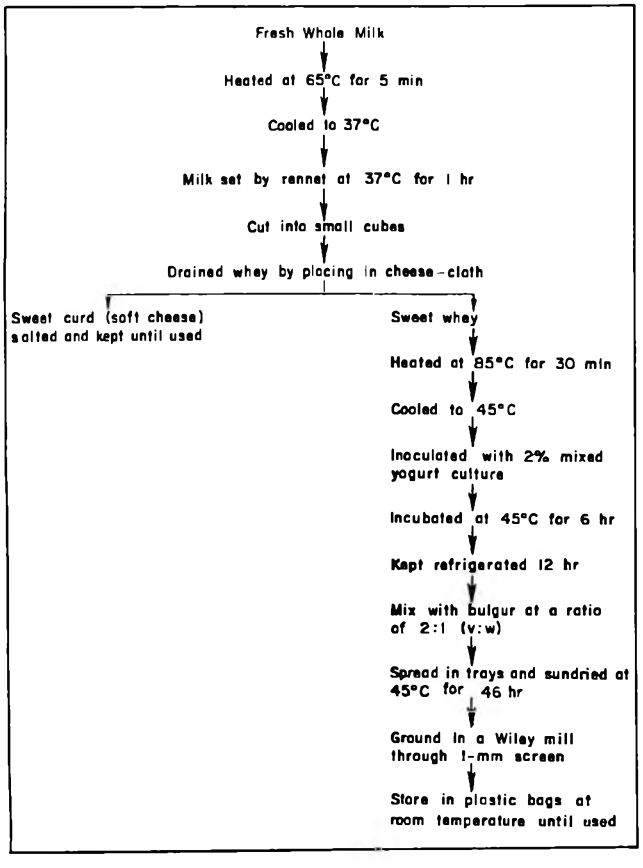

Fig. 1—Preparation of kishk.

Table 1—Formula for kishk soup

Ingredients[a]	Quantity
Kishk flour	132 g
Water	1185 ml
Salt	5 grams
Onion, cut in small pieces	80–85g
Olive oil	39g
Black pepper	To season

[a] Soak the flour (kishk) in the water for about 1 hr. Coat onions with olive oil and combine with the water-kishk mix and seasonings. Cook over medium heat for about 10 min.

Table 2—Percent moisture and protein and pH of yogurt and whey-kishk

Type and replication	Moisture %	pH		Total Crude protein (%)
		Before mixing[a]	After mixing[b]	
Yogurt-kishk-1	8.50	3.95	4.20	14.48
Yogurt-kishk-2	9.67	3.85	4.10	14.55
Yogurt-kishk-3	8.96	3.90	4.20	14.66
Mean	9.40	3.90	4.17	14.56
Whey-kishk-1	10.61	3.90	4.20	12.03
Whey-kishk-2	9.28	3.80	4.10	12.02
Whey-kishk-3	9.15	3.85	4.10	12.66
Mean	9.68	3.85	4.13	12.23

[a] pH of yogurt and whey
[b] pH of kishk

Table 3—Amino acid profile of yogurt and whey-kishk and percent free amino acids (w/w %)

	Amino acids yogurt-kishk		Free amino acids	Amino acids whey-kishk		Free amino acids
	Total	Free	%	Total	Free	%
Aspartic acid	0.97	0.014	1.44	0.81	0.012	1.48
Threonine	0.50	0.003	0.60	0.43	0.004	0.93
Serine	0.69	0.010	1.40	0.54	0.010	1.85
Glutamic acid	3.85	0.030	0.78	3.18	0.025	0.79
Proline	1.42	0.013	0.91	1.08	0.010	0.92
Glycine	0.49	0.003	6.12	0.43	0.004	6.98
Alanine	0.53	0.009	1.70	0.47	0.011	2.34
Cystine	0.16	–	–	0.18	–	–
Valine	0.75	0.005	0.67	0.56	0.008	1.43
Methionine	0.22	–	–	0.18	–	–
Isoleucine	0.60	0.002	0.33	0.48	–	–
Leucine	1.15	0.007	0.61	0.90	0.008	0.89
Tyrosine	0.47	0.003	0.64	0.32	0.003	0.94
Phenylalanine	0.69	0.002	0.29	0.53	0.003	0.57
Histidine	0.35	0.002	0.57	0.27	0.001	0.37
Lysine	0.62	0.005	0.81	0.44	0.002	0.45
Ammonia	0.49	0.009	0.20	0.41	0.012	2.93
Arginine	0.65	0.007	1.00	0.55	0.006	1.10
Tryptophan	–	0.010	–	–	0.009	–
Total	14.62	0.134	0.87	11.77	0.128	1.02
Crude protein	14.53					

The niacin content of the whey-based kishk was higher than that of the yogurt-based kishk (Table 4). Watt and Merrill (1963) reported niacin values of 0.1 mg/100g in yogurt and values of 4.2–4.5 mg/100g for dry bulgur wheat. Presumbly most of the niacin came from the bulgur wheat, but since the same bulgur wheat was used with both yogurt-based and whey-based kishks, fermented whey functioned as well as yogurt.

CONCLUSIONS

THIS RESEARCH demonstrated that an inexpensive whey-based kishk made into a soup was acceptable nutritionally. Because of its stability, whey-based kishk has many potential uses.

Table 4—Mean[a] of riboflavin and niacin contents of control and experimental kishk

Type of product	Replication	mg/100g sample	
		Riboflavin	Niacin
Yogurt-kishk	1	0.09	3.33
	2	0.08	3.03
	3	0.08	3.15
Mean		0.09	3.17
Whey-kishk	1	0.14	3.39
	2	0.14	3.39
	3	0.14	3.30
Mean		0.14	3.36

[a] N = 3. Dry weight base.

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ASEPTICALLY FILLED SWEET POTATO PUREE . . . From page 1132

sweet potato was rated highly acceptable as were those produced by the Harris kettle cook method.

The flavor of the souffles made from all the purees was highly acceptable (Table 4). The homogenized puree was judged to yield a souffle with a slightly less acceptable flavor than those produced from any of the other processing variables. This was due to the excessively smooth texture and increased separation of the whey from the solids during cooking. The processing temperature of the purees in the heat exchanger had no effect on the quality of the souffles into which they were incorproated.

CONCLUSIONS

IMPROVED QUALITY and storage stability of sweet potato purees were accomplished by flash sterilization at high tempratures followed by aseptic packaging. The use of innovative heat exchange equipment permitted processing of high solids purees without the burn-on problems encountered with conventional heat exchangers. Processing at the high temperatures involved in the flash sterilization process had no detrimental effect on puree quality.

The highest quality puree was produced by rapid starch conversion followed by pulping and flash pasteurization. A highly acceptable product which incorporated the fiber and thus reduces processing wastes was attained by colloid grinding rather than pulping. Starch conversion is possible in a relatively short holding period at 75°C if the sweet potatoes are sliced to facilitate heat transfer. This rapid conversion should allow continuous or semicontinuous processing.

Flash sterilization with aseptic packaging makes possible an institutional pack of sweet potato puree which does not require expensive refrigeration or the inconvenience of thawing.

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Sponge-and-Dough Bread: Effects on Fermentation Time, Bromate and Sponge Salt Upon the Baking and Physical Dough Properties of a Canadian Red Spring Wheat

K. R. PRESTON and R. H. KILBORN

ABSTRACT

The effects of fermentation time and bromate level with 0.15 and 1.0% sponge salt on the sponge-and-dough bread quality and physical dough properties of a Canadian red spring wheat sample (No. 1 CWRS-13.5) have been studied. At the higher sponge salt level, fermentation requirements were substantially reduced for each bromate level. The higher sponge salt level also reduced oxidation requirements and gave bread of acceptable quality over a much wider range of fermentation times. Changes in sponge heights during fermentation and dough energy requirements during mixing and sheeting suggested that the higher sponge salt level increased gas retention properties.

INTRODUCTION

IN NORTH AMERICA, the sponge-and-dough process continues to be the predominant method for the production of white pan bread. In addition to the excellent quality of the product, the process is favored for the reduced dough mixing requirements and for its tolerance to variations in processing conditions and ingredients (Merritt, 1950; Pyler, 1978). However, compared to other commercial processes (Continuous, Chorleywood, Canadian Short Process, etc.) the long fermentation time (4–6 hr) required with the sponge-and-dough procedure poses economic disadvantages with respect to increased space requirements and lower bread yields due to higher fermentation losses.

Utilizing a laboratory sponge-and-dough procedure, recent studies in our laboratory have shown that sponge fermentation time could be reduced from 4.5 hr to 2.5 hr by the addition of 1.0% salt (based on total flour) to the sponge without any loss in bread quality (Kilborn et al., 1981). The results also showed that the addition of increasing levels of salt to the sponge resulted in decreasing oxidation requirements. With the exception of slightly increased dough mixing requirements, no basic changes in ingredients or processing conditions were required.

Considering the possible economic benefits of the above findings, further studies concerning the relationship of sponge salt, fermentation time, and oxidation level have been undertaken. In the present paper the effects of adding 0.15% (standard procedure) and 1.0% sponge salt at three oxidation (bromate) levels over a wide range of fermentation times upon the bread quality of a Canadian hard red spring wheat flour has been investigated. In addition, changes in the physical properties of the doughs were monitored during processing to determine responses to the various treatments.

MATERIALS & METHODS

THE NO. 1 CWRS-13.5 wheat flour used in the present study was the same as previously described (Kilborn et al., 1981). It was laboratory milled from an average sample of No. 1 Canada Western Red Spring Wheat of 13.5% protein content (No. CWRS-13.5). The straight grade flour had a protein content of 12.7% (14.0% moisture basis), an ash content of 0.39% and a gassing power of 425 mm. Farinograph absorption was 65.6% and dough development time was 5.25 min.

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Baking

Baking was carried out using the G.R.L. sponge-and-dough procedure with 200g of flour as previously described (Kilborn and Preston, 1981a). Constant sponge ingredients included 140g flour, 4.0g yeast, 0.20g ammonium phosphate, 0.20g malt syrup (60°L) and water (absorption of 64% = 2% below final dough absorption). Variable ingredients included salt and potassium bromate. Salt was added to the sponge at levels of 0.15% (0.3g) and 1.0% (2.0g) based upon total flour. Final dough levels of 0, 10 and 20 ppm potassium bromate were used with 75% of the bromate added to the sponge Following mixing of the ingredients for 2.5 min at 160 rpm in the GRL-200 mixer, sponges were fermented at 27°C (90% R.H.) for periods ranging from 0–6.0 hr. After fermentation, the sponge and remaining ingredients [including flour (60g), salt to give a final concentration of 2.4% (4.5g), sucrose (10g), shortening (6.0g), skim milk powder (4.0g), 60L malt syrup (0.10g), water to give a final absorption of 66% and the remaining potassium bromate] were mixed to approximately 10% past peak consistency as judged from mixing curves. Doughs were then rested 15 min at 30°C, punched and rested a further 15 min. Following sheeting and moulding, doughs were proofed for 70 min at 37.8°C and baked for 30 min at 216°C. All experiments were carried out in duplicate on different days.

Loaf volumes were measured approximately 30 min after baking, by rapeseed displacement. Loaf appearance, crumb colors and crumb structures were measured after storing the bread 20 ± 4 hr in a bread cabinet. Total bread scores were determined by multiplying loaf volume x loaf appearance x crumb structure x crumb color ÷ 10,000, after correcting numerical values for such factors as greyness, age, openness etc. (Kilborn et al., 1981). The standard deviation of duplicates for loaf volume was ±30 cc. Duplicate values for individual bread scores were all within 0.3 units of the mean while corresponding values for total bread scores were within ±2 units of the mean.

Measurements of physical properties of sponges and doughs

Sponge heights of fermenting doughs were followed continuously by means of the GRL Dough Height Tracker (Kilborn and Preston, 1981a). Average curves were determined from at least four determinations carried out on different days. Dough sheeting properties, including dough length, average sheeting force and total sheeting work, were obtained with the GRL Sheeting and Moulding Property Indicator attached to the GRL Sheeter, as described previously (Kilborn and Preston, 1981b). Mixing energy requirements were obtained by means of a power input meter attached to the GRL-200 mixer as outlined by Kilborn (1979).

RESULTS

Effects of fermentation time and bromate level upon bread properties with 0.15% sponge salt

The effects of fermentation time and bromate level in the presence of 0.15% sponge salt upon loaf volume, external and internal bread characteristics and total bread score of sponge-and-dough processed bread are shown in Fig. 1, Table 1, and Fig. 2, respectively. With 0.15% sponge salt (Fig. 1) low loaf volumes were obtained at all bromate levels (0, 10 and 20 ppm) when fermentation was

omitted even though dough mixing requirements were satisfied. With the relatively short fermentation time of 0.45 hr, large increases in loaf volume were evident. However, further increases in loaf volume were less evident, until fermentation times of approximately 3.75 hr were utilized. The effect of bromate was most dramatic with the shorter fermentation times (0.75–3.75 hr) where higher levels resulted in much larger loaf volumes. At longer fermentation times differences in loaf volume were less evident due to the large loaf volume increases occurring in the absence of bromate. The latter results are probably related to the well known ability of fermentation to reduce bromate requirements.

Values for loaf appearance, crumb structure and crumb color are given in Table 1. At all three bromate levels, increasing fermentation time had the greatest effect upon loaf appearance. At shorter fermentation times all bread gave "green" characteristics associated with under-oxidation. In the absence of bromate "greenness" did not disappear until sponge fermentation time was increased to 6.0 hr. With 10 and 20 ppm bromate, fermentation requirements were reduced to approximately 4.5 hr and 3.75 hr, respectively, for the production of bread with satisfactory age characteristics. At longer fermentation times with higher bromate levels, slightly "old" characteristics due to over-oxidation

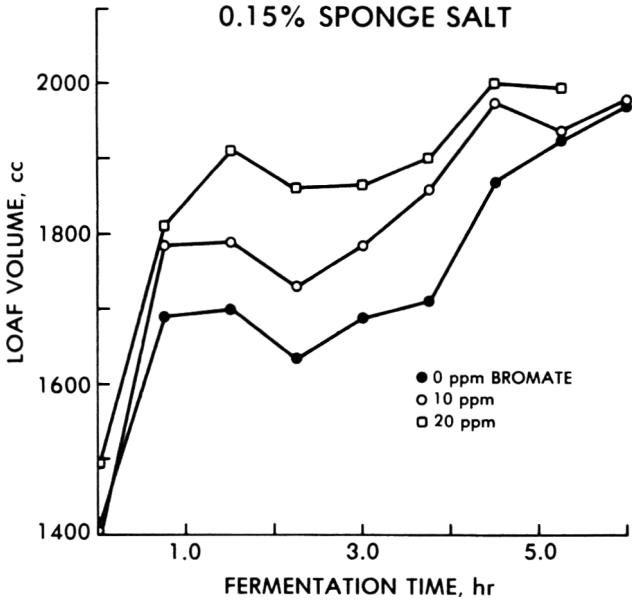

Fig. 1—Effects of fermentation time and bromate level on the loaf volumes of a No. 1 CWRS-13.5 wheat flour in the presence of 0.15% sponge salt.

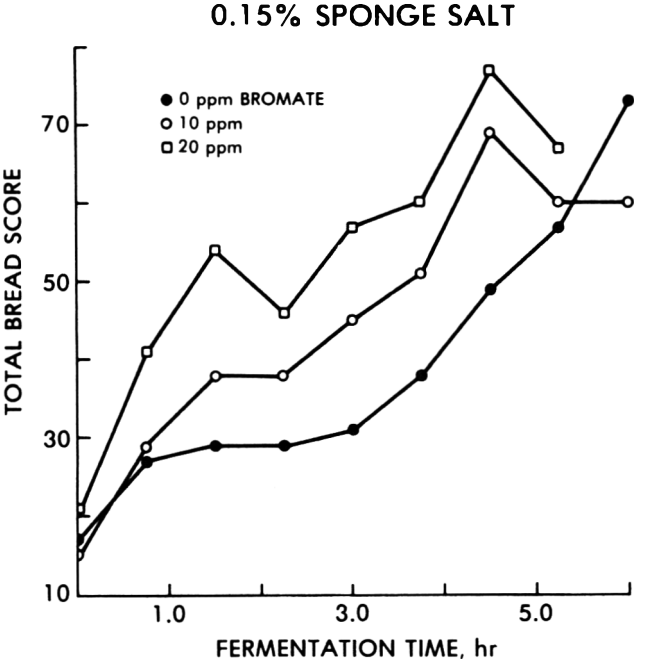

Fig. 2—Effects of fermentation time and bromate level on the total bread scores of a No. 1 CWRS-13.5 wheat flour in the presence of 0.15% sponge salt.

Table 1—Effects of fermentation time and bromate level on the loaf and crumb characteristics and mixing requirements of a No. 1 CWRS-13.5 wheat flour with 0.15% sponge salt[a]

	Fermentation time (hr)								
	0.00	0.75	1.50	2.25	3.00	3.75	4.50	5.25	6.00
0 ppm Bromate									
Appearance[b]	4.5-vg	5.1-g	5.2-g	5.1-g	5.5-g	5.6-slg	6.0-slg	6.4-slg	7.3
Crumb structure[c]	6.0	5.8-o	6.0-o	6.3-o	6.2-o	6.2-o	6.5-o	6.8-o	6.6-o
Crumb color	6.8	7.3	7.5	7.5	7.2	7.6	8.0	8.0	8.3
Mixing time (min)[d]	6.1	4.7	4.5	4.7	4.9	5.0	4.8	5.1	4.8
Mixing energy (Wh/kg)[d]	6.9	5.9	5.9	5.7	4.9	5.0	4.8	5.1	4.8
10 ppm Bromate									
Appearance	4.5-vg	5.3-vg	5.8-g	5.8-g	6.0-slg	6.4-slg	7.1	6.8	6.6-slo
Crumb structure	5.8-o	6.0-o	6.2-o	6.5-o	6.2-o	6.5-o	6.6-o	6.8-vo	6.8-o
Crumb color	6.6	7.9	7.8	7.8	8.0	7.8	8.3	8.0	8.0
Mixing time (min)	5.4	4.4	4.5	4.8	4.7	4.8	5.1	4.6	4.7
Mixing energy (Wh/kg)	6.2	4.7	5.2	5.8	4.9	4.8	4.8	4.8	4.3
20 ppm Bromate									
Appearance	5.1-vg	6.0-g	6.0-slg	5.8-slg	6.5-slg	6.6	7.5	7.0-slo	–
Crumb structure	6.0-o	6.3-o	6.5-o	6.3-o	6.5-o	6.6-o	6.8	6.0-o	–
Crumb color	7.3	7.9	8.6	8.0	8.5	8.1	8.4	8.2	–
Mixing time (min)	6.2	4.3	4.7	4.3	5.0	4.0	4.6	4.1	–
Mixing energy (Wh/kg)	6.9	4.6	5.1	5.4	4.6	4.2	3.8	4.5	–

[a] Mean of duplicate
[b] g = green; o = old; v = very; sl = slightly
[c] 0 = open
[d] Measured at peak consistency

were evident. Numerical values for loaf appearance determined from "break and shred" characteristics showed a tendency to increase with increasing fermentation time for all bromate levels then decrease with 10 and 20 ppm bromate at the longest fermentation times

The highest scores for crumb structure and crumb color were obtained when bromate levels and fermentation times were optimum for loaf appearance characteristics. These values were similar for each bromate level although fermentation times were different. At all bromate levels the lowest values for crumb structure and crumb color occurred with the shortest fermentation time. However, even with no fermentation, values for both these factors were still fairly high.

The major factors contributing to differences in total bread score (Fig. 2) were loaf volume and loaf appearance (including age characteristics). In general the effects of fermentation time and bromate level upon total bread score followed a trend similar to that of loaf volume. At shorter fermentation times, values increased more rapidly as bromate level was increased. Maximum bread scores with 10 and 20 ppm bromate were attained with 4.50 hr fermentation after which values decreased due to apparent over-oxidation. After 6.0 hr of fermentation, bread processed in the absence of bromate gave a total bread score similar to the maximum values achieved with 10 and 20 ppm bromate. From a commercial standpoint, it would appear that only the optimum values for each bromate level would be acceptable (bread scores > 70).

Effects of fermentation time and bromate level upon bread properties with 1.0% sponge salt

Fig. 3, Table 2, and Fig. 4 show the effects of fermentation time and bromate level upon bread properties in the presence of 1.0% sponge salt. As fermentation time was increased from 0 to 2.0 hr, loaf volumes (Fig. 3) showed very rapid increases at all bromate levels. After 2.0 hr fermentation, further increases in loaf volume were less evident. Increases in loaf volume occurred earlier at the highest bromate level although differences in bromate response were less evident than with 0.15% sponge salt. With the exception of the shortest fermentation times (0, 0.75 hr), loaf volumes in the presence of 1.0% sponge salt were much higher than corresponding values obtained with 0.15% sponge salt. With no bromate, differences were as large as 300 cc (3.00 and 3.75 hr fermentation) while with 10 and 20 ppm bromate, differences of approximately 200 cc were evident with fermentation times of 2.25–3.75 hr.

Values for loaf appearance, crumb structure and crumb color with 1.0% sponge salt are shown in Table 2. As in the case of the 0.15% sponge salt, increasing bromate levels reduced fermentation requirements for optimum loaf appearance characteristics. Compared with 0.15% sponge salt, the addition of 1.0% sponge salt resulted in earlier improvement in age and break and shred characteristics for each bromate level and, in addition, gave a wider range of fermentation times over which loaf appearances were acceptable. Similar trends were evident with crumb structure and crumb color. In general these characteristics showed improvement at earlier fermentation times with all bromate levels in the presence of 1.0% sponge salt compared to corresponding results obtained with 0.15% sponge salt.

With 1.0% sponge salt, total bread scores (Fig. 4) showed very rapid increases with fermentation times. These increases were most dramatic with the highest bromate levels (20 ppm) where the highest total bread scores were obtained after only 3.00 hr fermentation. From a commercial standpoint, acceptable bread was produced under these conditions after only 2.25 hr fermentation. After 4.75 hr, bread produced with 20 ppm had an old appearance, indicating "over-oxidation." With no bromate, bread of good quality was produced after only 3.75 hr fermentation with 1.0% sponge salt compared to the 6.00 hr required with 0.15% sponge. In addition to the shorter fermentation requirements with 1.0% sponge salt compared to 0.15% sponge salt, total bread scores were much higher with optimum fermentation times at all bromate levels. Bread of high quality was also produced over a much wider range of fermentation times at all bromate levels with 1.0% sponge salt.

—Continued on next page

Table 2—Effects of fermentation time and bromate level on the loaf and crumb characteristics and mixing requirements of a No. 1 CWRS-13.5 wheat flour with 1.0% sponge salt[a]

	Fermentation time (hr)								
	0.00	0.75	1.50	2.25	3.00	3.75	4.50	5.25	6.00
0 ppm Bromate									
Appearance[b]	4.8-vg	5.2-g	5.9-g	6.0-slg	7.0-slg	7.0	7.2	7.0	7.2
Crumb structure[c]	5.8-o	6.0-o	6.2-o	6.5-o	6.9-o	6.8-o	6.8-o	6.8-o	6.8-o
Crumb color	6.8	7.5	7.9	8.1	8.2	8.2	8.5	8.4	8.3
Mixing time (min)[d]	6.0	5.2	5.3	5.5	5.9	6.0	5.7	5.9	6.0
Mixing energy (Wh/kg)[d]	6.7	5.6	5.9	5.7	5.2	6.1	5.1	5.0	5.1
10 ppm Bromate									
Appearance	4.9-vg	5.5-vg	6.2-slg	7.2	7.2	7.5	7.8	7.0	7.0
Crumb structure	5.6-o	6.3-o	6.5-o	6.9-o	6.8-o	6.8-o	6.8-o	6.8-o	6.8-o
Crumb color	6.8	7.9	8.1	8.0	8.5	8.4	8.5	8.4	8.3
Mixing time (min)	5.4	5.0	4.8	5.3	5.6	5.9	6.0	5.9	5.4
Mixing energy (Wh/kg)	6.1	5.3	5.3	5.6	5.7	5.7	5.5	5.5	4.5
20 ppm Bromate									
Appearance	5.0-vg	6.0-slg	6.6	7.2	7.5	7.2-slo	7.2-o	7.2-o	–
Crumb structure	6.2-o	6.6-o	6.6-o	6.8-o	6.8-o	6.8-o	6.8-o	6.8-o	–
Crumb color	7.3	8.2	8.8	8.5	8.6	8.0	8.9	8.1	–
Mixing time (min)	5.9	5.0	5.2	4.7	5.9	4.9	5.3	5.1	–
Mixing energy (Wh/kg)	6.7	5.0	4.8	5.1	4.9	4.8	4.1	5.3	–

[a] Mean of duplicate
[b] g = green; o = old; v = very; sl = slightly
[c] 0 = open
[d] Measured at peak consistency

Physical properties of sponges and doughs

In addition to dough mixing requirements, the physical properties of the sponges and doughs were followed during processing with the GRL Dough Height Tracker (Kilborn and Preston, 1981b) and the GRL Sheeting and Moulding Property Indicator (Kilborn and Preston, 1981b). The former instrument was used to follow changes in sponge height during fermentation and dough height during proofing for selected samples. The latter instrument was used to study the reaction of the dough to sheeting, including measurements of dough length, average force and total work imparted. For the three sheeting, gaps of 8.7, 4.8 and 3.2 mm (width = 132 mm) were used for all doughs on the 1-lb National sheeting rolls.

Dough mixing requirements for development to peak consistency are given in Tables 1 and 2. With both 0.15% (Table 1) and 1.0% (Table 2) sponge salt both mixing energy (Wh/kg) and mixing time requirements (min) were greatest with no fermentation. Changes in mixing requirements were less evident as fermentation time was increased from 0.75 to 6.0 hr. With 0.15% salt there appeared to be a further significant decrease in energy mixing requirements at 3.0 hr at all bromate levels, which was not evident with 1.0% sponge salt. With both sponge salt levels increasing bromate levels reduced energy mixing requirements with the effect being most prominent at earlier fermentation times. Mixing requirements (energy or time) tended to be greater with the higher sponge salt level although this trend was not always consistent.

The effects of fermentation time, bromate level and sponge salt level upon sponge height are shown in Fig. 5. At all levels of bromate (0, 10 and 20 ppm) with 0.15% or 1.0% sponge salt, sponges increased in height up to about 2.0 hr then decreased to a minimum at approximately 2.5 hr after which sponge height increased again. The drop or "collapse" of the sponge after about 2.0 hr fermentation is well known commercially (Pyler, 1978; Garnatz et al., 1949). Bromate level did not appear to have much effect upon sponge height curves during fermentation with either salt level. However, with 0.15% salt, the initial peak height (after 2.0 hr) and sponge height after 4 hr were somewhat lower (significant at 5% level) with no bromate, compared to 10 and 20 ppm bromate. With 1.0% sponge salt the dough drop after 2.0 hr was much less evident than with 0.15% sponge salt. In addition the sponge heights increased more rapidly and to higher levels after the dough drop with 1.0% sponge salt. In contrast to differences occurring in sponge height with salt level, no differences occurred in the proofing rate of the doughs up to 70 min with either a 2.5 or 4.5 hr sponge fermentation (data not shown).

Total sheeting work imparted to the doughs for all three sheetings and sheeting work imparted to the doughs during the final (3rd) sheeting with 0 and 20 ppm bromate are shown in Fig. 6 and 7, respectively. With 0 ppm bromate total sheeting work showed an initial increase then a decrease up to approximately 3.0 hr of fermentation with both 0.15% and 1.0% sponge salt. Sheeting work imparted to the doughs during the final sheeting followed a trend similar to but less pronounced than total sheeting work with minimum sheeting requirements at both sponge salt levels evident after 3.0 hr fermentation. For both total and final sheeting, increased work was required to sheet the doughs which had been processed with 1.0% sponge salt at all fermentation times. With 20 ppm bromate, the effect of fermentation time on total and final sheeting work followed a trend similar to that obtained with 0 ppm bromate. However, the initial minimum work requirement occurred earlier with 20 ppm bromate (after 2.25 hr fermentation) and, following a slight increase at 3.0 hr sheeting, work requirements showed further decreases rather than the increases generally evident with 0 ppm bromate. For both total and final sheeting, differences in sheeting work re-

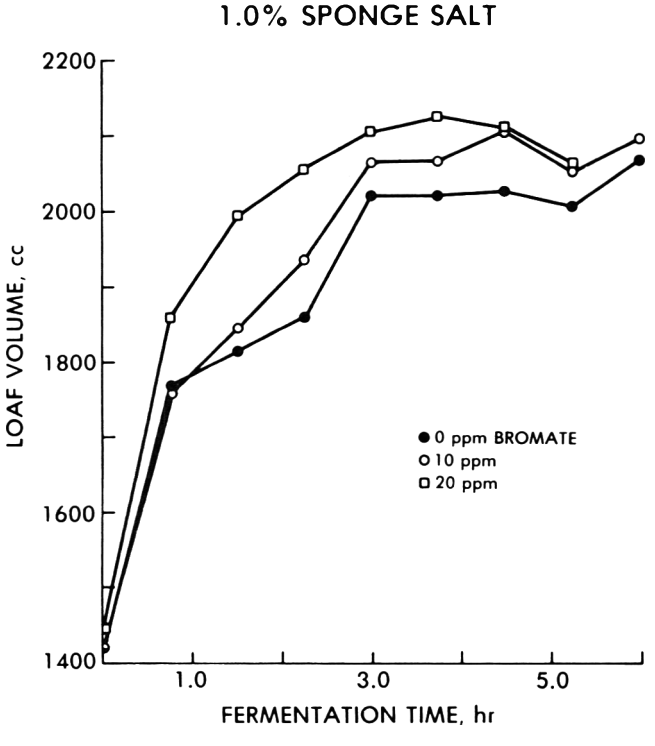

Fig. 3—Effects of fermentation time and bromate level on the loaf volumes of a No. 1 CWRS-13.5 wheat flour in the presence of 1.0% sponge salt.

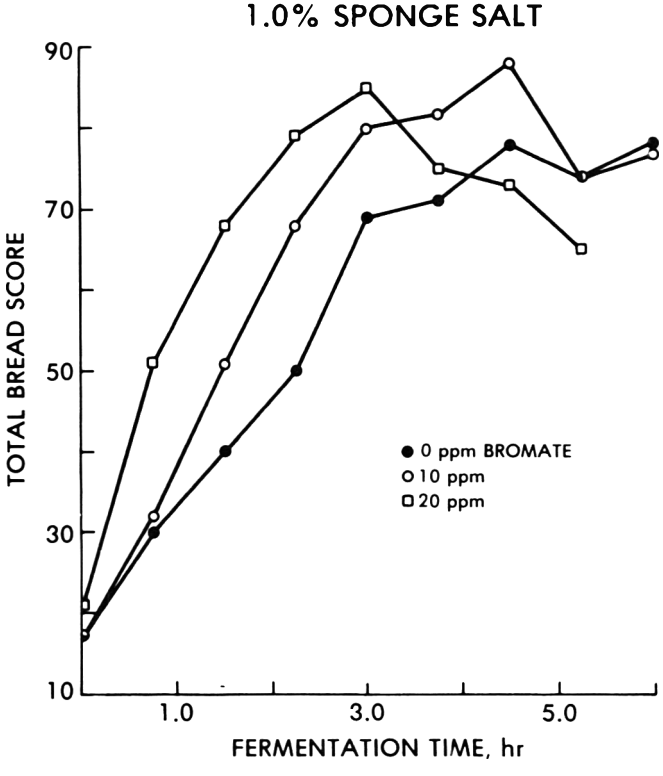

Fig. 4—Effects of fermentation time and bromate level on the total bread scores of a No. 1 CWRS-13.5 wheat flour in the presence of 1.0% sponge salt.

quirements with 0.15% and 1.0% sponge salt were much less evident with 20 ppm bromate than with 0 ppm bromate.

Although dough length showed large increases after each sheeting, fermentation time, bromate level and sponge salt level did not have any significant effects upon dough sheeting length after each sheeting (data not shown). Unpublished studies in our laboratory suggest that doughs which have "normal" handling properties (are not "bucky" or "slack") do not tend to vary in sheeting length. In the present study all of the doughs had "normal" handling properties as judged by an experienced operator.

Average sheeting force was also measured for all doughs. However, since dough sheeting lengths did not change with the various treatments, average sheeting forces were directly proportional to sheeting work. In other words, differences in sheeting work due to fermentation time, bromate level and sponge salt level were due almost exclusively to changes occurring in sheeting force.

DISCUSSION

IN A PREVIOUS PUBLICATION, it was shown that bread of high qualiy could be produced after only 2.5 hr sponge fermentation by increasing sponge salt level from the 0.15% (standard procedure) to 1.0% without an increase in oxidant requirements (Kilborn et al., 1981). In the present paper the effects of sponge salt and bromate level over a wide range of fermentation times upon the bread quality and physical dough properties of a No. 1 CWRS-13.5 wheat flour have been explored. The main conclusions of this study, which support and extend those of the previous publication, may be summarized as follows:

1. The inclusion of higher levels of salt (1.0%) in the sponge than is normally used commercially offers a means of substantially reducing fermentation requirements for the production of acceptable (bread scores of 70 or more) bread. The extent to which fermentation requirements can be reduced was also dependent on bromate level with higher levels being more effective.

2. Tolerance to fermentation was dramatically increased by the presence of 1.0% sponge salt as evidenced by the

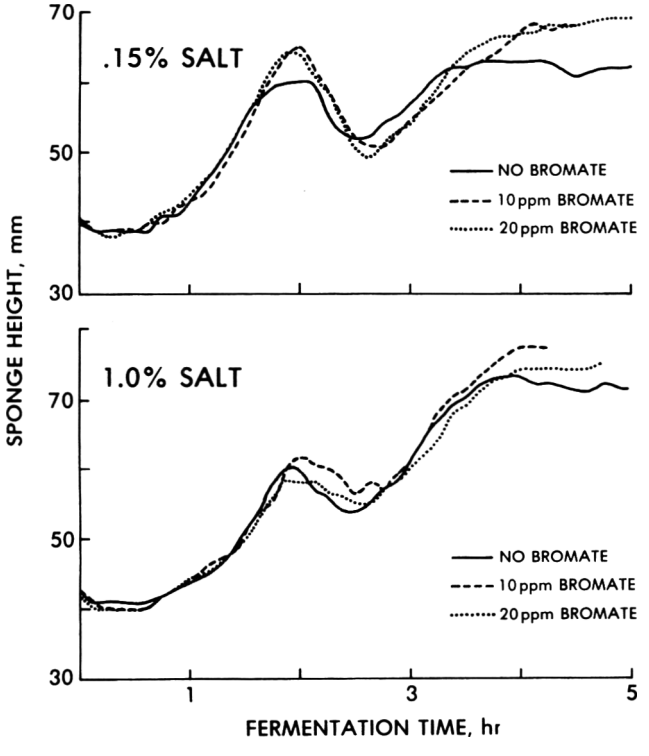

Fig. 5—Effects of fermentation time and bromate level on the sponge height curves of a No. 1 CWRS-13.5 wheat flour in the presence of 0.15% and 1.0% sponge salt.

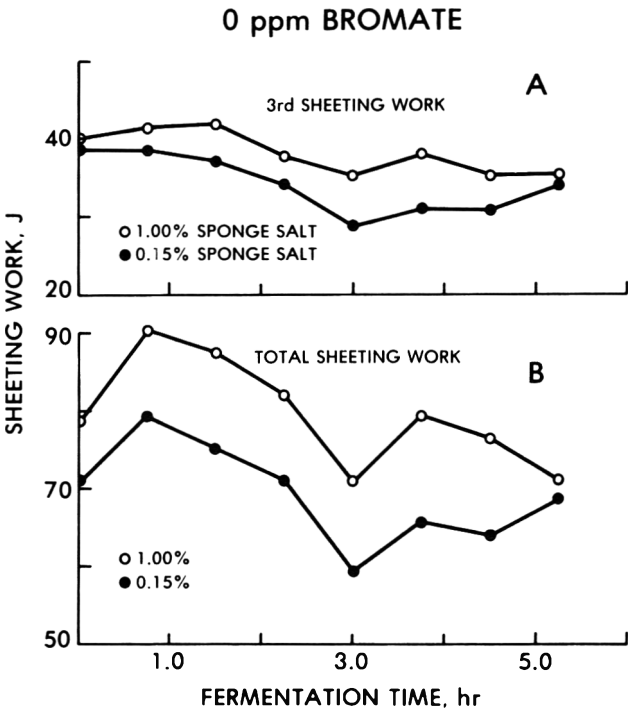

Fig. 6—Effects of fermentation time and salt level on the dough sheeting work requirements (per 100g flour) of a No. 1 CWRS-13.5 wheat flour in the absence of bromate.

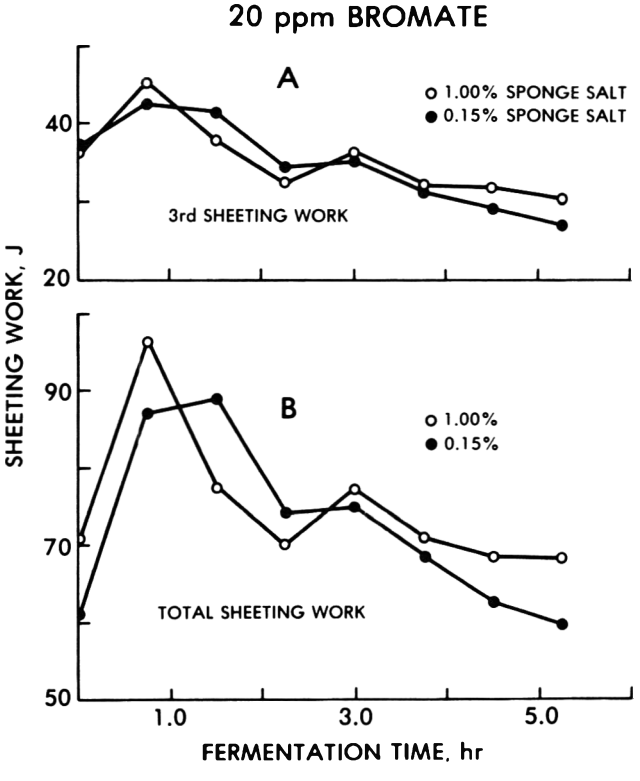

Fig. 7—Effects of fermentation time and salt level on the dough sheeting work requirements (per 100g flour) of a No. 1 CWRS-13.5 wheat flour in the presence of 20 ppm bromate.

wide range over which bread of acceptable quality was produced. Acceptable bread was produced with 2.25–4.5 hr fermentation with 20 ppm bromate, with 3.0 to at least 6.0 hr with 10 ppm bromate and with 3.75 to at least 6.0 hr with no bromate.

3. The inclusion of 1.0% salt in the sponge appears capable of reducing oxidation (bromate) requirements. This particular finding may be important in countries such as Japan where the use of bromate is not desired by the consumer.

4. Dough mixing requirements increased with sponge salt level. However, the extent of these increases does not suggest potential problems in the processing of the dough with the lower speed commercial mixers used in some bakeries. Recent studies in our laboratory with small commercial bar and spiral mixers have confirmed this view (unpublished data).

During sponge (or straight dough) fermentation, the properties of the gluten proteins are altered by the action of yeast fermentation products such as alcohols and acids and by the action of enzymes to give the proper balance of elastic and viscous properties required for optimum gas retention properties and bread quality (Pyler, 1978). In addition, the slow deformation and large strain on the sponge brought about by internal carbon dioxide pressure from yeast fermentation also is thought to play an important role in this "mellowing" process (Bloksma, 1962; Matsumoto et al., 1973). From the earlier and more rapid increase in loaf volumes and bread scores that were evident during fermentation with the 1.0% sponge salt compared to the standard 0.15% sponge salt, it is apparent that the gluten "mellowing" process occurred at a substantially greater rate with the higher salt level. Since salt used at this higher level is known to slightly reduce yeast activity (Miller et al., 1943), yeast fermentation products such as alcohols and acids and changes in enzyme activity would not have been expected to be responsible for this increased fermentation rate. However, as demonstrated by Matsumoto et al. (1973), compounds such as oxidants, which can decrease fermentation time requirements, can have large effects upon internal dough pressure.

Considering the above, it is postulated that the increase in fermentation rate achieved with the high sponge salt level may have been due to increases in the rate of deformation and strain on the gluten proteins in the sponge. An increase in strain rate with the higher salt level would be consistent with the known ability of salt to increase dough resistance as evidenced by extensigraph studies (Fisher et al., 1949). Thus salt may act in a manner similar to fast acting oxidants such as iodates which are also known to increase extensigraph resistance (Smith and Andrews, 1952) and to increase the internal gas pressure in dough (Matsumoto et al., 1973). In addition to the "oxidant-like" effect, salt, in contrast to oxidants, also is known to increase the extensibility of doughs (Fisher et al., 1949). This increase in extensibility may lead to larger rates of deformation due to carbon dioxide stress. Thus the strain on the dough with the higher salt level may be maintained over a longer period of time which would increase gluten development rate, since gas leakage associated with the rupture of the gluten membranes surrounding the gas cells would be reduced.

Although this hypothesis is highly sepculative, the physical dough data are consistent with this view. Studies by Marek and Bushuk (1967) have shown that the sponge drop is associated with a loss in gas retention properties and the release of a large amount of carbon dioxide. Thus, the reduced sponge drop with the higher sponge salt level is consistent with the maintenance of a higher stress level associated with a smaller loss in gas retention properties which may significantly increase the fermentation rate. It should be noted that large increases in loaf volumes and bread scores were obtained with 1.0% sponge salt during the fermentation period associated with sponge drop (1.5–3.0 hr) while with 0.15% salt, loaf volumes and bread scores showed little change. Sponge heights at later fermentation times (after 3.0 hr) also showed larger increases with the 1.0% salt indicating an improvement in gas retention properties compared to the 0.15% salt. In addition to the sponge height results, the doughs derived from the 1.0% salt sponges also required greater sheeting work (or average sheeting force) that those derived from the 0.15% salt sponge while sheeting dough lengths did not vary. These results suggest that during baking, the doughs derived from the 1.0% salt sponges would be capable of withstanding the higher forces associated with the rapid gas expansion during "oven rise" due to their increased elasticity (related to sheeting force) while maintaining their extensibility (related to dough length).

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Texture of Yeast Breads Containing Soy Protein Isolates: Sensory and Objective Evaluation

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ABSTRACT

Three soy protein isolates were substituted on an equal protein basis for soy flour in a yeast bread formula. The consumer texture profile technique was used for sensory evaluation of the breads. A compression cage attached to an Instron was used for objective evaluation of textural characteristics. Panelists noted differences in smoothness, uniformity of cell distribution, chewiness, greasiness and crumbliness among the soy-containing breads. Deviations from the perceived ideal bread were shown for most textural characteristics. Soy isolates influenced volume, specific volume, hardness, springiness, gumminess and chewiness of the breads.

INTRODUCTION

BAKERY PRODUCTS provide a means of improving dietary protein world-wide. Various soy protein products (e.g. toasted soy flour, chemically treated soy flour, lecithinated soy flour, full-fat soy flour, concentrates and isolates) have been produced for the baking industry (Hoover, 1979). Acceptable cookies have been prepared from wheat flour fortified with either 12% whole or 12% dehulled soybean products (Tsen et al., 1975). Soy flours have been used commercially in economy grade cakes for their water absorption characteristics and their ability to hold moisture during the shelf life of the product. These functional properties and the associated economics have created considerable interest among bakers in the use of soy flour as an extender of cake batter (Turro and Sipos, 1970).

Soy protein concentrates and isolates have been less widely promoted for use in bakery products. There are relatively few reports in the literature in which the possible use of these products in bakery applications is discussed. Soy protein concentrate has been used in specialty breads and in high protein cookies (Sipos et al., 1974). With the use of soy concentrates, the manufacture of specialty breads having protein contents of 13–14%, as compared with 8–9% for white bread, is possible, as is the manufacture of snacks with protein contents of 15% (Sipos et al., 1974). The use of soy protein isolate to improve the nutritional value of white bread has been investigated (Finney et al., 1963; Ranhotra et al., 1974). Onayemi and Lorenz (1978) gave additional information on the functional properties of soy protein concentrate and soy protein isolate in breadmaking. As breadstuffs are a major convenience food eaten daily and liked by large masses of the population, the objective of this study was to evaluate the performance of three soy protein isolates in yeast bread.

MATERIALS & METHODS

THREE SOY PROTEIN ISOLATES, Pro-Fam 90/LS, Promine-D and Supro 620, were obtained from Grain Processing Corp., Central Soya, and Ralston Purina Co., respectively. The bread was prepared according to the straight-dough formula and procedure of Marnett et al. (1973) except that soy isolate was substituted for soy flour on an equal-protein basis and the amount of water added was varied according to farinograph data obtained for flour-isolate mixtures (Elgedaily et al., 1982). A deep electric frypan with a thermostatic control was used as a water bath for maintaining dough temperature at 29°C during bulk fermentation. The dough was weighed (330g), shaped, placed in pans (19 X 9.2 X 5.5 cm) and proofed at 42°C and 85% relative humidity for 50–70 min (7-cm height) in an environmental chamber (Associated Testing Laboratories Inc., model HH-5127). The bread was baked at 224°C for 30 min in the electric rotary Despatch oven, then cooled for 30 min, wrapped in aluminum foil and frozen until the testing period.

Breads containing the three isolates were made and frozen as described above. Two loaves for each isolate were taken out of the freezer 3 hr before the evaluation. Each loaf was weighed and its volume was measured by rapeseed displacement. The end crusts of each loaf were removed and the loaf was cut into 9 slices. Slices 2, 3, 5, 6, 8 and 9 were 1.25 cm thick and used in sensory evaluation; slices 1, 4 and 7 were 2.5 cm thick and used in objective evaluation.

Sensory evaluation

The consumer texture profile technique (Szczesniak et al., 1975) was used to evaluate the breads. Twenty female and two male panelists ranging in age from 22 to 64 participated in the evaluation. The panelists had had experience as sensory panelists prior to participation in this study.

Each panelist was asked to describe an ideal bread on 0–5 scales where 0 indicated the absence of each of 15 characteristics and 5 indicated its presence to a very high degree. The characteristics (listed in Fig. 1) were selected from those listed by Szczesniak et al. (1975) that seemed to be applicable to bread. Uniform cell distribution, a frequently used bread descriptor, was added.

After describing the ideal bread, each panelist received one-half slice of each of the three breads to rate on like score cards. Order of presentation of the bread samples was randomized. Water was provided for rinsing the mouth between samples.

Panelists were seated at individual booths in a room with fluorescent lighting for testing. Testing was conducted in the morning and each judge described the ideal and evaluated the three breads in each of two replications.

Objective evaluation

Four cylindrical samples, 2.5 cm in diameter, were removed from slices 1, 4 and 7 of each of the two loaves per isolate per replication. A compression cage attached to an Instron, Model 1130, was used to compress each sample to 20% of its original height. The area of the compressing surface was greater than that of the sample. A 50-kg load cell was used with a range setting of 10 or 20, a cross head speed of 50 mm/min and a chart speed of 100 mm/min. The compression curve for each cycle was recorded. Parameters determined included hardness, springiness, cohesiveness, gumminess and chewiness (Bourne, 1978). Each sample was compressed twice and values for the four samples per slice were averaged. Correlation among different parameters used in evaluating the bread subjectively and objectively was studied.

RESULTS & DISCUSSION

Sensory evaluation

Mean sensory scores for the breads containing soy isolates are shown in Table 1. Analysis of variance showed that breads made from the three soy isolates differed significantly

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in chewiness and greasiness ($P < 0.05$), and smoothness, uniformity of cell distribution and crumbliness ($P < 0.01$). The results of the Student-Newman-Keuls test of the data for these properties also are shown in Table 1. The panelists differed in their evaluations of the bread; however, they generally were consistent from one replication to another. A significant negative correlation coefficient (−0.74, $P < 0.01$) for good and bad is an indication that the panelists used the scorecard as intended.

Fig. 1 shows the consumer texture profiles of yeast breads made from mixtures of wheat flour and the three soy protein isolates as compared with the ideal bread in the panelists' opinion. The profile for the ideal product is shown as a straight vertical line with an adjusted rating of zero for each characteristic. The profiles for the three isolates are shown as deviations from the scores used in describing an ideal product (Table 1). The range of deviations fell within ± 3 of the ideal score for each characteristic.

The profiles suggest that the soy-containing breads were firmer, drier, grainier, less tender and gummier than the panelists expected an ideal bread to be. Breads containing Pro-Fam 90/LS and Promine-D were closer to the concept of an ideal product than that containing Supro 620 with respect to smoothness and uniformity of cell distribution; breads containing Pro-Fam 90/LS and Supro 620 were closer to the panelists' ideal than that containing Promine-D with respect to chewiness, greasiness and crumbliness. Overall, bread made from wheat flour with Pro-Fam 90/LS tended to be slightly closer to the ideal product than those containing the other isolates.

Objective evaluation

Bread made from wheat flour with the three soy protein isolates showed significant differences in volume and specific volume. Bread made from wheat flour with soy isolate Pro-Fam 90/LS had a greater volume than bread made from wheat flour with Promine-D or Supro 620: the mean volumes of 1064, 878 and 994 cm^3, respectively, all differed significantly ($P < 0.05$) according to the Student-Newman-Keuls test, as did the corresponding specific volumes of 3.69, 3.04 and 3.46.

Means from the Instron evaluation of texture are shown in Table 2. Bread containing Pro-Fam 90/LS or Supro 620 was less hard, gummy and chewy and more springy than that containing Promine-D ($P < 0.05$).

Soy protein isolates with almost the same chemical composition differed in their performance in the bread system. This finding may be related to the functional properties of the soy isolates. Water absorption of Pro-Fam 90/LS was lower than that of Promine-D when investigated in a simple system (Elgedaily et al., 1982). However, when water absorption was studied with the farinograph in a system containing both wheat flour and isolate, flour with Pro-Fam 90/LS showed higher water absorption than did flour with Promine-D ($P < 0.05$). A soy protein isolate having a high water absorbing capacity in simple systems might be expected to make a relatively large contribution to standard farinograph consistency. This could prevent full hydration of wheat gluten proteins. Pro-Fam 90/LS with its lower hydration capacity, might therefore be expected to compete less with gluten proteins for water and thus perform relatively well in a bread system. This was supported when bread made from Pro-Fam 90/LS was somewhat superior overall to that made from the other isolates under the conditions of this study. *—Continued on page 1154*

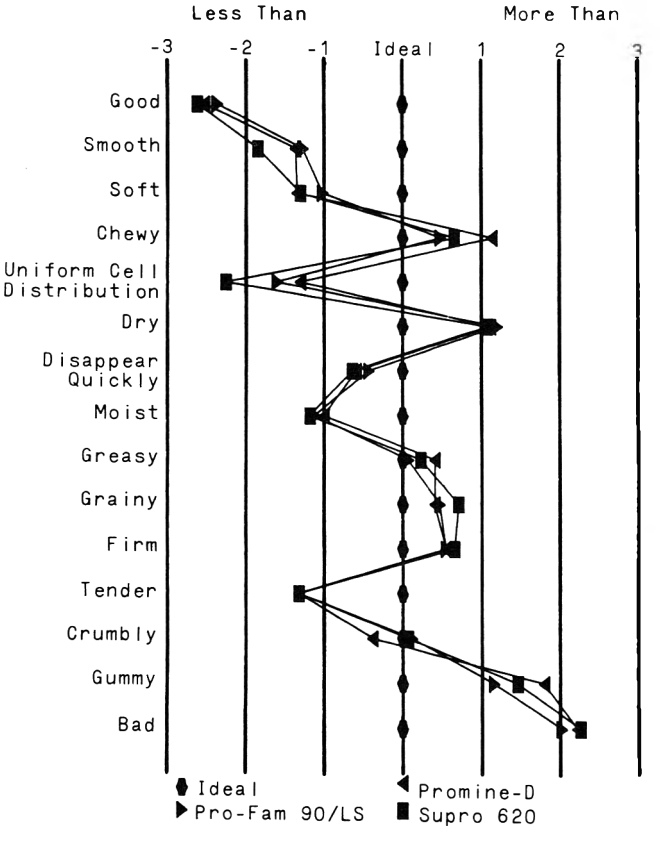

Fig. 1—Consumer texture profiles of yeast bread, with results represented as deviations from the perceived ideal product. (Each point is the mean for two replications of evaluation by 22 panelists.)

Table 1—Mean scores of sensory parameters for the perceived ideal bread and breads containing three soy protein isolates[a,b]

	Ideal	Pro-Fam 90/LS	Promine-D	Supro 620
Good	4.86	2.50	2.34	2.25
Smooth	3.50	2.23a	2.14ab	1.66b
Soft	3.48	2.45	2.14	2.18
Chewy	1.86	2.34c	3.00d	2.52c
Uniform cell distribution	3.98	2.39a	2.68a	1.73b
Dry	1.04	2.25	2.16	2.11
Disappear quickly	2.50	2.07	1.91	1.86
Moist	3.66	2.54	2.66	2.48
Greasy	0.25	0.32c	0.66d	0.48cd
Grainy	0.91	1.39	1.32	1.61
Firm	2.54	3.09	3.09	3.20
Tender	3.86	2.54	2.54	2.54
Crumbly	1.02	1.14c	0.64d	1.09c
Gummy	0.40	1.57	2.20	1.86
Bad	0.12	2.14	2.34	2.39

[a] 0 = the absence of the characteristic; 5 = the presence of the characteristic to a very high degree.
[b] Means within a row for the three isolate-containing breads followed by like letters do not differ: a, b, $P > 0.01$; c, d, $P > 0.05$.

Table 2—Objective texture parameters of yeast breads containing soy[a]

Soy	Hardness (kg)	Springiness (cm)	Cohesiveness	Gumminess (kg)	Chewiness (kg-cm)
Pro-Fam 90/LS	5.66a	0.77a	0.36	2.02b	1.54a
Promine D	13.60b	0.57b	0.33	4.43a	2.52b
Supro 620	7.09a	0.75a	0.34	2.44b	1.82a

[a] Means within a column followed by like letters do not differ ($P > 0.05$).

Development of Food Ingredients from Navy Beans *(Phaseolus vulgaris)* by Roasting, Pin Milling, and Air Classification

J. M. AGUILERA, E. W. LUSAS, M. A. UEBERSAX, and M. E. ZABIK

ABSTRACT

Eight Navy bean samples, roasted between 92 and 125°C in a particle-to-particle heat exchanger, were ground to produce whole bean and hull flours. Dehulled bean flours were also separated into high protein (HPF) and high-starch fractions (HSF) by air classification. Whole bean flours contained 1.92% fat and 25.8% protein on the average. Hull flours contained between 31.2 and 50.2% dietary fiber, of which approximately 60% was crude fiber. Dehulled bean flour containing 26.8% protein was fractionated into HSF and HPF with average protein contents of 15.6 and 43.1%, respectively. Greater protein shift may be further accomplished by finer grinding and adjustment of the cut point. Residual trypsin inhibitor activity of HPFs ranged from 25–108 TIA/mg protein, down from 116 units in the raw control HPF. Hemagglutinins were inactivated from 110 HA units/mg to values ranging from 2.4–105 HA units/mg.

INTRODUCTION

STEADY DECLINES in dry bean consumption in the U.S., and expansion of the ingredient market for convenience foods, foster development of instant bean ingredients. Tremendous growth has occurred in the ethnic foods market. Sales of Mexican-type foods are growing at rates exceeding 30% per year, and acceptance is moving from the Southwest to the Midwest and eastwards. Mexican foods rank "numero uno" in the West (39% of the ethnic food market), second to Italian in the South (30 and 36%, respectively) and third in the Midwest and the East, after Italian and Chinese foods (Wood, 1980). Good quality ingredients are required to sustain this growth (Jacobs, 1981).

Initial efforts to produce bean ingredients were aimed at keeping cell rupture at a minimum in order to retain the same texture, appearance and taste as conventionally prepared bean soups. Instant precooked bean powders have been prepared by soaking, cooking, slurring, and drum or spray drying (Bakker et al., 1973).

The second generation bean ingredients are based on fractionation of the two major components, starch and protein. Chang and Satterlee (1979) produced bean protein concentrates containing 72–81% protein by wet processing using water extraction techniques; Molina and Bressani (1973) prepared protein isolates containing about 90% protein, and starch products with almost 50% starch. Several disadvantages exist in using wet processing for fractionation and concentration: (1) significant energy is used in drying the final products; (2) waste by-products are produced which contain significant amounts of organic matter; and (3) yields are reduced through losses in wet by-product streams.

Enriched bean protein and starch fractions can be made by dry milling followed by air classification (Vose et al., 1976). Compartmentalization of protein in friable protein bodies, and starch in denser, less brittle granules, enables size reduction and fractionation of dry beans into a coarse fraction which contain most of the starch, and a fines fraction which contains most of the protein. Air classification has been applied to wheat (Stringfellow and Peplinski, 1964), oats (Cluskey et al., 1973), corn, sorghum and soy (Pfeifer et al., 1960), rice (Stringfellow et al., 1961) barley (Pomeranz et al., 1971), triticale (Stringfellow et al., 1976), and field peas and beans (Youngs, 1975).

It has been observed that, when raw legumes are ground without pretreatment, they develop undesirable odors and flavors which persist after cooking. Lipoxidases have been held responsible for the appearance of off-flavors by catalyzing formation of hydroperoxides from unsaturated fatty acids (Kon et al., 1970). The highest lipoxidase activity experienced in pulses and oilseeds occurs in soybeans. However, treatment with dry heat for 6–8 min at 104–105°C completely inactivates this enzyme (Smith and Circle, 1972).

Roasted Navy beans, prepared as reported by Aguilera et al. (1982), were used to develop four ingredients: whole bean flour, hull flour, a high-protein or fines fraction (HPF), and a high-starch or coarse fraction (HSF). Whole bean flour is of interest because of widespread use of beans as whole seeds in soups, as purees and potentially, in formulating composite flours. Current interest in new sources of dietary fiber prompted study of the hulls fraction for this application. Dehulled bean flour, with about 25% protein, is potentially fractionable into a high-starch portion similar in composition to cereal flours (10–12% protein), and a high-protein portion resembling defatted soy flour (around 50% protein) – both widely utilized food ingredients. This paper reports on processing, yields, composition of several bean ingredients, and on the fate of antinutritional factors.

MATERIALS & METHODS

Raw materials

Michigan prime handpicked Navy beans (*Phaseolus vulgaris*) from the Wolverine Bean Division, Bay City, MI, were roasted under eight different conditions in a particle-to-particle heat exchanger as described by Aguilera et al. (1982). Roasted product temperatures ranged from 92–125°C.

Processing

A flow diagram of the process is presented in Fig. 1. Roasted whole Navy beans, with moisture contents ranging from 7.5–9.4%, were cracked in a disc attrition mill (The Bauer Bros. Co., Springfield, OH) and hulls removed by aspiration. Hull flours were produced by grinding hulls in a swinging blade Model D6 Fitzmill (W.J. Fitzpatrick Co., Chicago, IL) using the impact edges for pulverization and a screen with 1/32 in (0.69 mm) round holes. Whole bean flours were prepared similarly.

Cracked cotyledons were finely ground in a Model 1602 Alpine Kolloplex stud impact mill (Alpine American Corp., Natick, MA) at 11,500 rpm. Flours were fractionated in a Model 100 MZR Alpine Multiplex air classifier at an air flow setting of 50 m^3/hr, classifying wheel speed of 5,000 rpm, and throughput rates of 4.5-7.0 kg/hr. After initial classification, the coarse fraction (C1) was remilled, and fractionated in the air classifier under identical conditions into a fines fraction (F2) and a coarse fraction (C2). Fines fractions from both classifications (F1 and F2) were combined to yield a high-protein flour; C2 became the high-starch flour. All

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products were stored in polyethylene bags or glass jars, and kept at 4°C until used.

Analyses

Moisture and ash contents were determined according to procedures described in AOAC (1975). Protein was analyzed by AACC (1976) Kjeldahl procedures, and crude fiber by method Ba 6-61, of AOCS (1978). Samples were evaluated for enzyme neutral detergent fiber (ENDF) by the method described by Robertson and Van Soest (1977). This method was modified to include 1 mg of amyloglucosidase for additional digestion of starch.

Trypsin inhibitor activity (TIA) was determined by the method of Smith et al. (1980), and results expressed in mg trypsin inhibited per g protein, instead of the arbitrary usual units of trypsin units inhibited per mg of sample; values reported are the means of three replicates.

For hemagglutinin analysis, a 4% red cell solution in saline was prepared using rabbit blood. Five grams of beans were ground to pass 100 mesh and were extracted under agitation with 50 ml of 1% NaCl solution. The extract was stored overnight at 4°C and centrifuged at 3020 x *g* for 10 min in a Sorvall refrigerated centrifuge. Several dilutions were then prepared and 1.0 ml each was mixed with 4.0 ml of blood cell solution, incubated in a 37°C bath for 1 hr, and observed for agglutination as compared to a control. The lowest dilution to show no signs of agglutination was used as a measure of hemagglutinin activity, and the result calculated by the formula.

$$HU/g = (D_b \times S)/V$$

where D_b = dilution factor, S = ml original extract/g flour (10) and V = volume of extract in test tube (1 ml).

A model TA Coulter Counter, manufactured by Coulter Electronics, Inc., (Hialeah, FL) was used for particle sizing analysis of fine fractions. The Coulter Counter sizes and counts particles, suspended in an electrolite solution (lithium chloride and methanol), by detecting change in resistance which results from current blockage as a particle passes through a small aperture (100 μm).

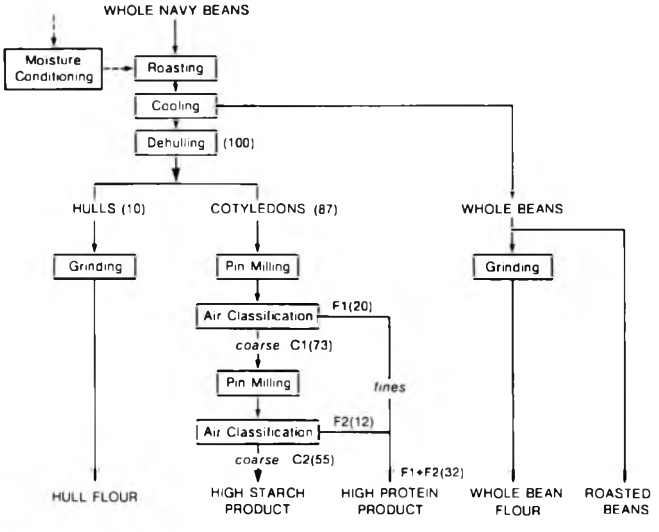

Fig. 1—Flow diagram for processing Navy beans. Figures in parentheses represent average values for a material balance.

RESULTS & DISCUSSION

A TYPICAL MATERIALS BALANCE for the process is presented in Fig. 1. One hundred parts of whole roasted beans typically yielded 7–13 parts of hull flour, 28–35 parts of high-protein flour, and 50–60 parts of high-starch flour.

Table 1 summarizes data on chemical composition of whole Navy bean flour, hull flour, and air classified fractions for the eight runs. Average values for whole navy bean flours were: moisture content, 8.8%; fat, 1.9%; protein 25.9%; ash, 4.6%; and dietary fiber, 7.6%. Flours had lower fat and higher ash contents than Navy bean flours prepared by Naivikul and D'Appolonia (1978), possibly due to genetic differences. Roasted flours had reduced antitryptic activity (22–92% of raw beans) and hemagglutinin activity (1–48% of the original). Detailed results of these findings, as well s some functinal properties, are reported elsewhere (Aguilera et al., 1982).

Variations in yield of hull flours were probably due to differences in loosening of hulls during roasting and to amounts of fines produced during cracking. Moisture averaged 8.5%; fat, 1.1%; protein, 14.9%; ash, 6.7%; crude fiber, 26.5% and dietary fiber, 40.4%. Contents of components other than moisture are probably affected more by efficiency of dehulling than by roasting itself. The high standard deviations for fat and protein contents may indicate large variations in the levels of cotyledon fines aspirated with the hulls. Hull flours contained 31.24–50.17% dietary fiber; that is, components not hydrolyzable by enzymes in the human digestive tract and not absorbable into the blood stream. About 60 percent of the dietary fiber was crude fiber, composed mainly of lignin and cellulose. Studies are now underway to evaluate functional characteristics of whole bean and hull flours, and their effect on food product properties such as appearance, flavor and texture.

Average composition of high-starch and high-protein fractions is presented also in Table 1. Protein content of HSF varied between 13.6 and 17.5%, averaging 15.6%. Extreme values correspond to the harshest and lightest roasting conditions. Apparently, roasting seems to improve separation of starch and protein, independent of the absolute level of protein in the HSF. Patel et al. (1980), using similar fractionation procedures but different equipment, were able to produce a HSF with 10.7% protein from Navy beans. Kon et al. (1977) studied effects of air velocity on protein shift in California small white bean flour, and found that higher velocities tend to improve separation of small starch granules from the light fraction; but the lowest protein content achieved was 15.3%.

Protein content of the HPF ranged between 39.7 and 47.6%, averaging 43.1%. Results are similar to those of Kon et al. (1977), who obtained fines fractions with 43.3–45.7% protein. These assays are lower, however, than those of Patel et al. (1980), who claimed to obtain 61.9% protein in their concentrate, starting fromflour with 30.4% protein, and those of Tyler et al. (1981), who reported combined protein fractions having 55.3 and 56.4% protein. Data in the literature suggest that air classification of bean flours is most efficient when the protein content of the HPF is

Table 1—Composition on Navy bean flour, hull flour and air classified fractions (dry basis) for eight runs[a]

Sample	Moisture			Fat			Protein			Ash			Crude B fiber			Dietary fiber[b]		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Whole flour	9.0–9.5	8.8	0.57	1.8–2.1	1.9	0.11	25.3–26.9	25.9	0.52	4.1–5.3	4.6	0.26	–	–	–	6.9–8.6	7.6	0.55
Hull flour	7.1–9.3	8.5	0.67	0.5–1.9	1.1	0.44	9.8–18.4	14.9	3.28	6.3–7.9	6.7	0.74	21.1–31.8	26.5	3.7	31.2–50.2	40.4	5.89
High-protein fraction	6.1–7.3	6.9	0.43	2.3–3.2	2.7	0.28	39.1–47.6	43.1	2.8	4.5–6.0	5.2	0.54	1.7–2.4	2.1	0.25	2.4–5.0	3.8	0.95
High-starch fraction	6.5–8.4	7.5	0.58	0.7–1.3	1.0	0.18	13.6–17.5	15.6	1.41	2.7–3.0	2.9	0.19	–	–	–	1.9–5.1	3.3	1.19

[a] The value for each run is an average of three determinations
[b] Moisture-free basis

double that of the original flour. Protein content of HPF was 1.5 to 1.8 times that of the original dehulled flour (26.8% protein).

Yields of fractions are fixed by relative proportions of starch and protein in the seed and by the cut size chosen for fractionation. Naivikul and D'Appolonia (1978) reported that dehulled Navy beans contain 51.6% starch and 24.7% protein on a moisture-free basis. Thus the ratio of starch to protein is very close to 2. Air-classified flours were produced by adjusting operational variables (air throughput and classifying wheel speed) until the target ratio of two parts of coarse to one part fines was obtained.

Regrinding and air classifying the coarse fraction obtained in the first pass (C1) was introduced by Vose et al. (1976) to lower the protein content and to reclaim more fines. Effects of this process can be followed in Table 2. C1 contained an average of 19.3% protein, or 28% less protein than the initial dehulled flour. The corresponding fines fraction (F1) was obtained in 1 to 3.5 ratio (w/w) to C1 (See Fig. 1), and contained up to 56.1% protein in one run. Protein content of the seven F1 fractions studied averaged 49.7% protein. F2, the fines fraction obtained in the second pass, had an average protein content of 37.1% and weighed about half of F1. Reclaiming fines from C1 reduced protein content of the final starch fraction to an average of 15.6%, but also decreased the protein content of the fines fraction (after F1 and F2 were combined) from 49.7% to 43.1%. Improvement of the fractionation procedure during the second pass is needed to attain high protein levels in the final protein fraction.

Information on the relationship between particle size and protein content of air-classified legume flours was not found in the literature. Experiences in air classification of wheat flour (Anonymous, 1961) showed that cuts with mean particle sizes larger than 45 μm had the same protein content as the original wheat flour (about 10.2%). Cuts with mean particles sizes between 18 and 45 μm had lower protein contents than original flour (between 4.5 and 6.2%), while cuts below 17 μm had increased protein contents (from 19 to 25% protein). Thus, a very sharp cut-off point exists in wheat at about 17 μm for optimum fractionation of starch and protein.

Particle diameters representing the 50% cumulative mass point were obtained from particle distribution curves for the eight runs and plotted against percent protein in the fines fraction. A regression line (r = −0.75), presented in Fig. 2, shows that finer flours having cumulative mass points at lower particle sizes contain more protein. From an operational standpoint, this means that finer grinding, followed by air classification at a lower cut point, is needed to achieve optimum protein shift.

Fractionation is also accompanied by partition of other components. Hulls present will concentrate in the starch

Table 2—Moisture and protein contents of intermediate fractions of air classified Navy bean flours[a]

	Fraction					
	F1		F2		C1	
Sample	Moisture	Protein[b]	Moisture	Probein[b]	Moisture	Protein[b]
Raw	10.0	43.3	10.0	35.4	11.0	18.9
3	8.4	50.7	7.9	37.6	9.3	19.6
4	7.7	56.1	7.7	36.2	9.1	17.9
5	7.4	53.4	7.4	38.7	7.9	18.2
6	7.0	44.2	7.2	33.8	7.7	19.2
7	7.6	50.7	8.0	42.4	9.4	19.9
8	7.7	42.5	8.0	35.7	9.1	21.3

[a] Average of three determinations
[b] Moisture-free basis

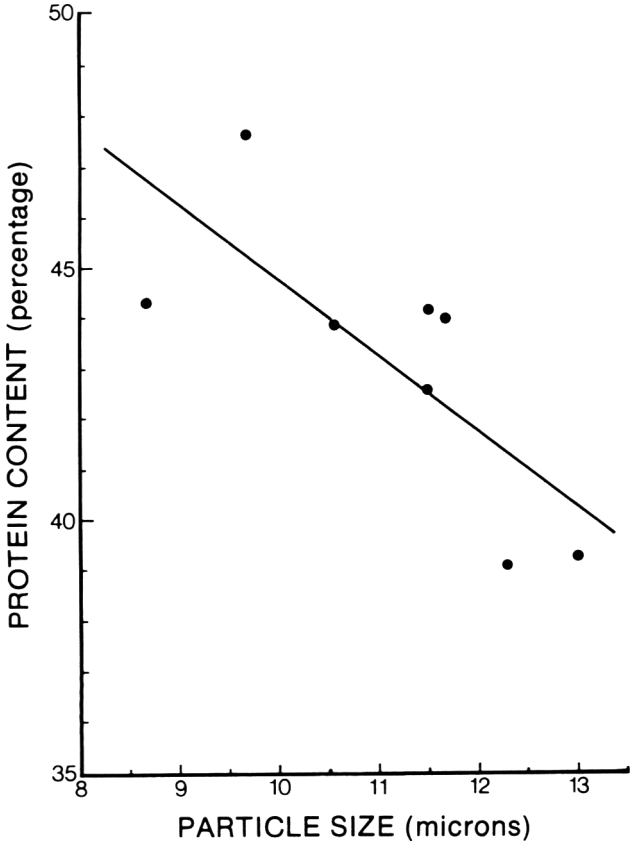

Fig. 2—Variation of protein content with particle size in high-protein fractions at 50% cumulative mass. Cumulative mass refers to the percent mass of particles larger than a certain particle size as determined from a particle size distribution curve.

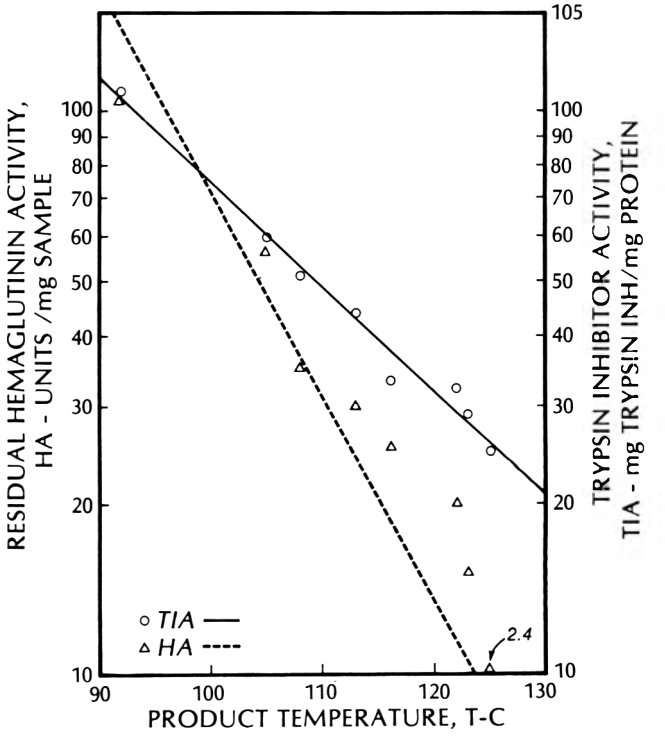

Fig. 3—Effect of roasting temperature on TIA and HA of high-protein fractions.

fraction due to their particle size. As seen in Table 1, fat tends to become more concentrated in the protein fraction; and so does bean flavor, according to Youngs (1975).

Since trypsin inhibitors and hemagglutinins are proteins, levels of these two antinutritional factors were determined only in the protein fraction and it was assumed that their activity in starch fractions was lower by a factor equal to the ratio of protein contents. Fig. 3 shows that highly significant linear correlation exists between TIA and residual HA, and roasting temperatures of the beans (r = 0.99 and 0.85, respectively). Residual activity of trypsin inhibitors ranged betwween 108 and 25 TIA/mg protein, down from the original 116 TIA/mg protein. Hemagglutinins were inactivated more rapidly than trypsin inhibitors. Residual activity of hemagglutinins varied from 105 to 2.4 HA units/mg sample (down from 110 HA units present in the raw fraction).

Roasted HSF and HPF are expected also to have longer shelf lives than raw fractions due to inactivation of thermolabile enzymes. The protein to starch ratio of HSF is expected to make this ingredient attractive for formulating snacks and gravies and as replacement for wheat flour. HPF is likely to find applications in extruded products and as a protein fortificant.

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SOY PROTEIN ISOLATES IN BREAD . . . From page 1150

All of the breads containing soy protein isolate were considered by the panel members to be inferior to their perceived ideal bread. However, panelists commented that the breads were acceptable, indicating the feasibility of their use when increasing dietary protein is an important consideration.

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Corn Distillers' Dried Grains with Solubles and Corn Distillers' Dried Grains: Dry Fractionation and Composition

Y. VICTOR WU and ARTHUR C. STRINGFELLOW

ABSTRACT

Corn distillers' dried grains with solubles (CDGS) and corn distillers' dried grains (CDG) at various moisture and fat contents were pin milled and screened. Fractions of CDGS and CDG contained from 11.4–45.7% and 12.5–49.6% protein, respectively (dry basis), compared with 30% for CDGS and 25% for CDG. The highest protein shifts were obtained when CDGS at initial moisture of 21% was ground twice at 14,000 rpm and CDG at initial moisture of 21% was ground once at 14,000 rpm. Fat, ash, dietary fiber contents, and amino acid composition of CDGS and CDG fractions were also determined.

INTRODUCTION

FERMENTATION of corn to make alcohol produces a protein-rich material (stillage) after alcohol is distilled. The starch in corn is consumed in the fermentation process, while other nutrients such as protein are concentrated as much as threefold. The stillage is fractionated by screening and/or centrifugation to yield distillers' grains and a solubles fraction. Most of the solubles fraction are combined with the grains, which then are dried and sold as corn distillers' dried grains with solubles (CDGS); but some corn distillers' dried grains (CDG) and corn distillers' dried solubles (CDS) also are marketed separately.

Feeding studies utilizing CDGS or CDG included beef cattle (Chen et al., 1977), dairy cattle (Loosli et al., 1961; Warner et al., 1957), calves (Schabinger and Knodt, 1948), swine (Wahlstrom et al., 1970; Thong et al., 1978), and chickens (Harms et al., 1969; Matterson et al., 1966). Satterlee et al. (1976) prepared protein concentrate from corn distillers' grains by extraction with alkali. Wu et al. (1981) have reported on the chemical composition and physical properties of the constituents of corn distillers' grains and solubles. This paper reports dry fractionation of CDGS and CDG by pin milling and sieving and presents the yield, protein, fat, ash, dietary fiber, and amino acid composition of the fractions.

MATERIALS & METHODS

CORN DISTILLERS' DRIED GRAINS with solubles and CDG were supplied by Hiram Walker & Sons, Inc. (Peoria, IL), and Brown-Forman Distillers Corp. (Louisville, KY), respectively. Each solid was adjusted to desired moisture levels by adding water and equilibrating overnight, by partially drying in an oven or was used as received. Some CDGS was defatted four times with hexane which lowered the fat content from 11.8 to 2.2% dry basis.

Corn distillers' dried grains with solubles and CDG were ground in an Alpine Model 160Z pin mill at 9,000, 14,000, or 18,000 rpm for 1–3 times (1X to 3X), and the resulting materials were sieved with 20, 35, 50, and 80 mesh screens. Some of the fractions that passed through the 80 mesh screen were further separated by 10XX, 13XX, and 17XX screens. The opening for the 20, 35, 50, 80 mesh, 10XX, 13XX, and 17XX screens is 841, 420, 289, 177, 130, 102, and 64 microns, respectively.

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Protein, fat, and ash determinations were described by procedure numbers 46-13, 30-26, and 08-16 of AACC Approved Methods (1971), respectively. Moisture was determined by heating (105°C) samples to constant weight. Protein content (nitrogen X 6.25) was calculated from the average of 4–6 micro-Kjeldahl nitrogen analyses. Fat and ash determinations were in duplicate. Dietary fiber was determined by the neutral detergent method (McQueen and Nicholson, 1979). Samples for amino acid analyses were hydrolyzed for 24 hr by refluxing in constant-boiling hydrochloric acid. A portion of the acid hydrolysate was analyzed on a Glenco MM-100 or a Beckman Spinco Model 121 amino acid analyzer, and the amino acid data were calculated with the aid of a computer (Cavins and Friedman, 1968). Tryptophan was determined by the method of Wooley and Sebrell (1945).

RESULTS & DISCUSSION

Milling yields

The particle size distribution after milling CDGS varies with different moisture levels and pin mill speeds (Table 1). As the initial moisture content of CDGS (before milling) is increased, larger particles resulted where pin mill is operated at the same speed. When the initial material was defatted before milling, the particle size decreases at both 1 X 9,000 and 1 X 14,000 rpm when compared with the full-fat CDGS (not shown in Table).

The milling yields of CDG at various moisture levels and speeds is shown in Table 2. Higher initial moisture content results in an increased particle size when pin-milling is conducted at the same speed compared with lower initial moisture content. The particle size decreases as the speed of the pin mill increases or as the sample is pin-milled more than once at the same speed.

Proximate analysis

Since composition data are important consideratons for any food or feed uses, a number of analyses are made. Protein, fat, ash, and neutral detergent fiber contents of

Table 1—Milling yields of corn distillers' dried grains with solubles at various moisture levels and speeds

% Moisture before milling	No. of passes through pin mill speed, rpm	Yield of mill fractions, % starting material dry basis mesh				
		on 20	20–35	35–50	50–80	thru 80
5.0	1 X 9,000	0.5	12.2	30.8	44.5	12.1
	1 X 14,000		2.2	8.5	56.9	32.5
9.8	1 X 9,000	8.1	30.8	22.4	31.6	7.1
	3 X 9,000		4.9	20.3	52.6	22.3
	1 X 14,000	3.8	22.2	16.4	38.8	18.8
	2 X 14,000		0.9	5.0	49.0	45.1
	1 X 18,000		2.8	9.9	44.4	42.9
15.0	1 X 14,000	23.9	25.1	18.9	23.0	9.0
	2 X 14,000		9.4	16.2	41.5	32.8
21.0	1 X 14,000	36.7	26.2	14.8	17.5	4.9
	2 X 14,000		41.4	17.3	26.3	15.0
31.0	2 X 14,000	18.5	23.2	13.2	20.8	24.3
	3 X 14,000		2.1	7.7	35.8	54.4

CDGS are 29.9, 11.8, 3.4, and 33.8% (dry basis), respectively. Protein contents of CDGS fractions increase with decreasing particle size and range from 11.4–45.7%, Table 3. The ash contents of CDGS fractions (not shown in Table) range from 3.2–5.6%. In general, ash content increases as particle size decreases. Fat contents of CDGS fractions (not defatted) are from 7.0–19.3%. The finest fraction (through 80 mesh) has the highest fat content. Neutral detergent fiber contents of fractions from 9.8% initial moisture and 1 X 14,000 rpm are 52.1, 48.0, 43.7, 34.6, and 27.6% for on 20, 20–35, 35–50, 50–80, and through 80 mesh fractions, respectively. The corresponding values from 21% moisture and 2 X 14,000 rpm are 41.5, 31.7, 23.7, and 21.2 for on 35, 35–50, 50–80, and thru 80 fractions, respectively. Neutral detergent fiber value decreases with decreasing particle size and is respectively lower at higher moisture. Moisture contents of CDGS fractions after milling and screening range from 7.1 to 13.6%. Fractions from initial high-moisture CDGS lose moisture, but those from initial low-moisture CDGS gain moisture after milling and screening.

Table 2–Milling yields of corn distillers' dried grains[a] at various moisture levels and speeds

% Moisture before milling	No. of passes through pin mill speed, rpm	Yield of mill fractions, % starting material dry basis mesh				
		on 20	20–35	35–50	50–80	thru 80
6.1	1 X 14,000		8.9	11.7	41.8	37.6
	3 X 14,000		7.3	5.4	46.8	40.5
	1 X 18,000		4.8	4.2	26.7	64.2
	2 X 18,000		3.0	3.1	18.5	75.4
21.0	1X 14,000	16.9	34.1	22.0	24.3	2.8
	2 X 14,000	2.1	12.1	16.0	43.0	26.8
31.0	1 X 14,000	21.2	35.6	21.6	18.5	3.1
	2 X 14,000	3.9	24.1	27.6	35.0	9.4

[a] Without solubles

Table 3–Protein content of mill fractions of corn distillers' dried grains with solubles obtained at various moisture levels and speeds

% Moisture before milling	No. of passes through pin mill speed, rpm	Protein content (N X 6.25), dry basis					Protein shift, %
		on 20	20–35	35–50	50–80	thru 80	
5.0	1 X 9,000	15.6	26.9	27.3	31.1	32.6	7
	1 X 14,000		27.0	29.3	30.3	32.2	4
9.8	1 X 9,000	20.2	25.4	28.9	32.9	37.1	13
	3 X 9,000		18.7	22.3	30.1	34.5	11
	1 X 14,000	13.4	17.1	24.5	33.4	38.3	24
	2 X 14,000		13.6	20.0	27.3	36.1	16
	1 X 18,000		17.7	17.9	25.8	37.4	22
15.0	1 X 14,000	12.3	22.6	33.9	41.8	41.9	36
	2 X 14,000		13.6	16.8	28.3	38.7	24
21.0	1 X 14,000	14.3	30.2	40.4	43.6	45.7	35
	2 X 14,000		13.4	14.6	41.8	45.0	50
31.0	2 X 14,000	11.4	19.8	27.6	39.1	41.3	36
	3 X 14,000		13.3	12.3	18.3	36.6	32

Table 4–Protein content of mill fractions of corn distillers' dried grains[a] obtained at various moisture levels and speeds

% Moisture before milling	No. of passes through pin mill speed, rpm	Protein content (N X 6.25), dry basis					Protein shift, %
		on 20	20–35	35–50	50–80	thru 80	
6.1	1 X 14,000		19.4	15.6	21.5	34.9	27
	3 X 14,000		19.3	19.1	17.3	33.6	31
	1 X 18,000		19.9	17.4	19.6	31.7	25
	2 X 18,000		18.1	19.7	17.9	27.4	13
21.0	1 X 14.000	12.9	17.3	28.3	40.3	49.6	39
	2 X 14,000	15.4	15.6	14.0	22.6	36.0	29
31.0	1 X 14,000	12.5	19.3	31.0	39.8	40.6	37
	2 X 14,000	15.6	13.4	14.1	26.9	43.3	34

[a] Without solubles

Corn distillers' grains has 25.3% protein, 10.8% fat, 1.6% ash, and 56.7% neutral detergent fiber on dry basis. Protein contents of CDG fractions range from 12.5–49.6% (Table 4). In general the protein content increases as particle size decreases for each pin mill speed. Fat contents of CDG fractions (not shown in Table) are between 6.0 and 12.9% and the through 80 mesh fraction has the highest fat content. Ash contents of CDG range from 1.3–2.7% and do not show any pattern with change in particle size. The ash content of CDG is considerably lower than that of CDGS. Neutral detergent fiber content of fractions from 6.1% initial moisture and 3 X 14,000 rpm are 59.6, 62.7, 69.4, and 49.7% for on 35, 35–50, 50–80, and through 80 mesh fractions, respectively. The corresponding values from 21% initial moisture and 1 X 14,000 rpm are 72.5, 59.9, 53.4, 35.1 and 26.6% for on 20, 20–35, 35–50, 50–80, and through 80 mesh fractions, respectively. Neutral detergent fiber values decrease with particle size for 21% moisture and 1 X 14,000 rpm, and they are higher than those of CDGS. Fractions from milling CDG of 6.1% initial moisture have 4.8 to 7.8% moisture after milling, whereas those from 21 and 31% initial moisture loss considerable water after milling and screening.

Protein shift

Protein shift is a calculated value to compare protein displacement and equals the sum of the amount of protein shifted into the high-protein fractions and out of the low-protein fractions as a percentage of the total protein present in the starting material (Gracza, 1959). Protein shift values of CDGS range from 4–50% (Table 3). This large difference emphasizes the importance of initial moisture content and speed of the pin mill. Decreasing the moisture content from original value of 9.8% to 5% decreases protein shift. Defatting CDGS before milling either has no effect or decreases protein shift (not shown in Table). Increasing initial moisture of CDGS from 9.8 to 15% increases protein shift. When initial moisture is increased from 15 to 21% for CDGS, there is a large increase in protein shift for 2 X 14,000 rpm from 24 to 50%, although there is no effect at 1 X 14,000 rpm. Protein shift reaches a maximum at 21% initial moisture content at 2 X 14,000 rpm; as a lower protein shift value is observed at 31% initial moisture content at 2 X 14,000 rpm.

Since protein shift for CDGS is dependent on moisture content, remoistening of the material after each grind to restore the initial moisture was tried. When CDGS was ground at 14,000 rpm and the moisture was adjusted to 31% before each grind, protein shift increased 3 and 8% for 2 X 14,000 and 3 X 14,000 rpm, respectively, compared with no remoistening between grinds. However, the protein shift for 2 X 14,000 rpm with remoistening to 21% moisture before second grind was not improved compared with no remoistening before second grind.

Protein shift value of CDG (Table 4) ranges from 13–31% for 6.1% initial moisture, compared with 4–7% at 5% initial moisture and 8–24% for 9.8% initial moisture for CDGS (Table 3). As the initial moisture content of CDG increases, protein shift value also increases and reaches a maximum of 39% for 1 X 14,000 rpm and initial moisture of 21%. The effect of initial moisture content and speed of pin mill on protein shift is less for CDG than CDGS.

Table 5—Yields and proximate analysis of corn distillers' dried grains with solubles (CDGS) and corn distillers' dried grains (CDG) by sieving the thru 80 mesh fraction

Sample	% Moisture before milling	No. of passes through pin mill speed, rpm	Fraction	% Dry basis		
				Yield, % starting material	Protein N X 6.25	Neutral detergent fiber
CDGS	9.8	3 X 14,000	8XX-10XX	12.3	33.7	
			10XX-13XX	10.4	35.8	
			13XX-17XX	13.8	35.3	
			thru 17XX	7.0	32.8	
CDG	6.1	3 X 14,000	8XX-10XX	9.2	35.3	53.8
			10XX-13XX	9.2	34.8	52.9
			13XX-17XX	13.6	33.6	46.9
			thru 17XX	8.5	30.3	46.4
CDGS	9.8[a]	1 X 14,000	8XX-10XX	22.1	34.5	
			10XX-13XX	17.4	35.7	
			13XX-17XX	17.2	35.0	
			thru 17XX	4.3	35.8	

[a] Corn distillers' dried grains with solubles was defatted with hexane to 2.2% fat, dry basis, before milling.

Table 6—Essential amino acid composition of corn distillers' dried grains with solubles (CDGS), corn distillers' dried grains (CDG) and their milled fractions (g/16g nitrogen recovered)

Amino Acid	CDGS, 21% moisture, 2 X 14,000					CDG, 21% moisture 1 X 14,000						NAS[a] (1980)
	CDGS	mesh				CDG	mesh					
		on 35	35–50	50–80	thru 80		on 20	20–35	35–50	50–80	thru 80	
Isoleucine	3.8	3.8	3.9	4.1	4.2	4.1	3.8	3.9	4.1	4.0	4.1	4.2
Leucine	12.2	10.4	12.3	13.4	13.6	12.3	7.9	8.5	11.5	12.4	13.5	7.0
Lysine	3.0	3.9	3.2	2.7	2.8	3.9	5.7	5.5	4.0	2.9	2.7	5.1
Methionine + cystine	3.3	2.4	3.3	3.5	3.6	4.1	2.7	2.8	3.8	3.9	3.9	2.6
Phenylalanine + tyrosine	9.5	9.1	10.3	10.7	11.0	9.6	8.4	8.5	10.1	10.5	10.8	7.3
Threonine	3.9	4.3	4.0	3.8	3.9	4.0	4.5	4.3	4.0	3.8	3.6	3.5
Tryptophan	0.4	0.9	0.6	0.3	0.4	0.5	0.9	0.7	0.5	0.4	0.4	1.1
Valine	5.1	5.4	5.3	5.4	5.3	5.6	5.9	5.9	6.0	5.5	5.3	4.8

[a] The National Academy of Sciences (1980) values for high-quality protein for human consumption.

Effect of sieving the through 80 mesh fraction

Since through 80 mesh fraction from both CDGS and CDG has the highest protein content for any initial moisture and pin mill speed, this fraction was further separated into four fractions to see if a further increase in protein can be obtained (Table 5). The spread in protein content for defatted CDGS is only 1.3%. The lowest protein content for both CDGS and CDG from 3 X 14,000 rpm is observed for the fraction with the smallest particle size (through 17XX), and the spread in protein content of the four fractions is 3 and 5% respectively. The respective spread in ash and moisture contents of the four fractions for each speed is less than 0.3 and 1.3% (not shown in Table 5). The fat content increases from 10 to 18% for CDGS and 8 to 15% for CDG as particle size decreases. Decrease of 7% in neutral detergent fiber content is seen as particle size decreases. Thus, there seems to be no advantage in sieving the through 80 mesh fractions further, at least as far as protein content is concerned.

Amino acid composition

The essential amino acid compositions of CDGS, CDG, and their milled fractions are listed in Table 6. Since both CDGS and CDG are primarily corn with only a small contribution from yeast, the amino acid compositions of CDGS and CDG are in general similar to that of corn (Wu and Sexson, 1976). Both CDGS and CDG meet or exceed the National Academy of Sciences (1980) pattern for high-quality protein for human consumption except for lysine and tryptophan.

Amino acids content of the fractions do not differ greatly from that contained in CDGS or CDG. There is an increase in leucine, methionine + cystine, and phenylalanine + tyrosine but a decrease in lysine and tryptophan with decreasing particle size for both CDGS and CDG. Both CDGS and CDG fractions meet or exceed the National Academy of Sciences (1980) pattern for high-quality protein except for lysine and tryptophan.

CONCLUSION

CORN DISTILLERS DRIED GRAINS with solubles at 21% initial moisture can be ground twice at 14,000 rpm and separated with a 50 mesh screen to obtain a high-protein fraction with 43% protein content in 41% yield compared with 30% protein for the parent CDGS. For CDG the best result is observed at 21% initial moisture and 1 X 14,000 rpm where protein content ranges from 13–50% compared with 25% for the starting CDG. Corn protein has a tendency to attract fat because of its hydrophobic nature. This relation was observed in CDGS and CDG fractions since those with highest protein content also were highest in fat. Fiber is more resistant to grinding than other components for both CDGS and CDG, therefore, fractions with larger particle size were observed to have

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Effects of Acidified Processing and Storage on Proteins and Lipids in Mung Bean Sprouts

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ABSTRACT

Fresh mung bean sprouts contained 5.3g protein/100g fresh weight which decreased slightly on blanching and on bottling and canning. Storage of bottled seedlings for 6 months at 35°C caused the largest loss in total protein. The protein contained the essential amino acids tryptophan, valine, leucine, phenylalanine, arginine, lysine and histidine. Total lipids of fresh mung bean sprouts were 350 mg/100g fresh weight and were not greatly affected by blanching nor by canning or bottling. When the bottled seedlings were stored at 10°C and at RT, the canned seedlings at RT, there was some effect on total lipids in that only bottled seedlings at 35°C showed a significant decrease in lipids. After storage at the three temperatures there were increases in glycolipids and decreases in phospholipids, due mainly to increases in monogalactosyldiglyceride and decreases in phosphatidic acid respectively.

INTRODUCTION

THE CHINESE developed the sprouting of mung beans centuries ago and sprouting mung beans are now one of the principal ingredients in chop suey, chow mein and other Chinese dishes. With the growing popularity of Chinese food many western countries, including the U.S.A., are becoming familiar with mung beans which have been shown to contain valuable food nutrients (Fordham et al. 1975; Kylen and McCready, 1975). Farhangi and Valadon (1981) have shown that the sprouts contain varying amounts of vitamins A and C and that although during blanching some of these vitamins are lost, there are still some left after acidified processing and storage for 6 months. One of the reasons for studying the effect of acidified processing on these sprouts is that since they are perishable, most of those produced in the United States are processed and seldom are fresh shoots found in the retail market (Hsuen et al., 1972).

Mung beans are known to contain trypsin inhibitors which can be inactivated by heating and this causes an improvement in protein utilization (Wolf and Cowan, 1975). However, other activities e.g. Maillard reactions, also take place which may reduce the amount of available protein during processing and storage. The object of this study was to investigate mung bean sprouts as a cheap source of proteins and amino acids, and the effect of blanching, processing (canning and bottling) and storage on these very important food constituents. Furthermore, heat treatment (blanching) and the storage conditions of processed vegetables are known to influence other nutritive compounds as well, e.g. lipids. Very little is known concerning the fate of small amounts of lipids in a vegetable such as the beansprout. It would therefore be most informative to ascertain what happens to such small amounts during processing and storage. This is discussed in the present study.

EXPERIMENTAL

GERMINATION, blanching, bottling in glass jars and canning were exactly as described previously (Farhangi and Valadon, 1981).

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Storage

The bottled sprouts were stored for 6 months at three different temperatures: 10°C, room temperature (RT) of approximately 25°C, and 35°C.

The canned sprouts were stored for the same period of time at RT only.

Protein extraction and determination

The sprouts were incubated with 10 ml N NaOH for 12 hr at 20°C (Fraser and Loening, 1974). The solution was centrifuged at 20,000 rpm for 20 min at −5°C and 3M trichloroacetic acid (TCA) was added to the supernatant to precipitate the proteins which were then estimated by the Folin-phenol reagent of Lowry et al. (1957). The results were expressed as g/100g fresh weight.

Determination of amino acids

Free amino acids and total amino acid composition were extracted with modifications of methods used by Russell (1944) and Naguib (1964).

Free amino acids

The bean sprouts were dried at 70–80°C overnight and ground to a fine powder, some of which was also used for determining tryptophan. To 50 mg of this powder was added 5 ml of 2% phenol and 10 ml of 30% TCA, the mixture was left overnight and then filtered through filter paper which then contained protein. The pH of the solution containing free amino acids was adjusted to 2 ± 0.1 with NaOH.

Protein amino acids

The filter paper containing the precipitate was kept at 50°C overnight. Five mg of the dried residue was collected to which 5 ml of 10N HCl was added. This was hydrolyzed in a boiling water bath for 5 hr, filtered and the pH of the solution adjusted to 2 ± 0.1 with 5N $NaOH_4$. This then contained acid-stable protein amino acids. Tryptophan, which is labile under acid hydrolysis, requires a separate method of analysis.

Tryptophan estimation

An aliquot of the fine powder (see under free amino acids) containing approximately 4 mg tryptophan was weighed accurately and 8.4g barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$) added. Sixteen ml of boiled water was then added and the whole heated at 120°C for 8 hr. This was cooled, acidified with conc HCl to pH 3–4, diluted to 50 ml and the solution filtered through filter paper (Osborne and Voogt, 1978).

The separation and identification of the amino acids were carried out on a Joel Model JLC 6AH fully automatic amino acid analyser. The amount of amino acid in each sample was calculated by comparison of peak areas with those obtained using a calibration mixture as described by Eveleigh and Winter (1960). The results are expressed as mg/100g protein.

Lipid extraction

The seedlings were macerated in hot methanol for 2–3 min to inactivate phospholipases, extracted further with chloroform: methanol (2:1 v/v) and then washed with 0.2% of its volume of 0.73% NaCl according to Folch et al. (1957). Total lipids were stored at −20°C in benezene: amyl alcohol:chloroform (1:1:1 v/v) with 0.01% butylated hydroxytoluene (BHT) added to prevent oxidation (Deven and Manocha, 1975).

Separation of lipid classes

Lipid classes were separated on thin-layer chromatography (TLC) on 0.25 mm Polygram silica gel G/UV 254 (Macherey-Nagel and Company, Düren, Germany).

For the separation of simple lipids, plates were developed in the solvent system of hexane:diethylether:formic acid (80:20:2 v/v) when the complex lipids stayed at the origin.

Complex lipids were separated by the use of (1) two-step single-dimensional TLC using petroleum ether:acetone (3:1 v/v) as the first solvent which removed the faster-moving simple lipids, and chloroform:methanol:acetic acid:water (170:25:25:6 v/v) which actually separated the complex lipids, and (2) two-dimensional TLC using chloroform:methanol:7N ammonium hydroxide (65:30:6 v/v) in the first run and chloroform:methanol:acetic acid:water (170:25:25:6 v/v) in the second run.

Identification of lipid classes

Lipid classes were identified by their migration characteristics relative to authentic standards that were chromatographed simultaneously alongside the samples under investigation or cochromatographed with them. Lipid spots were detected by specific spray reagents (Christie, 1973)–ninhydrin for amino phosphatides, molybdenum-blue sulphuric acid for phosphatides (Dittmer and Lester, 1964), acid ferric chloride for sterols and their esters, α-naphthol for glycosides and iodine vapour for neutral lipids.

For quantitative determination of lipid classes, the developed TLC plates were sprayed with 3% cupric acetate in 8% phosphoric acid heated at 180°C for 25 min (Fewster et al., 1949) and the resulting dark color estimated by using a Joyce-Loebl Chromoscan densitometer (Gasbarro, 1972). Results with the densitometer scans were generally reliable as they compared favourably with those of the weighing method.

Total fatty acids and free fatty acids

To obtain the fatty acids of total lipids, the latter were hydrolyzed for 2 hr under reflux in a solution of 5% KOH in methanol containing 20% benzene and 2% water. The alkaline solution was then acidified with dilute H_2SO_4 and extracted with diethylether to yield total fatty acids (Asselineau and Montrozier, 1976). Free fatty acids were extracted by the method of Draper (1969). Fatty acid methyl esters were prepared by using BF_3-methanol reagent according to Metcalfe and Schmitz (1961).

Sterol analysis

The total lipid fraction was saponified at room temperature in 12% KOH in absolute EtOH under N_2 for 20 hr. Steroids were extracted with ether after dilution with water. The ether layer was washed with water to remove the alkali, dried with Na_2SO_4, evaporated to dryness and weighed. This extract containing sterols was then dissolved in a small volume of $CHCl_3$:MeOH (2:1 v/v) and analyzed.

Gas-liquid chromatography (GLC) of fatty acid methyl esters and of sterols

The fatty acid methyl esters were analyzed using a PYE gas chromatograph. The 1.5m (i.d. 4 mm) glass column was packed with 10% PEGA on Chromosorb WAW DCMS 60-80 mesh (Pye-Unicam). The column temperature was programmed for 75–180°C (8°C min^{-1}). N_2 flow was 30 ml min^{-1}. Methyl esters and sterols were identified by comparing their retention times with those of authentic standards and by combined GC-MS and quantified by the peak area method.

Table 1–Total protein (g/100g) of fresh, blanched, canned and bottled mung bean seedlings, and protein percentage retention compared to fresh seedlings over a 6-month storage period under various temperature conditions

Seedlings	g/100g	% Retention
Fresh	5.3 ± 0.1	100
Blanched	5.0 ± 0.2	94
Immediately after bottling	4.8 ± 0.2	90
Bottled 10°C	2.4 ± 0.1	45
Bottled RT	2.0 ± 0.1	38
Bottled 35°C	1.5 ± 0.2	28
Immediately after canning	4.6 ± 0.3	87
Canned RT	1.9 ± 0.1	36

GC-MS of fatty acid methyl esters

The apparatus used was a Kratos MS25 mass spectrometer interfaced to a Perkin Elmer Sigma 3 gas chromatograph. Mass spectral data were obtained on a Kratos DS-50S computer data system. The instrument was operated with an ionizing current of 100 μA at 70 eV electron energy in Electron Impact mode, with a source temperature of 250°C, and the GC interface (all-glass jet separator) at 250–270°C. The 1.5m (i.d. 4 mm) glass column used was filled with 10% PEGA on Chromosorb WAW DCMS 60-80 mesh (Pye-Unicam), with the carrier gas helium at 30 ml min^{-1}.

All the experiments were repeated several times and the results are the average ± s.d. of at least three determinations.

RESULTS & DISCUSSION

Effect of blanching on proteins

Blanched seedlings of mung beans lost about 6% protein compared with fresh ones which contained 5.3 g/100g fresh weight (Table 1). This result agrees very well with those of Adam et al. (1942) who have reported a slight loss of protein (average 4%) after water-blanching in a wide range of vegetables, which could be due to hydrolysis of protein into free amino acids that may couple with carbohydrates, especially reducing sugars, to form brown pigments. However, heating during the blanching process tended to make the protein more susceptible to hydrolysis during further processing.

Effect of blanching on amino acids

Blanched seedlings of mung beans had lost about 33% free amino acids compared with fresh ones which contained 135.8 mg/100g protein (Table 2). Eighteen amino acids were identified in fresh bean seedlings and they all decreased after blanching, with glycine disappearing totally (Table 2). Blanching therefore has a marked effect on individual free amino acids from the extremely labile glycine to histidine which was retained the most (95%).

The total amino acids from hydrolyzed protein of blanched seedlings were 99.1 g/100g protein compared to 99.7g of fresh seedlings (Table 3). So, very little protein amino acid was lost, if at all, and this was reflected in individual amino acids where no significant loss was observed except possibly for cystine. These results are rather different to those of Kulesza and Gertig (1976) who showed that 92% total amino acids were retained after blanching, with glycine the most susceptible amino acid.

Our results showed that the loss of free amino acids was greater than that from hydrolyzed protein, i.e. there may be more leaching of free amino acids than of protein into the blanching water. On the other hand, since the blanching water was not investigated, the loss of free amino acids could be accounted for by their involvement in the first stage of the Maillard reaction (Bender, 1978).

Effect of bottling and canning on total proteins

Immediately after the sprouts were bottled and canned, the concentrations of total proteins were not very different from those of blanched sprouts (Table 1).

Effect of bottling and canning on amino acids

Immediately after the sprouts were bottled and canned, the concentrations of free amino acids decreased much more in cans (41% retention) than in bottles (56% retention) compared to those of fresh sprouts (Table 2). Individual amino acids all decreased, some more than others. Immediately after canning more severe decreases were observed in the following: alanine, valine, arginine and leucine which were retained less than 41% of the corresponding amino acids in fresh seedlings. The same four amino acids together

with tyrosine and phenylalanine decreased significantly immediately after canning. Histidine was less labile as 95% was retained after canning and bottling.

Total protein amino acids decreased to 90% and 87% immediately after bottling and canning respectively (Table 3). Although there was a decrease compared to fresh seedlings this was not as marked as with the free amino acids. Individually too there were decreases in certain amino acids but not in all: canning and bottling did not have any effect on threonine but argnine was lost after canning. On the other hand, aspartic acid and alanine showed the most decrease after processing, while there were increases in proline.

Effect of storage on protein

There were actual overall losses in total proteins of bottled and canned seedlings, ranging from 55% to 72% under the different temperature conditions (10°C, RT and 35°C for bottled seedlings, and RT for canned seedlings) over the 6-month period (Table 1). The percentage retention of protein in bottled and canned mung bean seedlings is highly dependent on the storage temperature regardless of the container. Compared to fresh seedlings there was an overall loss of 55% in bottled seedlings stored at 10°C, increasing to approximately 63% at RT (both for bottled and canned seedlings), and to a very high 70% at 35°C (Table 1). Under the conditions of bottling and canning and of storage, brown discoloration occurred due to the Maillard reaction and the largest effect was observed in the bottled seedlings stored at 35°C for 6 months. The Maillard reaction involves condensation between the amino groups of amino acids (either in the free form or in protein) with glycosidic and with reducing sugars (Bender, 1978). The results obtained here therefore agree with the suggestion that the Maillard reaction is positively dependent on high temperatures (Meyer, 1969; Priestley, 1979).

Effect of storage on free amino acids

The total free amino acids of bottled seedlings stored for 6 months at 10°C were 41 mg/100g protein, for bottled and for canned at RT approximately 31 mg/100g (Table 2), retaining 31%, 23% and 23% respectively compared to fresh seedlings. The absence of free isoleucine suggests that it may have been converted into leucine during storage, while the least retained free amino acid of processed seedlings was threonine, closely followed by proline and by tryosine. There was a considerable loss in lysine, histidine, arginine, valine and phenylalanine but not as much as in the previous three. The free amino acid most retained in bottles stored at 10°C and at RT, and in cans at RT was aspartic acid (approximately 61% respectively in all three cases), while for bottles stored at 35°C, aspartic acid and methionine were the most retained compared to those of fresh seedlings.

Effect of storage on protein amino acids

The total amino acids of hydrolyzed protein on a per 100g protein basis for bottles stored for 6 months at 10°C, RT and at 35°C, and cans at RT, were approximately 98% (Table 3).

Serine was the least retained amino acid under all four conditions of storage followed very closely by aspartic acid, alanine, cystine and leucine. The most retained amino acids on the other hand were glutamic acid, proline and valine which appeared to be increasing on a percentage basis (Table 3). It should be noted that as the temperature of storage increased from 10°C, through RT to 35°C, the destruction rate of free amino acids and protein amino acids increased regardless of storage containers. Overall there was very little difference in amino acid composition between seedlings stored in cans or in jars at RT.

When the bottles and cans were stored for 6 months at different temperatures proteins decreased. Contrary to expectations decreases in protein also resulted in decreases in amino acids. An explanation could be that amino acids obtained from the breakdown of protein were free to react with reducing sugars through the Maillard reaction or by other reactions, including hydroperoxides formed with unsaturated fat (Carpenter and Booth, 1973). The reaction between oxidized fat and protein is temperature-dependent and may explain why processed seedlings stored at 35°C lost the most protein.

Although there are 18 amino acids in the free form and in proteins of mung bean shoots, 16 appeared in both, while methionine (an essential amino acid) present in free

Table 2—Free amino acids contained in fresh, blanched, bottled and canned mung bean seedlings as mg/100g protein, and bottled and canned seedlings stored at different temperatures over a 6-month period

Amino acid	Fresh	Blanched	Immediately after Bottling	Immediately after Canning	Bottled 10°C	Bottled RT	Canned RT	Bottled 35°C
Lysine	5.7	4.0	3.9	3.5	1.0	0.8	0.8	0.6
Histidine	1.9	1.8	1.8	1.8	0.7	0.4	0.4	0.2
Ammonia	17.0	10.6	8.9	8.4	8.2	8.0	8.0	4.0
Arginine	9.4	6.0	3.8	3.1	3.0	1.4	1.4	1.2
Aspartic acid	13.2	10.0	7.9	8.0	8.2	8.0	8.0	4.0
Threonine	9.4	8.0	7.4	4.8	0.8	0.2	0.2	0.2
Serine	11.3	8.0	6.8	5.9	2.4	2.0	2.0	1.6
Glutamic acid	13.2	12.0	10.2	7.8	7.0	4.0	4.1	2.0
Proline	5.7	4.0	3.8	3.0	0.8	0.4	0.2	0.2
Glycine	1.9	T	0.0	0.0	0.0	0.0	0.0	0.0
Alanine	5.7	2.0	1.9	1.7	1.4	1.4	1.4	1.0
Methionine	1.3	0.9	0.6	0.6	0.4	0.4	0.4	0.4
Valine	13.2	8.0	4.9	2.4	2.2	1.4	1.2	1.2
Isoleucine	9.4	6.0	5.3	0.0	0.0	0.0	0.0	0.0
Leucine	7.5	4.0	3.0	1.8	2.2	1.6	1.6	1.2
Tyrosine	3.8	2.0	1.8	1.1	1.2	0.4	0.4	0.2
Phenylalanine	5.7	4.0	4.0	1.8	1.4	0.6	0.6	0.6
Tryptophan	0.5	0.4	0.4	0.4	0.3	0.2	0.2	0.1
Total (mg/100g protein)	135.8 ± 3.1	91.3 ± 1.8	76.6 ± 2.0	56.1 ± 1.6	41.2 ± 0.7	31.2 ± 1.6	30.9 ± 1.1	18.7 ± 0.8

T = Trace

amino acids was replaced by cystine in the protein amino acids. Over the range of experiments carried out it can be seen that certain amino acids were more labile than others, e.g. glycine, alanine, valine and leucine, the last three being members of the pyruvic acid family (Lapedes, 1977). The protein of mung bean contains the essential amino acid tryptophan, which is also present in the free form, while methionine, another essential amino acid, although not present as protein amino acid, is found in the free form.

Finally, all essential amino acids from hydrolyzed protein were affected during storage, some more than others. During storage at RT the containers had very little effect on the essential amino acids present. Further, apart from phenylalanine and tryosine which had decreased the most compared to processed seedlings stored at 10°C, the other essential amino acids did not decrease so much. Storing processed seedlings for six months at RT is therefore only slightly detrimental to the essential amino acids compared to those stored at 10°C, and much better than those stored at 35°C. Because of the cost of storing at the lower temperature it may be more economical to store the processed seedlings at RT.

The second part of the paper deals with the fate of small amounts of lipids (0.35%) in beansprouts after heat treatment. Ghanem and Hassan (1970) have reported no significant changes of total lipids for a variety of pickled vegetables and Fricker et al. (1975) have shown an increase of monogalactosyl-diglyceride compared to digalactosyl-diglyceride in processed spinach.

In the present study there were no significant changes in lipids during blanching or immediately after bottling and canning of mung bean seedlings and these will not be discussed further. However, there were certain differences in lipids due to the effect of storage of bottled and canned seedlings under the same conditions reported previously.

Effect of storage on total lipids

The total lipids of fresh mung bean seedlings were 350 mg/100 fresh weight and did not change after blanching and after bottling and canning (Table 4). These results are in agreement with those of Ghanem and Hassan (1970). After storage for 6 months at 10°C and at RT in jars and at RT in cans, total lipids did not differ significantly from those of fresh seedlings. However, at the highest storage temperature of 35°C there was a decrease in total lipids of about 30%.

Effect of storage on simple lipids

The simple lipids identified in fresh mung bean seedlings were mono-, di- and tri-glycerides, free fatty acids, fatty acid esters and the sterols, stigmasterol and β-sitosterol and their esters (Table 4).

The percentage of monoglycerides with respect to total simple lipids in fresh seedlings was 7% and it increased under all conditions tested (Table 4). In the case of the diglyceride, its percentage of total neutral lipids in fresh seedlings was 7% and there were very similar increases under three conditions of storage, and a decrease only in bottled seedlings at 35°C. Fresh seedlings contained triglycerides 9% total simple lipids which did not vary at all under three conditions of storage while in jars at 35°C there was a definite decrease to 5%.

The percentage of free fatty acids of total simple lipids was 15% in fresh seedlings while it increased significantly to 28% in both jars at 10°C and cans at RT, and to 33% in jars at RT, the greatest increase to 51% being in jars stored at 35°C. On the other hand, fatty acid esters were found in smaller amounts, about 1% under all conditions tested and in the controls.

There was 36% β-sitosterol of simple lipids in fresh seedlings which decreased to approximately 13% under all four conditions. Stigmasterol made up only 10% of total simple lipids in fresh seedlings. This decreased to a similar value (approximately 6%) under three of the conditions used, and again the greatest decrease (3%) was observed for jars at 35°C.

Effect of storage on complex lipids

Phospholipids made up 77% of total complex lipids in fresh seedlings which decreased under all storage conditions. Jars stored for 6 months at 10°C and cans at RT contained very similar decreased amounts of this group of complex lipids (67%), whereas both jars at RT and 35°C contained much smaller amounts of approximately 60%.

The main phospholipid of fresh seedlings was phosphatidylcholine which made up 32% total complex lipids and

Table 3—Amino acids composition of protein of fresh, blanched, bottled and canned mung bean seedlings, and bottled and canned seedlings stored at different temperatures over a 6-month period. Results are expressed as mg/100g protein

Amino acid	Fresh	Blanched	Immediately after bottling	Immediately after canning	Bottled 10°C	Bottled RT	Canned RT	Bottled 35°C
Lysine	4.8	4.7	4.5	4.9	6.7	7.0	6.6	6.9
Histidine	1.9	1.9	1.9	2.1	1.9	2.0	1.9	1.7
Ammonia	2.9	2.8	3.1	3.1	5.0	5.0	4.8	5.0
Arginine	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Aspartic acid	23.1	23.1	19.1	19.5	16.3	16.5	16.1	15.9
Threonine	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0
Serine	8.7	8.7	9.3	9.9	5.0	5.7	5.7	6.1
Glutamic acid	15.4	15.4	16.9	15.3	22.0	23.0	22.8	23.1
Proline	3.8	3.8	4.2	4.6	6.7	6.3	5.7	5.7
Glycine	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Alanine	14.4	14.4	12.8	12.3	11.5	11.6	11.4	11.0
Cystine	6.7	6.6	7.4	8.1	4.8	5.2	4.7	5.3
Valine	1.9	1.9	2.2	2.3	2.9	3.1	2.8	3.1
Leucine + Isoleucine	6.7	6.7	6.0	6.2	4.9	4.7	4.7	5.1
Tyrosine	2.9	2.9	3.3	3.4	3.8	3.8	3.8	3.5
Phenylalanine	2.9	2.9	3.1	3.1	4.8	3.9	3.9	4.1
Tyrptophan	3.3	3.0	3.0	3.0	2.8	2.1	2.2	1.6
Total (mg/100g protein)	99.7 ± 2.4	99.1 ± 2.7	97.0 ± 1.9	97.9 ± 2.3	99.1 ± 1.0	99.9 ± 1.3	97.1 ± 1.7	98.1 ± 1.8

which was reduced significantly to 20% for jars stored at 10°C and to a low 12% under the remaining three conditions. The other two phospholipids worth noting were phosphatidyl-ethanolamine and phsophatidic acid. Both made up 12% complex lipids but whereas the former did not change very much over the conditions of storage, the latter disappeared completely when the processed seedlings were stored at RT and at 35°C.

Glycolipids together with phospholipids made up total complex lipids and since phospholipids decreased on storage, the reverse applied to glycolipids (Table 4). The individual glycolipids were found in small amounts, never exceeding 8% total complex lipids. The two to note however were monogalactosyl-diglyceride (MGDG) and digalactosyl-diglyceride (DGDG) which were found as 5% and 3% complex lipids in fresh seedlings respectively but storage affected these compounds differently. Whereas DGDG stayed fairly constant under the conditions of storage, MGDG increased greatly to reach a high 29% in jars stored at 35°C. Therefore, glycolipids of processed seedlings increased mostly due to increases in MGDG the higher the temperature of storage the greater was the increase in MGDG. Fricker et al. (1975) working on spinach at temperatures of up to 100°C suggested that lipid fractions were affected by heat treatment; with increasing heat MGDG increased and DGDG decreased. This relationship however was not stoichiometric and it was possible that further reactions were leading to other unidentified products. Similar results were obtained in the present study and there was no doubt that the small decrease in DGDG could not by itself account for the large increases in MGDG. It has been suggested recently that MGDG and DGDG may be on separate pathways but may be formed from the same precursor 1,2-diglyceride. It is possible that under the conditions of the present experiment 1,2-diglyceride could combine with free galactose (Farhangi, 1980) to give rise to MGDG. Fricker et al. (1975) have also suggested that the influence of heat may change plant cells and their membranes in such a way that lipids not accessible to the solvent in the fresh product become more readily extractable. This would account for the large increases in MGDG in processed mung bean seedlings without the corresponding large decreases in DGDG.

Individual fatty acid components of free fatty acids

The major fatty acid components of fresh seedlings were 16:0, 18:0, 18:2 and 18:3 (Table 5). In terms of μg/g fresh weight there was an increase in all fatty acid components at all conditions of storage compared to controls, the greatest being in 18:2 and 18:3. Total free fatty acids in jars (332 μg/g) was higher than in cans (291 μg/g) when both were stored at RT. The fatty acid components that varied a great deal under all conditions of storage were the saturated 16:0 and the unsaturated 18:2 and 18:3, but all these values were significantly higher compared to controls. On the other hand 14:0, 18:0 and 18:1 did not vary significantly from one another under three conditions of storage although once again these values were significantly higher than those of controls, while under the fourth condition i.e. jars at 35°C, all three fatty acids were significantly higher than under the other conditions of storage. The essential fatty acids, linoleic acid (18:2) and the other C_{18} unsaturated compounds 18:1 and 18:3 increased after

Table 4—Simple and complex lipids of fresh mung bean seedlings, and of those packed and stored at three temperatures over 6 months. Results are expressed as % total unless otherwide stated

	Fresh	Bottled 10°C	Bottled RT	Canned RT	Bottled 35°C
Simple lipids					
Sterol esters	15 ± 1.5	9 ± 1.0	9 ± 1.8	9 ± 1.3	10 ± 1.1
Fatty acid esters	1 ± 0.4	1 ± 0.2	1 ± 0.2	1 ± 0.1	1 ± 0.3
Triglycerides	9 ± 0.4	9 ± 1.0	9 ± 1.1	9 ± 0.8	5 ± 1.1
Free fatty acids	15 ± 0.7	28 ± 1.1	33 ± 1.1	29 ± 0.8	51 ± 2.8
Stigmasterol	10 ± 0.5	6 ± 1.0	7 ± 1.1	6 ± 1.2	3 ± 0.9
β-Sitosterol	36 ± 0.5	14 ± 1.1	13 ± 1.2	14 ± 1.8	12 ± 1.2
Diglycerides	7 ± 0.5	12 ± 1.1	11 ± 1.0	9 ± 1.2	5 ± 1.0
Monoglycerides	7 ± 0.1	21 ± 1.0	15 ± 1.2	23 ± 1.2	13 ± 1.8
Total (mg 100g)	33 ± 0.8	33 ± 1.1	43 ± 1.2	34 ± 0.8	62 ± 1.7
Complex lipids					
Sterol glycoside	8 ± 0.5	8 ± 1.5	8 ± 1.2	8 ± 1.1	5 ± 0.9
Cardiolipin	8 ± 0.6	–	–	–	–
Phosphatidic acid	12 ± 0.6	6 ± 0.8	–	–	–
Monogalactosyl diglyceride	5 ± 0.4	16 ± 1.1	22 ± 1.3	17 ± 1.2	29 ± 1.7
Ceramide monohexoside	6 ± 1.1	4 ± 0.8	3 ± 1.0	3 ± 0.6	2 ± 1.2
Phosphatidyl glycerol	5 ± 0.2	12 ± 1.9	15 ± 1.8	15 ± 1.9	14 ± 1.8
Phosphatidyl ethanolamine	12 ± 0.8	12 ± 0.6	9 ± 1.0	10 ± 0.6	8 ± 0.6
Digalactosyl diglyceride	3 ± 0.2	2 ± 0.4	2 ± 0.5	1 ± 0.2	1 ± 0.3
Sulpholipid	1 ± 0.5	1 ± 1.6	2 ± 1.1	2 ± 1.1	1 ± 1.2
Phosphatidyl inositol	2 ± 1.0	1 ± 1.8	1 ± 1.1	2 ± 1.2	1 ± 1.8
Phosphatidyl choline	32 ± 0.5	20 ± 1.2	12 ± 1.1	13 ± 1.4	10 ± 1.7
Lysophosphatidyl ethanolamine + Lysophosphatidyl inositol	–	1 ± 1.1	3 ± 1.8	3 ± 1.7	2 ± 1.8
Ceramide	–	2 ± 1.2	3 ± 1.1	4 ± 0.9	4 ± 1.2
Lysophosphatidyl choline	6 ± 0.1	15 ± 1.1	20 ± 1.2	22 ± 1.2	23 ± 1.1
Total (mg 100g)	317 ± 4.1	302 ± 2.1	264 ± 3.4	286 ± 4.1	182 ± 2.9
Simple % of total lipids	10 ± 1.1	10 ± 0.8	14 ± 1.1	11 ± 1.2	26 ± 2.1
Complex % of total lipids	90 ± 1.1	90 ± 0.8	86 ± 1.1	89 ± 1.2	74 ± 2.1
Phospholipids % of complex	77 ± 1.2	67 ± 1.1	60 ± 0.5	66 ± 1.2	58 ± 1.0
Glycolipids % of complex	23 ± 1.2	33 ± 1.1	40 ± 0.5	34 ± 1.2	42 ± 1.0
Total lipids (mg/100g)	350 ± 20	335 ± 10	307 ± 24	320 ± 16	244 ± 22

Table 5—Major free fatty acids of fresh 4-day-old dark-grown mung bean seedlings, and of those bottled and canned which were kept at various temperatures over 6 months. Results are expressed as μg/g wet weight

Fatty acids	Fresh	Bottled 10°C	Bottled RT	Canned RT	Bottled 35°C
14:	4.4 ± 0.3	19.8 ± 2.8	26.6 ± 3.7	23.2 ± 3.7	35.8 ± 4.4
16:0	29.6 ± 1.9	50.9 ± 2.7	63.1 ± 3.6	46.6 ± 4.6	102.4 ± 4.1
18:0	25.2 ± 1.8	34.0 ± 3.6	36.5 ± 5.0	34.9 ± 3.9	51.3 ± 5.4
18:1	20.7 ± 1.9	25.5 ± 3.3	26.5 ± 3.6	23.2 ± 2.4	30.7 ± 2.4
18:2	38.5 ± 2.7	79.2 ± 2.7	93.0 ± 5.4	90.3 ± 1.2	158.7 ± 5.7
18:3	29.6 ± 4.0	73.6 ± 4.4	86.3 ± 2.7	72.8 ± 3.8	133.1 ± 5.5
Total FFA (μg/g)	148.0 ± 6.8	283.0 ± 10.9	332.0 ± 11.9	291.0 ± 8.4	512.0 ± 27.7

processing and under the three temperatures of storage, reaching a maximum of 292.5 $\mu g/g^{-1}$ at 35°C compared to 88.8 in fresh seedlings. Therefore during processing and storage there is an increase in essential fatty acids due to the hydrolysis of other lipids. It may be advantageous then to use stored processed seedlings since some of the essential fatty acids are now present in the free form and are therefore more readily available to consumers.

CONCLUSIONS

BLANCHING had very little effect on proteins, protein amino acids and on lipids of mung bean seedlings while free amino acids were greatly affected. Immediately after bottling and canning total protein differed little from that of blanched seedlings. However, free amino acids decreased much more than did protein amino acids.

Storage temperature had a marked effect on proteins of processed mung bean seedlings and this was possibly aided by browning due to the Maillard reaction; the higher the temperature the more browning was observed. Protein amino acids although showing losses after storage at various temperatures were not as severely affected as were free amino acids. Further, during storage at RT the containers had very little effect on the essential amino acids present. Apart from phenylalanine and tyrosine which had decreased the most compared to seedlings stored at 10°C the other essential amino acids did not decrease so much. Although storage at 19°C was therefore better for certain amino acids, the cost of storage at the lower temperature would make it more attractive to store the processed seedlings at RT.

Total lipids of processed seedlings over the 6-month storage period were unaffected compared to blanched seedlings. There was an increase in simple lipids and a decrease in the more complex ones, especially phospholipid, as phosphatidic acid disappeared completely at all storage temperatures above 10°C. However, as the storage temperature increased from 10°C to 35°C, simple lipids increased at the expense of complex lipids which decreased under the same conditions. The simple lipid increases were due to a substantial increase in free fatty acids of processed seedlings. Finally, glycolipid increases were due mainly to increases in MGDG which could have arisen by 1,2-diglyceride combining with free galactose which is found in fairly large amounts under the conditions of the present study.

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Aseptically Packaged Papaya and Guava Puree: Changes in Chemical and Sensory Quality During Processing and Storage

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ABSTRACT

Effects of storage temperature and time on the quality of aseptically processed, "bag-in-box" packaged guava and papaya puree were investigated. During aseptic processing of guava puree there was virtually no loss of ascorbic acid (AA) and flavor but significant losses in color. After 6 months ambient storage the AA loss was about 30% and further color changes and flavor losses occurred. Samples stored at 38°C for 3 months showed an AA loss of about 47% and losses in color and flavor. For papaya puree AA losses of about 6% and 56% occurred during aseptic processing and after 6 months ambient storage, respectively. Color changes during aseptic processing and the first month of storage was characterized by a hypsochromic shift of the carotenoids' absorption spectra. After the first month of storage further color changes were attributed to the products of nonenzymatic browning. Papaya flavor was stable during both aseptic processing and 6 months ambient storage. Flavor of papaya puree stored at 38°C for 3 months changed significantly and AA retention was 39%.

INTRODUCTION

ASEPTIC PROCESSING has been used in the food industry for many years but is generating renewed interest because of rising energy costs and development of bag-in-box packaging (Orbell, 1980). Tropical fruit products for transoceanic shipments from lesser developed areas to industrially developed markets are likely candidates for adoption of aseptic processing since refrigerated transport and storage are not needed with this system. Presently, guava and papaya purees are processed in Hawaii and shipped frozen in 35- to 40-lb containers to distant markets. Because freezing, frozen transportation and storage are becoming more costly, an alternative bulk packaging system is desirable. The bag-in-box aseptic packing system is attractive because it requires no product refrigeration and because the container is disposable. This latter consideration is important because it eliminates costs incurred in returning containers to the processor. However, success of an aseptically processed puree packaged in a low oxygen permeable container is predicated on retention of color, flavor, and aroma quality as well as nutrient content. This study delineates changes in ascorbic acid, carotenoids, alcohol soluble color and sensory quality during aseptic processing and storage.

MATERIALS & METHODS

GUAVA PUREE, 2,500 lb, from a commercial source (Suisan Company) was prepared by the method described by Boyle et al. (1957). Papaya puree, 2,500 lb, was prepared by the same processor using an improved processing method (Brekke et al., 1972). The puree had been acidified to pH 3.9 with citric acid and had a soluble solids content of 13.5%. Both guava and papaya purees were frozen in polyethylene-lined containers at −18°C and shipped transoceanic and overland to the Cherry-Burrell Company in Cedar Rapids, IA, where test packs were conducted in the company testing facilities. The purees were defrosted and divided into two batches of 1,200 lb each prior to heating in a Cherry-Burrell Thermutator at 93°C. The guava puree was pumped into the Thermutator at 14–21°C. The first batch at 93°C was held for 26 sec and the second for 38 sec before cooling in a scraped surface heat exchanger to 24°C. Papaya puree was heated in a like manner at 93°C with the exception that product-in temperature was 4–8°C and holding times were 60 and 120 sec.

The thermal process schedule for guava puree of 26 sec at 93°C was selected as being sufficient to ensure commercial sterility based on information from The National Food Processors Association (personal communication, 1979). For papaya puree the thermal process of 60 sec at 93°C was based on the heat inactivation of papaya pectin esterase (Aung and Ross, 1965; Nath and Ranganna, 1981). The longer holding times of 36 sec for guava and 120 sec of papaya were used to determine the purees' tolerance to longer heat treatments.

A Scholle aseptic filler (Model Auto-fill X-1) was used to fill 1-gal bags. The multi-ply, metallized, polyester/evapolyethylene bags (Scholle #804AM) were previously sterilized by the supplier (Scholle Corporation) using gamma irradiation. The filling operation reported by Orbell (1980) is described as follows: The filler was first steam-sterilized at 120°C for 30 min after which sterile-heated air was introduced into the filling-head chamber. The heated air activated chlorine which was constantly misted around the filling-head area. The chlorine mist provided a sterile wash for the oustide of the bag while it was in the chamber. The bag cap was removed inside the chamber and the filling-head was then inserted into the bag spout. After the bag was filled the filling-head was removed from the spout, and the cap replaced. The machine then ejected the bag from the filling chamber. The filled bags were then placed in fiberboard cartons and airfreighted to Honolulu, Hawaii.

Upon arrival in Honolulu, an initial evaluation was conducted and the remaining samples stored at ambient temperature (24–31°C) and at 38°C (accelerated storage). Ambient samples were tested after 1, 3, and 6 months and the accelerated storage samples after 1, 2, and 3 months. Tests included visual color evaluation of the puree, taste tests on nectars made from the puree, physical and chemical measures of color, ascorbic acid, total carotenoids, total acids and Brix. All samples were compared with frozen puree (control) from the same lot used for the aseptic packs.

pH, total acid, and ascorbic acid assay

pH was determined with a glass-electrode pH meter; total titratable acidity was determined by Association of Official Analytical Chemists (1980) method no. 22.061 and the results were expressed as percent citric acid. The colorimetric method of Loeffler and Ponting (1942) with slight modifications was used to assay ascorbic acid (AA). The method was modified by increasing the metaphosphoric acid concentration to 3% which stabilized the extracted AA.

Carotenoids

Carotenoids were extracted from the purees as follows: 30g of puree were mixed with 5g of Hyflo Supercel (Johns Manville Co.) and 75 ml of 70% methanol (v/v), and filtered in vacuo through Whatman No. 2 filter paper. The residue was extracted two more times with 75 ml of acetone: petroleum ether (1:1) (v/v). The extracts were transferred to a 500 ml separatory funnel containing 25 ml of 10% KOH in methanol (w/v), and allowed to stand for 0.5–1.5 hr. Partition was achieved by adding 75 ml of petroleum ether and 100 ml of 20% NaCl (w/v), and mixing gently. The hypsophasic layer was discarded. The epiphasic layer was washed three times with water, passed through anhydrous Na_2SO_4, and made up to 250 ml with petroleum ether. Absorption spectra in the visible region, 350–750 nm, were run with a Perkin-Elmer 550

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Spectrophotometer. Total carotenoid values were calculated from the absorption maxima using $E^{1\%}_{1\ cm}$ = 3,450 at 468 nm for guava and $E^{1\%}_{1\ cm}$ = 2,370 at 445 nm for papaya.

Physical color readings

Color changes were measured by presenting a polystyrene petri dish (100 x 15 mm) containing 70 g of puree to the viewing port of the Hunter Colorimeter D25M-3. Results were expressed in Hunter *L, a, b* values.

Alcohol soluble color (ASC) index

100 ml of 70% methanol were mixed with 40g of puree and 1–2g of Filtercel (Johns Manville Co.) for 3 min. The mixture was filtered in vacuo through a layer of Filtercel on Whatman No. 2 filter paper.

The filtrate was brought to 200 ml with 70% methanol. Absorbance was measured at 400 nm with a Perkin-Elmer 550 Spectrophotometer using 70% methanol as a blank. The alcohol soluble color (ASC) index was calculated by dividing the absorbance (x 1000) at 440 nm by the grams of sample.

Results of simple alcohol extraction methods such as this correlated well with nonenzymatic browning in many foods (Maier and Schiller, 1960; Hendel et al., 1950; Stadtman, 1948; Nury and Brekke, 1963).

Sensory evaluation

Color of aseptically packaged puree was compared with that of the frozen control by an 11-member sensory panel. An identified control served as a reference against which the coded aseptic puree and an unidentified coded control were compared. Papaya samples were scored more orange (+1 to +3), same as (0), or less orange (−1 to −3) than the reference. Using the same scale, guava samples were scored more or less pink. One hundred-gram portions were placed in petri dish bottoms (100 x 15 mm) for viewing with illumination provided by a Haluk grading light.

At each storage interval drinks were prepared which contained 20% puree. Sucrose and citric acid were added to adjust Brix to 12° an titratable acidity to 0.3 and 0.4% for papaya and guava, respectively. The drinks were chilled to 5°C and served in 50-ml beakers to subjects seated in individual booths with red illumination. Subjects received a reference sample which was a drink prepared from the frozen control puree. In addition, the control drink and the drink prepared from aseptic puree were presented as coded samples. Coded samples were rated as having more papaya or guava flavor (+1 to +3), same as (0), or less papaya or guava flavor than the reference (−1 to −3). Each treatment was evaluated three times by the subjects.

RESULTS & DISCUSSION

Effect of aseptic processing and storage on pH, total acids, Brix

There were no significant changes in pH, total acids or Brix of guava or papaya puree during processing or storage. This lack of change in acid and sugar content coupled with lack of observed odor or gas production *indicated* that the aseptically processed puree was commercially sterile.

Effect of aseptic processing and storage on ascorbic acid in guava puree

Ascorbic acid retention during aseptic processing and subsequent storage at ambient temperature and at 38°C is shown in Fig. 1. Analysis of the frozen control and aseptically treated samples showed virtually no change due to processing. After 1 month storage at both ambient and 38°C, all of the aseptically treated guava samples retained more than 88% of their original AA. And after 2 months at 38°C the samples heated at 26- and 38-sec retained 76 and 71%, respectively, of their original AA. After storage for 3 months, the ambient temperature samples retained at least 72% of their AA and samples heated at 26- and 38-sec, respectively, then stored at 38°C retained 62% and 53%. After 6 months ambient storage, AA retention was 70.2% and 43.2% for the samples heated at 26- and 38-sec, respectively.

Brekke et al. (1970) reported an AA retention of 65% after 5 months frozen storage in guava puree concentrate. They also reported that guava concentrate preserved with sorbate and stored at 45°C lost most of its AA during th the first month of storage. Hence, aseptic processing and bag-in-box packaging appears to provide a comparable if not superior system in terms of AA retention.

Effect of aseptic processing and storage on guava puree color

Absorption at 400 nm of the alcohol extracts of puree was used as an indicator of browning. Results are shown in Fig. 2. Alcohol soluble color indices for the control and aseptically processed samples were similar at 0 month storage.

After 1 month storage, samples stored under accelerated conditions (38°C) showed a 53% increase in the ASC index which was quite significant when compared to samples stored at ambient temperatures whose ASC indices increased by about 11%. For the 38- and 26-sec aseptically treated samples stored at 38°C for 2 months, ASC indices increased nearly 135% for the 26-sec sample and appeared to level off for the 38-sec sample. For samples stored at ambient temperatures, the ASC index increased about 100% after 3 months and appeared to remain constant throughout the 6 months storage. Hunter readings reflected color changes during processing and storage. However, values were nearly identical for the 26-sec and 38-sec samples. Aseptic processing appears to cause a lightening of the guava puree as reflected in an increase in Hunter *L* values from 43.2 for the control to 45 for the aseptically processed samples (Fig. 3). However, upon storage at 38°C, Hunter *L* values decreased continuously with storage time indicating a darkening in color. In contrast, samples stored at ambient temperatures did not darken significantly for the first month of storage. After 3 months ambient storage, color darkened to about the same degree of darkness as the control at 0 month storage.

Aseptic processing also caused a considerable decrease in both *a* and *b* values indicating a loss in chroma which was

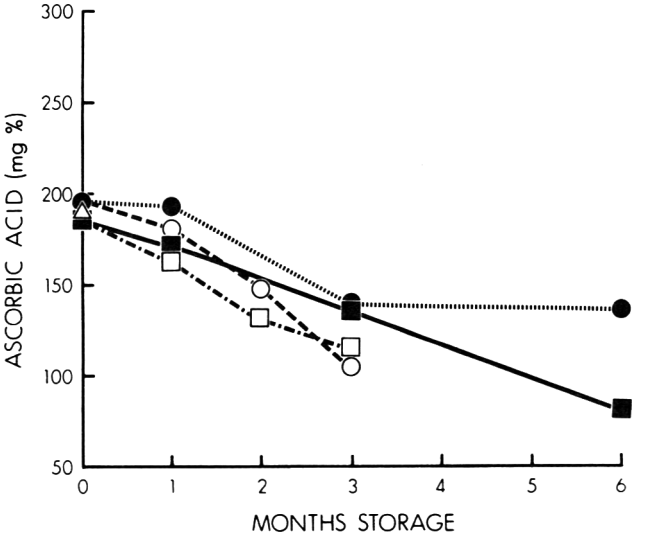

Fig. 1—Effect of aseptic processing and storage on ascorbic acid retention in guava puree: △ control; ambient storage, (■ — ■ processed at 93°C for 38 sec), (● - - - ● processed at 93°C for 26 sec); accelerated storage, 38°C, (□ - - - □ processed at 93°C for 38 sec), (○ - - - ○ processed at 93°C for 26 sec).

also observed by the sensory panel as a loss of pink color. During storage, Hunter *a* values decreased and *b* values increased (Fig. 4) indicating a change in hue to less red and more yellow. The change in color during storage in terms of Hunter *L*, *a*, *b* values was more rapid at 38°C than at ambient temperatures. This indicates that color stability of guava puree during storage is highly temperature dependent. Interestingly, after 3 months ambient storage, *L* values had leveled off indicating no further darkening up to 6 months storage. This lack of further darkening from 3 to 6 months is confirmed by the lack of increase in ASC.

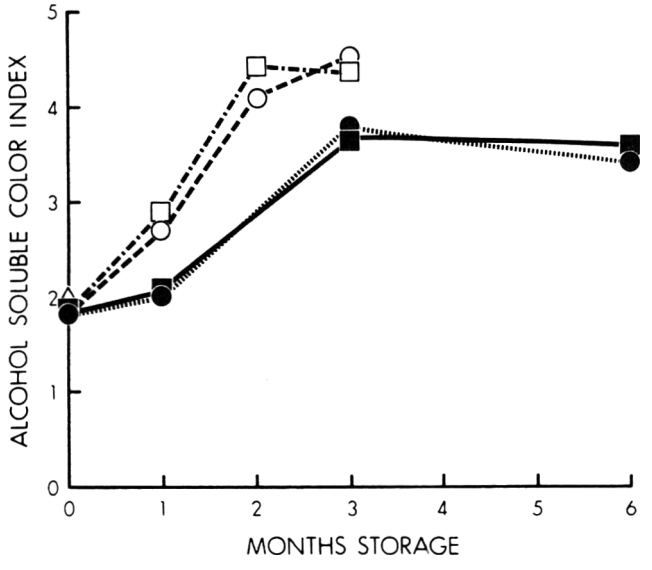

Fig. 2—Effect of aseptic processing and storage on the alcohol soluble color index in guava puree: Δ control; ambient storage, (■ — ■ processed at 93°C for 38 sec), (● - - - ● processed at 93°C for 26 sec); accelerated storage, 38°C (□ - - - □ at 93°C for 38 sec), (○ - - - ○ processed at 93°C for 26 sec).

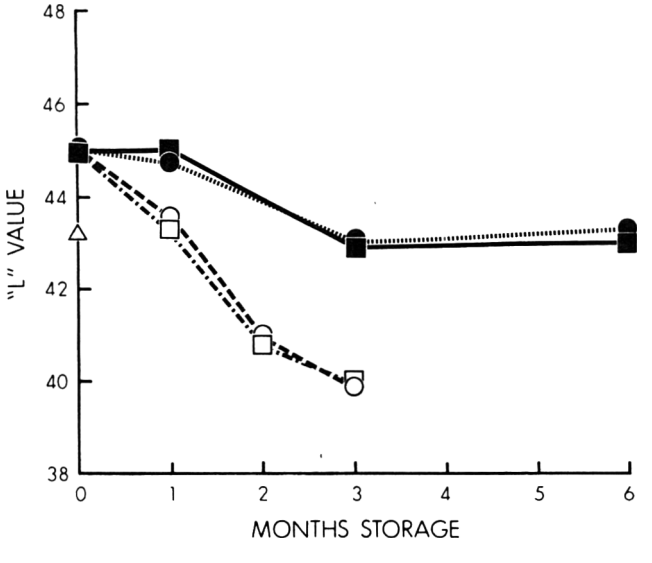

Fig. 3—Effect of aseptic processing and storage on Hunter "L" value in guava puree: Δ control; ambient storage, (■ — ■ processed at 93°C for 38 sec), (● - - - ● processed at 93°C for 26 sec); accelerated storage, 38°C, (□ - - - □ processed at 93°C for 38 sec), (○ - - - ○ processed at 93°C for 26 sec).

Effect of aseptic processing and storage on carotenoids in guava puree

Total carotenoids, as determined from absorbance of carotenoid extracts at 468 nm, decreased from an initial value of 4.42 mg % to 4.02 mg % and 3.91 mg %, respectively, for the 26- and 38-sec, aseptically processed guava purees (Fig. 5). After 1 month ambient storage, both samples showed increases in absorbance of the carotenoid extracts at 468 nm. The sample processed at 38-sec after storage at 38°C for 1 month also showed an increase in carotenoids. The reason for this unexpected increase remains unexplained as no spectral shifts were observed. This increase in carotenoids was not reflected in either Hunter color values or sensory color scores.

Effect of aseptic processing and storage on sensory quality of guava puree

Aseptic processing had considerable impact on guava color whether processing time was 26- or 38-sec. In both cases the puree was less pink than the frozen control ($P = 0.01$) (Fig. 6). These results were also confirmed by Hunter values (Fig. 4). Results of the sensory panel suggest that after processing, only a slight loss of pink color occurred during 6 months at ambient temperature. However, when the puree was stored at 38°C, loss of pink color progressed more rapidly than at ambient and was much greater after 3 months at 38°C than after 6 months at ambient temperature (Fig. 6).

Flavor was not as greatly affected as color. There was no statistically significant initial effect of processing on guava flavor with either heating time (Fig. 7). However, after only 1 month at ambient temperature, some loss of guava flavor was observed. This flavor loss remained constant for the 26-sec treatment throughout the 6-month storage period. However, puree processed for 38 sec showed some additional flavor loss after 6 months of ambient storage. Flavor loss after 3 months at 38°C was comparable with the loss after 6 months at ambient. Initial color changes resulting from aseptic processing as reflected in Hunter *L*, *a*, *b* values and panel scores appear to be due to changes in carotenoids and not to appearance of browning products. During storage, however, color changes appear to be due to both changes in carotenoids and appearance of browning products. Flavor changes were the result of storage and not processing.

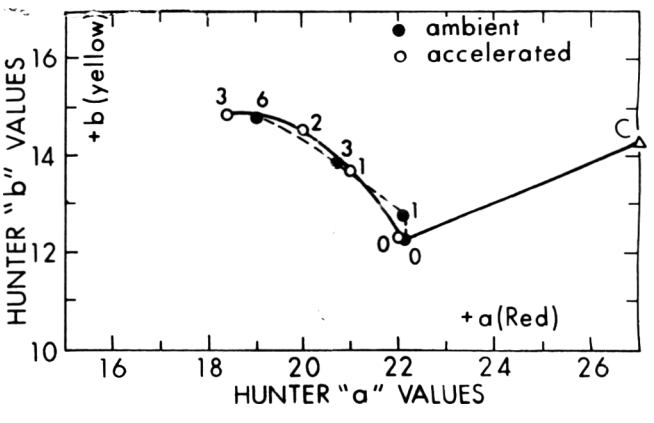

Fig. 4—Effect of aseptic processing and storage on Hunter a and b values in guava puree: ΔC, control; ambient storage, (●^{0} - - - ●^{1}, ●^{3}, ●^{6}); accelerated storage, 38°C. (○^{0} — ○^{2}, ○^{3}; superscripts designate 0, 1, 2, 3, and 6 months storage, respectively).

Effect of aseptic processing and storage on ascorbic acid (AA) in papaya puree

Ascorbic acid retention during aseptic processing and subsequent storage at ambient temperature and at 38°C is shown in Fig. 8. Analysis of the frozen control sample and aseptically treated samples showed an AA retention of 95.6% and 94.2% during aseptic processing at 93°C for 60 and 120 sec, respectively. After 1 month storage at both bient and 38°C, all the aseptically treated samples with the exception of the 60-sec sample stored at ambient temperature showed little or no further loss in AA. However, after 2 and 3 months storage at 38°C, AA levels showed a sharp but nearly linear decline resulting in only a 35.5 and 39.3% AA retention after 3 months for the 60- and 120-sec samples, respectively. The samples stored under ambient conditions fared better; after 3 months, the 60-sec and 120-sec samples had retained 59 and 56% of their AA contents, respectively. After 6 months in ambient storage the AA retention was 45.8% and 44.5% for the 120- and 60-sec samples.

Effect of aseptic processing and storage on the alcohol soluble color (ASC) in papaya puree

Absorption at 400 nm of alcohol extracts of puree was used as an indictor of browning; results are shown in Fig. 9. ASC indices for the control and aseptically processed samples were relatively similar at 0 month storage. After 1 month storage ASC indices increased about 0.6–0.7 units for both samples stored at ambient and the same was true for both samples stored at 38°C. After 2 months storage at 38°C, the ASC index had increased about 100% over initial values. After 3 months, samples stored at ambient temperatures attained a maximum ASC index which remained constant thereafter.

Effect of aseptic processing and storage on papaya puree color

Effects of aseptic processing and storage conditions on the Hunter L values are shown in Fig. 10. Aseptic processing caused a slight decrease in L values indicating some darkening due to the process. All samples processed at 120 sec and stored at ambient temperature progressively darkened after 1 month storage. Samples stored at 38°C showed an accelerated decrease in L values compared to samples stored under ambient conditions. Aseptic processing of both the 60- and 120-sec samples caused lower a values than for the control (Fig. 11). This decrease in a values continued during storage under both ambient and accelerated storage (38°C). Hunter b values for the samples followed a similar trend. Decreases in b values were of about the same order of magnitude as in a values indicating a loss in chroma and very little change in hue. There appears to be a regression of the a and b values towards the achromatic gray of the Hunter L, a, b color solid. Of all treatments, the 60-sec sample stored under ambient conditions showed the least amount of change in Hunter values indicating that color stability of papaya puree is dependent on both the severity of heat processing and storage temperature.

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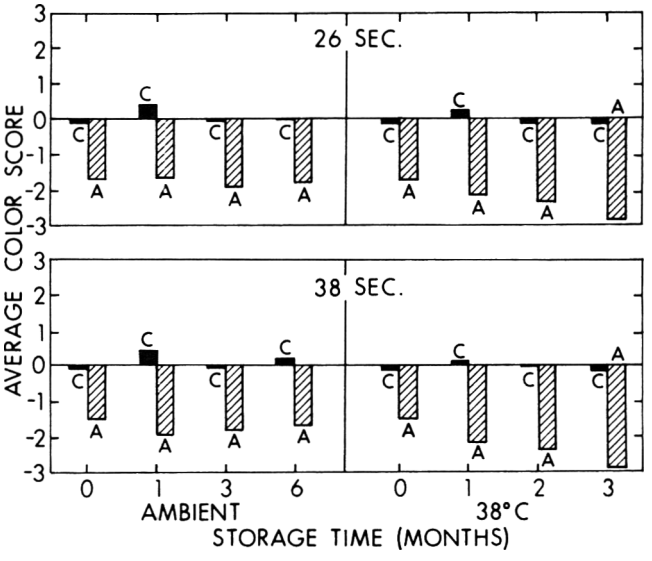

Fig. 6—Effect of aseptic processing on the average color scores for guava puree processed at 93°C for 26 and 38 sec (A) and control (C).

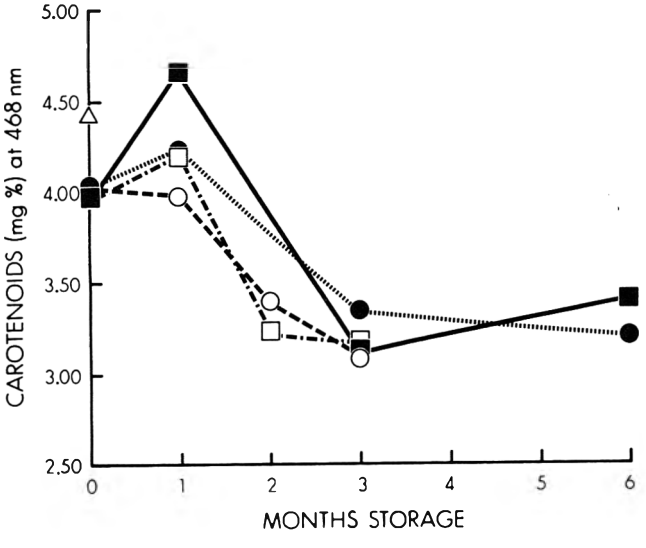

Fig. 5—Effect of aseptic processing and storage on total carotenoids in guava puree: △ control; ambient storage, (■ — ■ processed at 93°C for 38 sec), (● - - - ● processed at 93°C for 26 sec); accelerated storage, 38°C, (□ - - - □ processed at 93°C for 38 sec), (○ - - - ○ processed at 93°C for 26 sec).

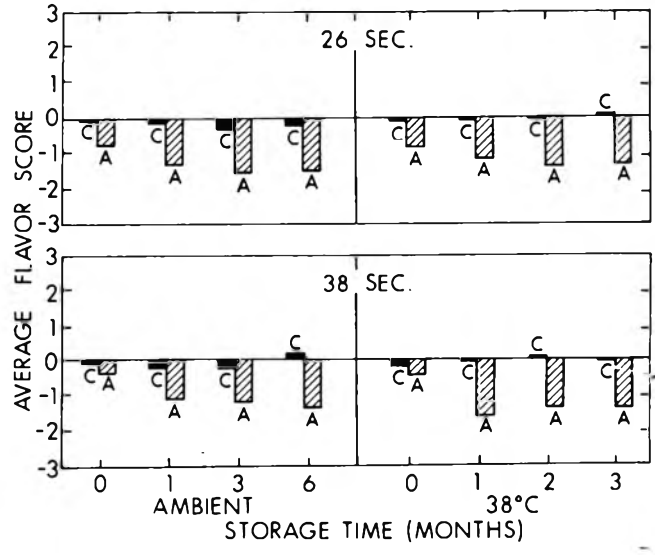

Fig. 7—Effect of aseptic processing and storage on average flavor scores for guava puree processed at 93°C for 26 and 38 sec (A) and control(C).

Effect of processing and storage on carotenoids in papaya puree

Total carotenoids, as determined from absorbance of carotenoid extracts at 445 nm decreased from an initial value of 2.43 mg % to 2.04 mg % and 1.95 mg %, respectively, for the 60- and 120-sec samples. During both ambient and accelerated storage carotenoids appeared to decrease further (Fig. 12). However, as shown in Fig. 12, loss in carotenoids can be explained as a hypsochromic shift in the absorption spectra: absorption increased at 423 nm, decreased at 445 nm as reflected in the increase of the absorbance ratio 423 nm/445 nm. The hypsochromic effect in processed papaya products has been reported by Chan et al. (1975) as due to the acid-catalyzed isomerization of 5,6-monoepoxycryptoxanthin to 5,8-monoepoxycryptoxanthin. Because of the hypsochromic effect, decreases in carotenoid values should not be construed as destruction of carotenoids, but rather as a change in composition. Hence, initial color changes during aseptic processing and after the first month of ambient and accelerated storage appear to be predominantly influenced by the hypsochromic shift of the carotenoid absorption spectra as there was very little progressive change with time in absorption ratio (Fig. 12). However, after this same period the ASC index increased considerably (Fig. 9) indicating that after the first month of ambient and accelerated storage, color changes are mainly due to the products of nonenzymatic browning.

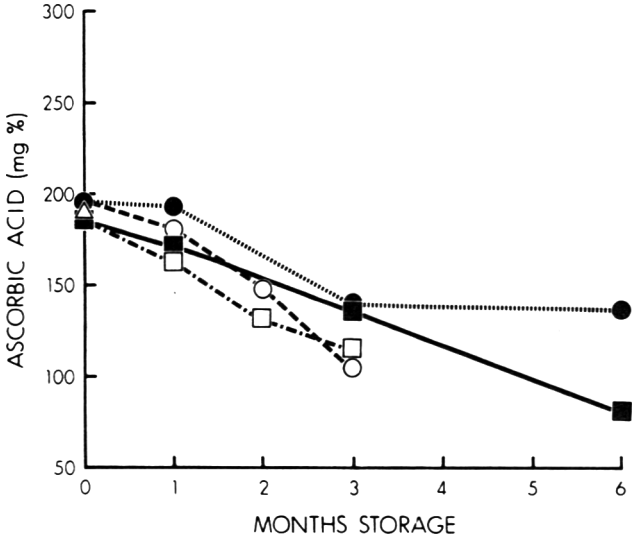

Fig. 8—Effect of aseptic processing and storage on ascorbic acid retention in papaya puree: Δ control; ambient storage, (■ — ■ processed at 93°C for 120 sec), (● - - - ● processed at 93°C for 60 sec); accelerated storage, 38°C, (□ — □ processed at 93°C for 120 sec), (○ - - - ○ processed at 93°C for 60 sec).

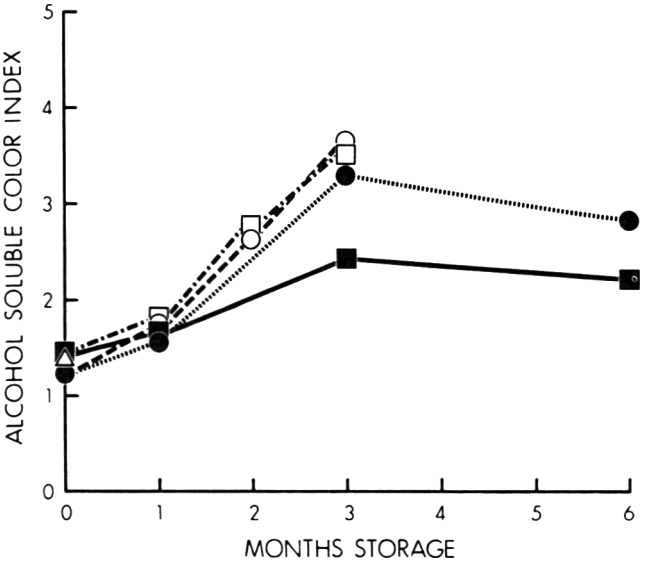

Fig. 9 -Effect of aseptic processing and storage on the alcohol soluble color index in papaya puree: Δ control; ambient storage, (■ — ■ processed at 93°C for 120 sec), (● - - - ● processed at 93°C for 60 sec); accelerated storage, 38°C, (□ — □ processed at 93°C for 120 sec), (○ - - - ○ processed at 93°C for 60 sec).

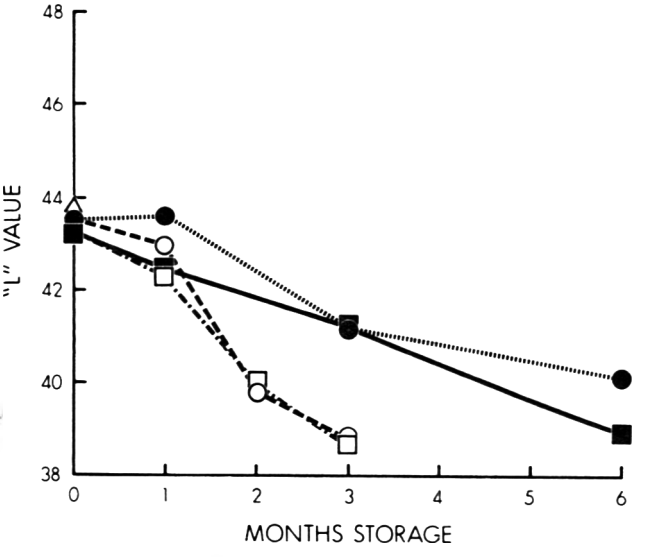

Fig. 10—Effect of aseptic processing and storage on Hunter "L" value in papaya puree: Δ control; ambient storage, (■ — ■ processed at 93°C for 120 sec), (● - - - ● processed at 93°C for 60 sec); accelerated storage, 38°C, (□ — □ processed at 93°C for 120 sec), (○ - - - ○ processed at 93°C for 60 sec).

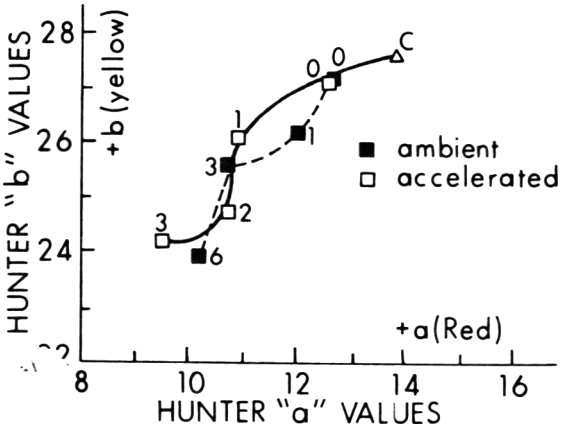

Fig. 11—Effect of aseptic processing and storage on Hunter a and b values in papaya puree: ΔC, control; ambient storage, (■^{0} - - - ■^{1}, ■^{3}, ■^{6}); accelerated storage, 38°C (□^{0} — □^{1}, □^{2}, □^{3}; superscripts designate 0, 1, 2, 3, and 6 months storage, respectively).

Effect of aseptic processing and storage on sensory quality of papaya puree

Aseptic processing resulted in a papaya puree that was significantly less orange ($P = 0.01$) than the frozen control (Fig. 13). During storage at ambient temperature, no difference in orange color was observed between the control and aseptic sample after 1 month but significant changes ($P = 0.01$) were seen after 3 and 6 months. The same general observations were made for samples stored at accelerated conditions (38°C) except that loss of orange color was greater. The 60- and 120-sec samples reacted similarly. No loss in flavor could be attributed to aseptic processing (Fig. 14). Furthermore, flavor of the aseptic packs was stable for 6 months at ambient temperature. The only flavor change observed was the significant loss ($P = 0.01$) after 3 months storage at 38°C.

The adoption of the concept of aseptically packaged puree would depend on a process which would provide enough product stability to allow nonrefrigerated transport of puree to distant manufacturers for reprocessing into finished products.

It is expected that an aseptically packaged puree would be reprocessed soon after its arrival since most manufacturers would prefer to avoid the additional costs of storage. When compared with the shelf-life required for a finished consumer product, the shelf-life requirement for aseptically processed puree to be used for remanufacture is relatively short. A shelf-life of 6 months is estimated to be sufficient for delivery in an orderly market structure. Papaya puree, aseptically processed and packaged by the method presented here appears to be stable enough to meet this 6-month requirement. Guava puree can also meet this shelf-life requirement if color is not a prime concern to the end user.

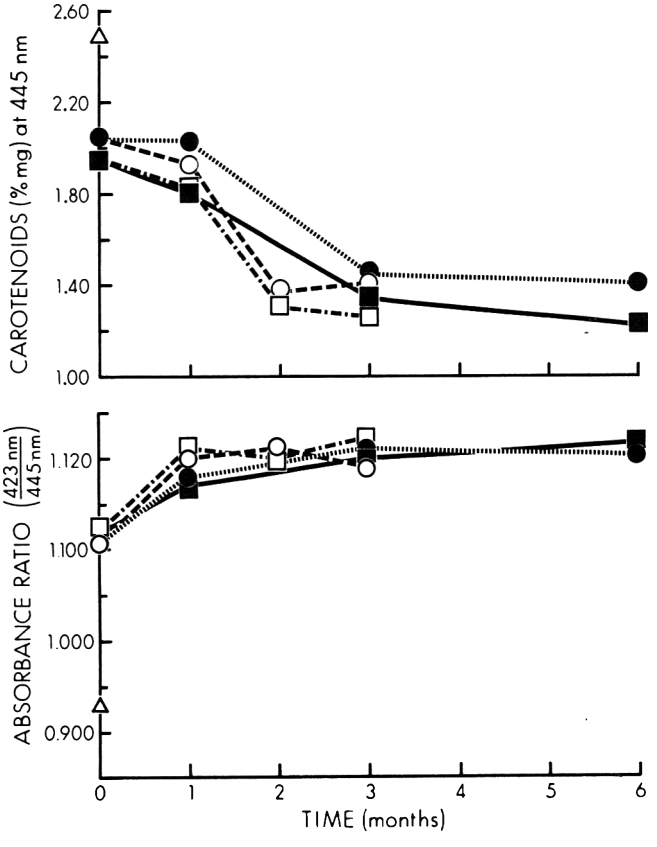

Fig. 12—Effect of aseptic processing and storage on changes in absorption ratio 423 nm/445 nm of papaya carotenoids and changes in total carotenoids at 445 nm; △ control; ambient storage, (■ — ■ processed at 93°C for 120 sec), (● - - - ● processed at 93°C for 60 sec); accelerated storage, 38°C, (□ — □ processed at 93°C for 120 sec), (○ - - - ○ processed at 93°C for 60 sec).

SUMMARY

ASEPTIC PROCESSING of guava puree had no effect on its AA content and flavor but did cause a significant loss of color. After 6 months ambient storage, AA loss was small, 30%, and slight additional color and flavor changes occurred.

Papaya flavor was stable during both aseptic processing and 6 months ambient storage. However, small losses of AA occurred during processing and larger losses, 56%, after 6 months ambient storage. Initial color changes in papaya puree during aseptic processing and the first month of storage were attributed to changes in carotenoids. Further color changes were attributed to the product of nonenzymatic browning. *—Continued on page 1174*

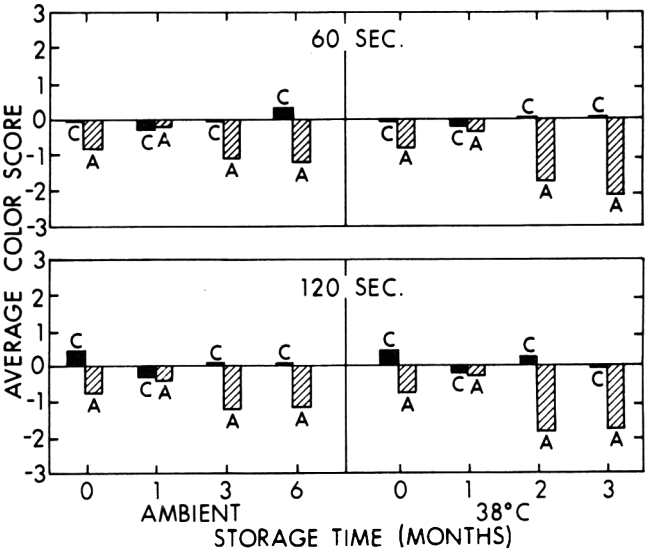

Fig. 13—Effect of aseptic processing and storage on average color scores for papaya puree processed at 93° C for 60 and 120 sec (A) and control (C).

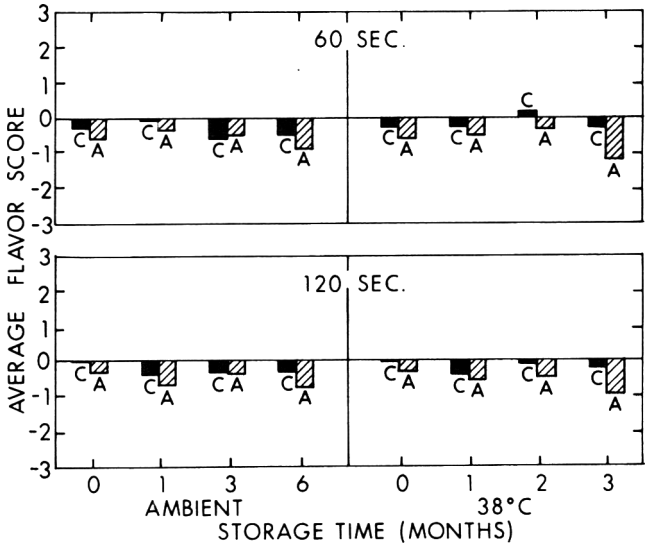

Fig. 14—Effect of aseptic processing and storage on average flavor scores for papaya puree processed at 93°C for 60 sec and 120 sec (A) and control (C).

Sulfur Amino Acid Stability. Effects of Processing on Legume Proteins

H. F. MARSHALL, K. C. CHANG, K. S. MILLER, and L. D. SATTERLEE

ABSTRACT

Four legume protein sources: peanuts, dry beans, alfalfa and soybeans were processed in laboratory, pilot plant and commercial processing facilities. Raw ingredients, intermediate and final protein products were analyzed for total and reactive cysteine/cystine (Cys), total methionine, methionine sulfoxide and methionine sulfone. Lysine, total and reactive, was determined on each legume protein sample. Processing of soybeans, peanuts and alfalfa into protein products caused no significant loss of lysine or the sulfur amino acids. The production of a protein concentrate from dry beans resulted in a significant loss of Cys. One commercial soy isolate was found to contain 51% of its total methionine as methionine sulfoxide, yet its Cys was unaffected.

INTRODUCTION

THE NUTRITIONAL QUALITY of a food protein is determined by the quantity of each of the essential amino acids (EAA) available from that protein, with the first limiting EAA being the greatest determinant of that protein's quality. It is known that several key AA (methionine, cysteine/cystine and lysine) can exist either partially or completely in biologically unavailable forms within proteins in their native state, as well as in proteins having undergone processing (Pusztai and Watt, 1974; Baldi and Salamin, 1973; Kunachowicz et al., 1976). Oxidized Cys, i.e., cysteic acid, has been shown to be inherent in two isolectins found in unprocessed Haricot dry beans (Pusztai and Watt, 1974). Endogenous methionine sulfoxide, S-methyl cysteine and S-methyl cysteine sulfoxide have been found in kidney beans and several other cultivars of *Phaseolus vulgaris* (Zacharius, 1970; Baldi and Salamini, 1973). The presence of γ-glutamyl methionine sulfoxide in seeds other than dry beans has been reported by Kasai et al. (1972).

Kunachowicz et al. (1976) and Strange et al. (1980) have shown that the processing of soy isolate, casein and meat (frankfurters) transforms some of the methionine and Cys in these protein sources to unavailable forms. Heat, hydrogen peroxide and lipid produced hydroperoxides can result in the oxidation of both methionine and Cys to partially or completely oxidized forms (Slump and Schreuder, 1973; Tannenbaum et al., 1969; Schnack and Klostermeyer, 1980). The formation of lysinoalanine and the racemization of amino acids, both induced by alkaline treatment of proteins, can also reduce the bioavailability of Cys and lysine (Whitaker, 1980; Masters and Friedman, 1980). Lysine losses can also result from the nonenzymatic browning reaction (Maillard), possibly the most frequent cause for the processing loss of an EAA (Carpenter and Booth, 1973; Feeney, 1980). In the early stages of the Maillard reaction the ε-amino group on the lysine residue becomes blocked by a sugar, yielding a biologically unavailable lysine residue. Yet this residue is identified as available lysine by several chemical assays, i.e., amino acid analysis.

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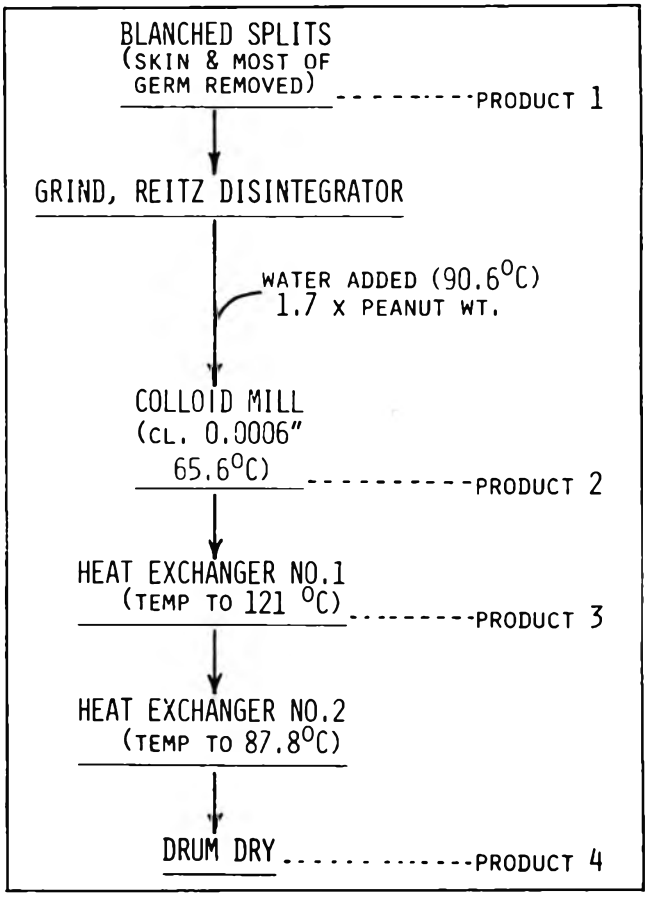

Fig. 1—Clemson process used to prepare peanut samples.

The bioavailability of the various oxidized forms of methionine and Cys differ, depending upon their degree of oxidation. Cysteic acid and methionine sulfone, as well as S-methyl cysteine, are completely unavailable to the rat, whereas L-methionine sulfoxide is partially utilized by the rat (60% as effective as L-methionine), (Block and Jackson, 1932; Anderson et al., 1976). Protein bound methionine sulfoxide has been suggested to be as available as protein-bound methionine (Slump and Schreuder, 1973; Sjoberg and Bostrom, 1977). The discrepancy between results reported by the above researchers indicates further research needs to be done to confirm the bioavailability of methionine sulfoxide in rats. The variation within the data reported may be due to different methods employed for determining bioavailability, as well as the strains and age of rats used in the bioassay for methionine sulfoxide. Evans and Bauer (1978) noted that the methionine and Cys in autoclaved Sanilac beans were only 40–50% available, while Sgarbieri and coworkers (1979) found that only 30–40% of the methionine was available in four other cultivars of *Phaseolus vulgaris* tested.

The bioavailability of the methionine, Cys and lysine residues in a protein is determined by two factors; (1) the degree to which the protein can be digested by proteases and peptidases, a measure of the total quantity of essential

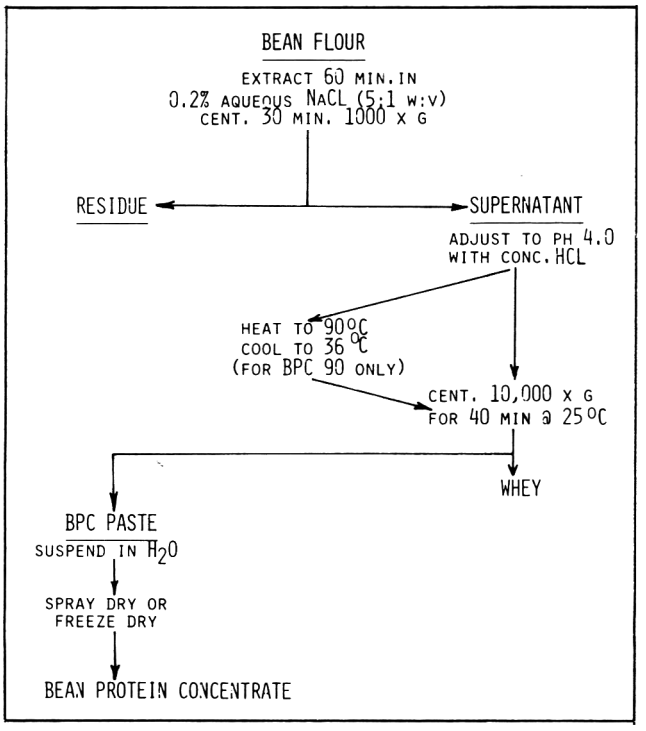

Fig. 2—Laboratory and pilot plant process for the production of bean protein concentrate.

amino acids released via digestion and (2) the chemical structure of the amino acids (i.e., oxidized, reduced, complexed with sugars, phytate) released is also a determinant of their bioavailability for use in synthesizing new tissue protein. Methionine sulfoxide, a very unstable compound, may in fact be rapidly reduced to methionine in the digestive system, which would account for its high degree of bioavailability.

Recent data discussed by Scrimshaw and Young (1978) indicate that soy isolates free of antinutrients (lectins and trypsin inhibitors) have a higher protein nutritional quality when measured in humans, than when measured in the rat. The variation in protein quality assessment between man and the rat could be the result of their differing abilities to use altered (oxidized in this case) sulfur amino acids. Since methionine is the first limiting EAA in soy products, any oxidation of methionine and Cys (Cys can partially make up a methionine deficiency) would lower the nutritional quality of those soy products. This is especially true if the species being fed (the rat) could only partially utilize some of the intermediate methionine oxidation products (i.e., methionine sulfoxide).

It is the objective of this study to characterize the chemical forms of methionine, Cys and lysine present in four legume sources: soy protein, bean protein concentrate from dry beans, processed peanut protein and leaf protein from alfalfa. Secondly, any changes that may occur in these amino acids will be determined at each step throughout the processing of the four seed and plant source into finished protein products.

MATERIALS & METHODS

Protein samples

Legume protein samples used in this study were either prepared in the laboratory, a pilot plant, or obtained from a full scale commercial facility. Protein products from peanuts were prepared and processed in the pilot plant facilities in the Dept. of Food Science, Clemson Univ., Clemson, SC. Bean protein concentrate (BPC) was isolated in the laboratory and pilot plant using the process described by Chang and Satterlee (1979). Leaf protein samples were obtained from a commercial processor. Soy protein products were supplied

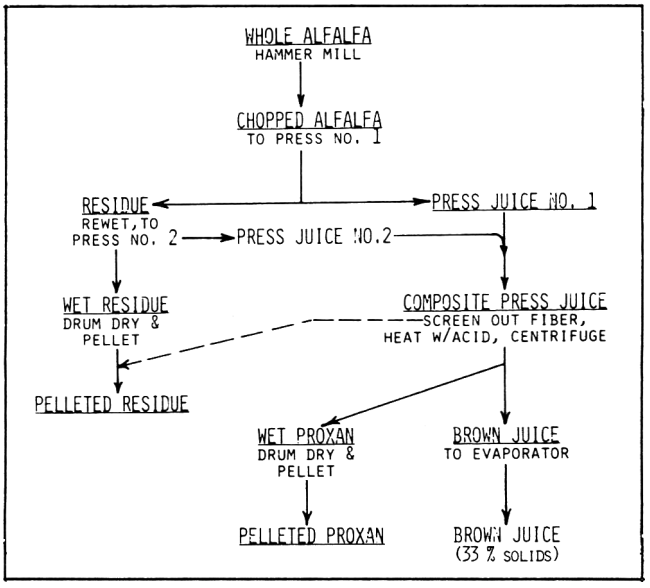

Fig. 3—Commercial process for fractionating alfalfa.

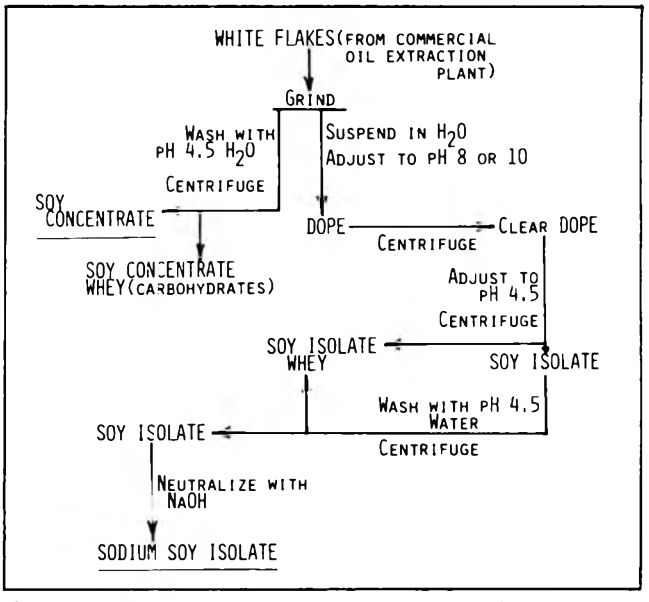

Fig. 4—Laboratory process used to prepare soy samples.

by two industrial sources, as well as being prepared in the laboratory. The flow diagrams (Fig. 1–4) illustrate the methodologies used for the preparation of the laboratory, pilot plant, and industrial samples.

Instrumentation

A Beckman Model 120 C Amino Acid Analyzer was used for all amino acid analyses. Spectrophotometric measurements were performed on a Hitachi Perkin-Elmer Model 139 UV-Vis spectrophotometer.

Sulfur amino acid determinations

The procedures for the identification and quantitation of reactive Cys, and the oxidized forms of Cys and methionine are described in detail in the preceding paper (Chang et al., 1982).

Lysine determinations

Total and reactive lysine was measured with the amino acid analyzer. For each sample, total lysine was determined from an acid hydrolysate (Moore and Stein, 1963). Reactive lysine for a sample was measured as homoarginine following guanidation with o-methylisourea (MIU) of those lysine residues having free ε-amino groups (Creamer et al., 1976). Lysine, determined as homoarginine was performed on a 9 cm column containing Beckman PA-35 resin. The elution buffer was 0.8N Na citrate, pH 7.0 with a flow rate of 70 ml/hr.

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Table 1—Total and reactive cysteine/cystine; total methionine, methionine sulfoxide; total and reactive lysine for sample obtained from the processing of peanuts

	Cysteine/Cystine g/16gN		Methionine g/16gN		Lysine g/16gN	
Sample[a]	Total	Reactive	Total	MetSO	Total	Reactive
Blanched peanut splits	1.35	1.40	1.47	0	3.32	2.96
Peanut slurry (65.5C)	1.35	1.46	1.65	0	3.38	2.99
Heated slurry (121C)	1.38	1.49	1.48	0	3.42	3.10
Drum dried product	1.34	1.46	1.21	0	3.36	3.08

[a] For processing details see Fig. 1.

Initially, the dye-binding procedure of Hurrell and Carpenter (1974) was used for the analysis of reactive lysine; however, reactive lysine values obtained were substantially higher than total lysine values for most of the samples studied, therefore the MIU procedure was used to determine reactive lysine in each sample.

RESULTS & DISCUSSION

Peanut processing

The Clemson peanut process has no detrimental effect on the Cys residues in the proteins of the starting material, intermediate and final products studied (Table 1). Total and reactive Cys were equal and no oxidized forms of methionine were detected. The reactive lysine content of materials and all products remained unchanged throughout the process. The process, as illustrated in Fig. 1, involves a restructuring of the protein via grinding in the presence of H_2O, cooking and drying. Under these conditions of abundant moisture and short periods of high temperature, Maillard, oxidation and condensation (lysinoalanine formation) type reactions would not be expected to occur to any large degree.

Dry bean processing

Results obtained for the analysis of processing of dry beans are presented in Table 2. Reactive Cys is slightly lower than the total Cys in the original bean flour and appears to be substantially lower in the laboratory and pilot plant produced BPC products. The lower concentrations of reactive cys in the bean protein concentrate (BPC) products is consistent; however, errors obtained when measuring these small quantities of Cys (total and reactive) could lead to the variability in Cys values seen here. In an examination of this process, it is apparent that there are not any specific steps that should cause losses of any of the amino acids. This is also indicated by the methionine and lysine data, which show little or no losses. Yet we do see appreciable losses of reactive Cys in all BPC products, a result for which we have no good explanation at this time. It should be noted here that for all four processes studied, the only oxidized form of methionine present was methionine sulfoxide (MetSO). Methionine sulfone ($MetSO_2$) was never detected in any of the food protein samples examined.

Alfalfa processing

Analysis of the leaf protein samples for reactive Cys was inconclusive, as color interference from the leaf pigments gave consistently unreliable results (Table 3). The total Cys and methionine content of the samples varied to a small degree; this variation most likely was due to the difficulty of oxidizing these samples with performic acid and the subsequent acid hydrolysis. Analysis of whole alfalfa shows it to contain a small amount of methionine sulfoxide. Generally the methionine sulfoxide tends to be equally distributed into the various products formed by processing with the greatest percentage of oxidized methionine in the brown juice fraction. This results from the lower total methionine concentration of this fraction and does not reflect an increase in methionine oxidation due to processing or an accumulation of methionine sulfoxide residues in this fraction.

There were some losses of lysine in those products that underwent heat treatment, with the protein in the pelleted residue fraction appearing to be more heat stable than that of the Proxan fraction. A possible reason is that protein in the residue fraction is protected by being encapsulated within a fibrous matrix whereas the Proxan fraction consists of cytoplasmic protein and chloroplasts, both of which have lysine vulnerable to losses due to heat treatment.

The brown juice component is contained within the composite press juice fraction. Reducing sugars and the enzyme polyphenol oxidase are present in brown juice both which can enhance EAA losses. A resulting highly reactive environment exists in the brown juice and would contribute losses of methionine and lysine observed for this fraction.

Soybean processing

The reactive lysine content for all soy fractions and products is approximately 93–100% of their total lysine content (Table 4). Measurable lysine losses resulting from Maillard reactions or LAL formation would not be expected from the process as it is shown. Over a period of several years, we have analyzed numerous soy products in our laboratory and have found lysinoalanine in food proteins on rare occasions. When detected in soy samples, LAL was in very small quantities.

Appreciable losses of reactive Cys and methionine to oxidation did not occur, with the exceptions of the whey fractions resulting from the soy concentrate and isolate production (Table 4). Soybean protein fractions (enzymes, protease inhibitors) that are rich in sulfur amino acids and peptides containing oxidized sulfur amino acids are present in the whey fractions (Catsimpoolas, 1969; Weil et al., 1969). Green gram seeds (Kasai et al., 1972) and kidney beans (Zacharius, 1970) have been shown to contain peptides possessing oxidized sulfur amino acid residues. The peptides are also present in the whey fractions. Close analysis of our data indicates that the small amount of methionine sulfoxide present in the raw soybeans and white flakes is concentrated into the whey fraction during processing. There is no evidence present to show that measurable losses of methionine and Cys occur at any one step in soybean processing, at least when using processes similar to those described in this manuscript.

The commercially processed soy flours, concentrates, and isolates show no significant losses of the sulfur amino acids, with the noteable exception of the product listed as soy isolate #1. This isolate has about 90% of the total Cys content present as reactive Cys and yet over 50% of the total methionine exists as methionine sulfoxide. The model protein studies described in Part I of this paper show similar results for the oxidation of proteins. Low concentra-

Table 2—Total and reactive cysteine/cystine; total methionine, methionine sulfoxide; total and reactive lysine for sample obtained from the processing of dry beans

Sample[a]	Cysteine/Cystine g/16gN		Methionine g/16gN		Lysine g/16gN	
	Total	Reactive	Total	MetSO	Total	Reactive
Bean flour	1.07	0.92	1.57	0	7.57	7.83
Laboratory						
BPC 25	0.91	0.45	1.74	0.05	71.4	7.22
BPC 90	0.91	0.49	1.63	0.06	7.06	7.05
Pilot plant						
BPC 25	0.86	0.60	1.54	0.10	7.77	7.21
BPC 80	0.81	0.57	1.47	0.06	7.72	7.29

[a] For processing details see Fig. 2.

Table 3—Total and reactive cysteine/cystine; total methionine, methionine sulfoxide; total and reactive lysine for sample obtained from the processing of alfalfa

Sample[a]	Cysteine/Cystine g/16gN		Methionine g/16gN		Lysine g/16gN	
	Total	Reactive	Total	MetSO	Total	Reactive
Whole alfalfa	1.22	N.A.	2.05	0.38	5.98	6.09
Press juice #1	1.45	N.A.	1.77	0.36	6.40	N.D.
Press juice #2	1.23	N.A.	1.69	0.44	6.52	N.D.
Composite press juice	1.39	N.A.	1.86	0.35	6.26	5.30
Wet resideu	1.31	N.A.	1.99	0.69	6.53	5.73
Pelleted residue	1.05	N.A.	1.62	0.38	4.84	4.66
Wet proxan	0.96	N.A.	2.19	0.30	6.79	6.23
Pelleted proxan	1.05	N.A.	2.23	0.48	6.48	5.30
Brown juice	1.69	N.A.	0.85	0.37	4.89	4.04
Brown juice w/50% solids	1.68	N.A.	0.82	0.32	4.87	N.D.

[a] For processing details see Fig. 3.
N.A. - because of color interference, not analyzed.

Table 4—Total and reactive cysteine/cystine; total methionine, methionine sulfoxide; total and reactive lysine for sample obtained from the processing of soybeans

Sample[a]	Cysteine/Cystine g/16gN		Methionine g/16gN		Lysine g/16gN	
	Total	Reactive	Total	MetSO	Total	Reactive
Raw soybeans	1.49	1.43	1.59	0.04	6.21	6.40
White flakes	1.52	1.32	1.48	0.06	6.41	5.97
Commercial						
Soy flour 1	1.50	1.28	1.44	0	6.29	5.88
Soy flour 2	1.40	1.32	1.46	0	6.28	5.97
Soy flour 3	1.41	1.26	1.48	0	6.17	6.15
Laboratory						
Soy conc.	1.15	1.14	1.44	0	6.06	5.84
Whey-Soy conc.	3.26	2.36	1.55	0.35	7.02	7.41
Commercial						
Soy conc. 1	1.36	1.16	1.43	0	6.28	6.00
Laboratory						
Na soy isol. pH 10	1.23	1.09	1.47	0	5.93	5.87
Whey-Na soy isol. pH 10	3.36	2.51	1.59	0.35	6.80	7.53
Na soy isol. pH 8	1.12	1.12	1.41	0	5.66	5.84
Whey-Na soy isol. pH 8	2.94	2.29	1.41	0.54	7.30	7.52
Commercial						
Soy isolate 1	1.13	1.00	1.37	0.70	6.11	5.91
Soy isolate 2	1.09	1.04	1.32	0	6.12	5.79
Soy isolate 3	1.16	1.10	1.34	0	6.30	6.11
Soy isolate 4	1.07	1.18	1.28	0	6.16	6.06

[a] For processing details see Fig. 4.

tions of H_2O_2 (0.25%), result in appreciable oxidation of methionine residues to methionine sulfoxide, while the Cys residues remain relatively unchanged. It is entirely possible that the procedures employed to produce this isolate involve a mild oxidation step that results in the preferential oxidation of methionine residues in contrast to Cys. Perhaps this may be the addition of an oxidizing agent to remove off-colors from the final product.

CONCLUSIONS

THE PROCESSES that were described in this manuscript have no adverse effects on sulfur amino acids, except the processing of dry beans to produce protein concentrates appears to result in the oxidation of a significant number of Cys residues, a result for which we have no explanation. The high levels of methionine sulfoxide in the whey fractions obtained from the laboratory processing of soybeans have been shown to be due to a concentration of the inherent sulfoxide from the raw bean into these fractions, and not an increase in oxidation of methionine due to processing. The commercial soy products showed little or no oxidized sulfur amino acids to be present, except for one soy isolate. The loss of sulfur amino acids in this soy isolate sample was similar to the losses seen for the casein sample oxidized with 0.25% H_2O_2 at 40°C. This suggests that when processing soy beans to produce this isolate, a mild oxidation step or environment was employed.

There were no significant losses of lysine in any samples tested. Therefore, the use of available (reactive) lysine assays to indicate general essential amino acid damage or possible damage due to processing is questionable.

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Functional Properties and Food Applications of Rapeseed Protein Concentrate

L. U. THOMPSON, R. F. K. LIU, and J. D. JONES

ABSTRACT

Rapeseed protein concentrate (RC), prepared with 2% hexametaphosphate, was tested for its functionality and performance in some foods. The RC had good nitrogen solubility, fat absorption, emulsification, and whipping capacities but poor water absorption and gelling properties. It increased the emulsion stability, and protein but lowered the fat content of wieners. It also increased the cooking yield, reduced the shrinkage and tenderized meat patties. Results were similar to soybean isolate except for the poorer color and flavor. The cooking yield of RC supplemented wieners was less than the all-meat control and soybean-supplemented wieners. A 9% RC dispersion mixed with an equal volume of eggwhite produced a meringue of comparable stability and texture to that of eggwhite alone.

INTRODUCTION

THOMPSON and co-workers (1976, 1982) prepared a protein concentrate (RC) from rapeseed flour (RF) using 2% sodium hexametaphosphate (SHMP). The nitrogen yields were either higher than or similar to those obtained by other investigators (Sosulski and Bakal, 1969; Owen and Chichester, 1971; Eklund et al., 1971; Girault, 1973). Also, the color of SHMP-RC was much lighter than the brown rapeseed isolates commonly prepared by alkali extraction-acid precipitation methods and has a higher protein efficiency ratio than casein without adverse effects on growth and thyroids of the rats (Liu et al., 1982) when fed diets at 10% protein level.

In order for new proteins such as the SHMP-RC to be adopted by the food industry, however, they should maintain or enhance the quality of the food in which they are used. They should not only have satisfactory nutritional properties, but also acceptable color, flavor and texture (Kinsella, 1976). In addition, new proteins should possess specific functional properties for incorporation into various products. Therefore, the objective of this study was to determine the functional properties of RC and its potential as a functional ingredient in wieners, beef patties and meringues.

EXPERIMENTAL

Materials

Dehulled, solvent extracted Tower RF was provided by the Food Research Institute, Ottawa, Ontario. RC was prepared from it using SHMP as described in an earlier report (Liu et al., 1982). Promine-D, a commercial soy protein isolate (SI), was obtained from Central Soya Corporation and fresh eggs, lean ground beef and seasoning from a local supermarket. All the basic wiener ingredients were provided by Griffiths Laboratories Limited, Scarborough, Ontario.

Functional properties evaluation

Nitrogen solubility was determined according to AACC (1969). One percent sample dispersion was stirred for 3 min, adjusted to pH 7 by addition of 0.1N NaOH or 0.1N HCl and stirred with a magnetic stirrer for 2 hr. Following centrifugation, aliquots of the supernatant were analyzed for nitrogen by microkjeldahl method. Water absorption was measured by the centrifugation method of Sosulski (1962). The sample (0.5g) was dispersed in 3.0 ml distilled water for 30 min with occasional stirring, followed by centrifugation, decanting of the supernatant, removal of excess moisture by draining for 25 min at 50°C, and reweighting of the sample. The percent water absorption was equal to the increase in sample weight divided by the sample weight times 100. Water absorption was determined by the method of Hagenmaier (1972) and expressed as percent weight increase after exposing the sample to an environment of 84% relative humidity. Fat absorption was measured by the technique of Lin et al. (1974). The sample (0.5g) was mixed with 3.0 ml corn oil in a 15 ml graduated centrifuge tube for 1 min. After a holding period of 30 min, the tube was centrifuged and the volume of free oil was read. Percent fat absorption was the amount of corn oil bound per 100g sample.

Oil emulsifying capacity was measured by titrating 75 ml of 1 or 2% sample dispersions with colored corn oil until the emulsions collapsed (Marshall et al., 1975) and expressed as ml oil per g sample. To determine emulsifying stability, a 1% sample dispersion (75 ml) was blended ina Waring Commercial Blendor at a high speed for 1 min. A volume of oil equivalent to 80% of the emulsifying capacity was then added and the mixture blended for another 10 sec. The emulsion was then transferred to a 100 ml cylinder and the ml water which separated per given time, up to 35 hr, was recorded.

Whipping capacity was expressed as percent volume increase of 50 ml 3 or 5% sample dispersions whipped with a high speed Sunbeam electric mixer for 6 min, and foam stability as volume of foam remaining after a standing period of up to 120 min (Lawhon and Cater, 1971). Color lightness (Y_{CIE}) was measured by reflectance spectrophotometry (Clydesdale and Francis, 1969), and bulk density was expressed as volume per gram sample.

Sample dispersions (10%) were blended at a high speed for 1 min, centrifuged at a low speed to remove entrapped air, and measured for viscosity at 25°C using a Brookfield-LVT viscometer with a standard #3 spindle rotating at 12 rpm. To test the gelling properties, the dispersions were heated in an 80°C water bath for 30 min and cooled to 25°C. The viscosity of the undisturbed dispersions was measured using the same viscometer with a T spindle 'C' and Helipath stand.

All functional property tests were carried out in triplicate with standard deviations of not more than two percent. Fresh eggwhite and/or SI were used as controls.

Preparation of wieners

Two experimental formulations of wieners (Table 1) were prepared. Formulation II contained a higher percentage of lean meat, and additional liquid smoke and paprika compared to Formulation I. Both meat block formulations were adjusted to provide approximately 26% fat in the final product. The unsupplemented wieners contained only the basic ingredients while the protein supplemented wieners contained both the basic and additional ingredients. RC and SI were added in sufficient amounts to raise the protein content of the wieners by about 2%. The RC and SI were hydrated with cold water at 1:2 and 1:3 ratios, respectively; preliminary runs showed these ratios to be optimum.

In processing and evaluating wieners, the procedures outlined by Sosulski et al. (1977) and Lin et al. (1975) were followed with slight modifications. The wiener ingredients were chopped and blended in a laboratory silent cutter for 15 min with a final emulsion temperature not exceeiding 18°C. The emulsions were stuffed into 2.3 cm synthetic casings (Visking Nojax, striped, E-Z peel) and

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machine-linked to 12.5 cm length. The wieners were weighed and then heated and smoked in a commercial smokehouse for 1½ hr to a final temperature of 89°C. The processed weiners were cooled by a water shower, drained, reweighed and refrigerated. Two batches of each wiener formulation were processed for statistical analysis of the data.

Evaluation of processed wieners

Wiener emulsions were sampled and tested immediately for pH and emulsion stability 10 min after they were formed. Emulsion stability, expressed as percent of total fat bound by the emulsion after heating at 70°C for 30 min, was determined by the method of Saffle et al. (1967). Processing shrinkage was calculated as percent weight loss after the wieners were heated, smoked, and cooled in the smoke-house. Firmness of peeled wieners was measured using a Precision Universal Penetrometer equipped with a 150g load and expressed as mm penetrating distance. Peelability of the casing and color of the peeled wieners were evaluated by a 12-member panel using a 7-point scale (7 = extremely easy to peel, 1 = extremely difficult to peel; 7 = like very much, 1 = dislike very much). Color lightness (Y_{CIE}) was measured by reflectance spectrophotometry (Clydesdale and Francis, 1969). Proximate analyses of the processed wieners were conducted according to standard procedures (AOAC, 1975).

Evaluation of cooked wieners

The methods developed by Lin et al. (1975) were used to evaluate the cooking yield, cooking loss (total, nonfat and fat) and water absorption of the cooked wieners. Firmness of boiled wieners was measured with a penetrometer and reported as mm penetrating distance. For organoleptic evaluation, peeled wieners were boiled in water for 10 min, drained, and served warm to a 12-member panel. Color, texture, flavor and overall acceptability were evaluated as above by the panel.

Preparation of meat patties

Ground beef (ca 16% fat) and seasoning (Club House Season All, containing salt, MSG, onion, red pepper, annato, $MgCO_3$ and spices) were the basic meat patty ingredients. The all-meat patties contained 98% ground beef and 2% seasoning. The protein concentrate/isolate extended patties consisted of 87.5% ground beef, 3% of either RC or SI, 7.5% water to hydrate the proteins, and 2% seasoning. The appropriate ingredients were mixed in a Hobart mixer for 3 min and made into patties (110g, 10 cm diameter, 1.3 cm thick) using a K-tel Pattie Stacker. The patties were wrapped in polyethylene freezer bags and kept frozen (−18°C) until studied. No weight loss occurred during frozen storage.

Table 1—Formulation of experimental wieners

Ingredients	Formulation, g/100g basic ingredients I	II
Basic:		
Meat block		
Lean beef chuck	11.5	13.5
Lean pork trim	11.5	13.5
beef plates	12.1	13.5
beef hearts	10.1	13.5
beef tripe	6.8	0
back fat	15.5	13.5
Cold water	24.2	24.2
Salt	0.8	0.8
Spice[a]	0.7	0.7
Curing agent[a]	0.4	0.4
Binder[a]	6.5	6.5
Liquid smoke[a]	0	0.03
Paprika[a]	0	0.13
Additional[b]:		
RC (or SI[c])	3.8 (3.3)	3.8 (3.3)
Cold water	7.6 (9.9)	7.6 (9.9)
Salt	0.13	0.13
Spice[a]	0.13	0.13

[a] Griffith's Laboratories Ltd., Toronto
[b] Added to RC or SI supplemented wieners only.
[c] Promine D from Central Soya Corp.

Evaluation of cooked patties

The patties were weighed and then cooked in the frozen state in an oven (Harwick Gas Oven) at 200°C for 7.5 min on each side. After cooking to room temperature, the patties were reweighed and the percent cooking yield determined. Moisture, protein and fat contents of the cooked patties were determined by standard methods (AOAC, 1975). Percent retentions of fat and moisture in the cooked patties were calculated by multiplying the percent cooking yield by the percent fat or moisture in cooked patties and dividing the resulting product by the percent fat or moisture of the raw patties (Anderson and Lind, 1975). Changes in diameter and thickness of the patties were measured with a pair of vernier calipers 45 min after cooking. The mean of six measurements was taken as the representative diameter and thickness of each patty. The firmness of cooked patties was measured using a Precision Universal Penetrometer with a 150g load; the mean of ten measurements per patty were reported.

The appearance, texture, flavor and overall acceptability of cooked patties were evaluated by a 12-member panel according to a 7-point hedonic scale (7 = like very much; 1 = dislike very much). The cooked patties were evaluated with and without catsup (15g Heinz Catsup per 100g patty) spread on the top.

Preparation and evaluation of meringue

Soft meringues were prepared from egg white, 9% RC dispersion in water and 1:1 mixture of egg white and 9% RC dispersion. Fifty ml of each protein sample was beaten 6 min to produce soft peaks followed by gradual, sequential addition of 48.8g sugar and 0.5g salt throughout a total beating time of 12 min. Preliminary experiments indicated that these conditions were optimum for producing meringues. Each whipped sample (ca 75g) was weighed and spread on a perforated aluminum pan (20 x 13 x 3 cm) supported by another pan and baked in an oven at 218°C for 7 min. After a 10 min cooling period, the total weight loss and the liquid that dripped from the meringue during baking were measured. The stability of the baked meringue was determined as weight of the liquid lost within a 2-hr period following baking. The volume, color, sheen, texture and flavor of the cooked meringue were evaluated subjectively.

RESULTS & DISCUSSION

Functional properties

Some functional properties of RC, RF and SI are presented in Table 2. The nitrogen solubility of the samples was determined as a function of pH (Table 2) since it provides a good index of the potential or limitation of a protein as a functional ingredient. It often relates to many other functional properties such as emulsification, foaming etc. (Kinsella, 1976) and is perhaps one of the most practical index of protein denaturation. The samples were least soluble at their isoelectric pH i.e. about pH 4 for RF and SI and pH 2–4 for RC. The lower solubility values for RC at pH 2–4 was probably due to SHMP-protein interactions. From pH 7–10, both RC and RF had similar solubilities suggesting that their proteins are relatively undenatured. The good nitrogen solubility of RC at pH 6–7 indicates that it may be useful in processed meat products and milk-type beverages which have similar pH's.

The water absorption capacity (Table 2) was determined by mixing the sample with excess water followed by centrifugation and decanting of the supernatant. The water absorbed was calculated by measuring the difference between the sediment and sample weight. This method, however, does not account for the soluble proteins that are decanted with the supernatant. Therefore, samples containing different proportions of soluble to insoluble protein cannot be accurately compared as to water absorption

capacity (Quinn and Paton, 1979). Because of the relatively high solubility of the samples (Table 2), water adsorption was also determined using the humidity equilibrium method of Hagenmaier (1972). This method is not affected by solubility and, therefore, data from both methods will give a better estimate of the water-holding capacity of the samples. The water absorption of RC is half that of RF and three to four times less than that of SI; it appears that the insoluble proteins in both RF and SI swell and retain more water than those in RC. The water adsorption of RC, however, is only slightly poorer than SI. This suggests that if water is not added in excess, the RC has just about as good water-holding capacity as SI. Ability to hold water is an important function of proteins in foods such as sausage meats, processed cheese, doughs, etc.

The RC has a better fat absorption capacity than RF and SI (Table 2). The relatively higher bulk density of RC could have contributed to the better fat absorption since the centrifugation method of assessing fat absorption measures the amount of oil physically entrapped by the protein. It had been shown that increasing the bulk density of a protein by chemical modification concomitantly enhances fat absorption (Franzen, 1975). In addition, the RC probably has more exposed hydrophobic groups than RF and SI. Sosulski et al. (1976) reported that rapeseed flour, concentrate and isolates were superior to the corresponding soy products in fat absorption.

The 1% RC dispersion emulsified more oil than RF but less than SI. The RC emulsion was more stable than that of RF but less than the SI emulsion. Increasing the protein sample concentration to 2% did not improve the oil emulsification of RC, decreased that of RF and resulted in an SI emulsion too thick to determine the titration end-point. On the contrary, the emulsion stability of RC was improved and was more stable than the 1% SI emulsion.

Using 3% aqueous dispersions, RC showed higher whipping volumes than RF and SI but lower than egg white. A volume higher than that of egg white was obtained when a 5% RC dispersion was whipped. Considering that fresh eggwhite had about 11% protein, on a protein basis, the 5% RC dispersion appeared to be a better whipping agent. In addition to the natural ability of rapeseed protein to whip, the complexed and entrained SHMP probably helped in the formation of a good foam. SHMP has been used as an additive to improve the performance of egg albumen in angel cakes (Chang, 1973).

The RC and egg white foam characteristics differed at the above concentrations. RC had a soft, flowing, cream-colored foam with medium-size air bubbles while egg white had hard, brittle white foam with small-size air bubbles. The stability of the RC foam was comparable to that of egg white.

The viscosity of unheated 10% dispersion of RC was half that of RF and about 30 times less than SI (Table 2). When heated, the RC and RF did not show the firm gelation characteristics of SI. The heated RC dispersion did not gel upon cooling but formed a viscous paste. The viscosity of each heated sample increased towards the base of the container; hence there was a range of viscosity values.

As previously noted (Thompson et al., 1976), the color of RC was slightly darker than RF or SI, but much lighter than the brown RC prepared by the common methods involving alkali extraction and acid precipitation. The discoloration can be attributed to the phenolic constituents previously identified by Kozlowska et al. (1975).

In general, except for the water and fat absorption capacities, RC appeared better in functionality than the previously tested rapeseed protein isolates and concentrates (Sosulski et al., 1976). It was also definitely better than the RF from which it was prepared except for the water adsorption and absorption properties. The high emulsification, water and fat absorption capacities suggest the possibile use of RC in processed meat products. The good whipping properties point to its potential use as egg white replacements in products like meringue. The performance of RC in wieners, beef patties and meringue was therefore investigated in this project.

Evaluation of wieners

In the preparation of wieners, two formulations were investigated. A second formulation was tried on the assumption that a better quality meat block (more lean meat) with additional coloring agent (paprika), and flavor (liquid smoke, spice and salt) would improve the acceptability of the RC-supplemented wieners. The all-meat and SI-supplemented wieners were used as controls. Both RC and SI were added on an equal protein basis. The characteristics of wiener emulsions and processed wieners with and without protein additives are presented in Table 3.

The pH of the emulsions were determined immediately after sampling since the pH of a system affects the func-

Table 2—Functional properties of rapeseed flour (RF) and concentrate (RC), and soybean isolate (SI)

Functional property	RF	RC	SI[a]	EW[b]
Nitrogen solubility, %				
pH 2	39.8	2.6	72.2	
pH 4	29.2	5.4	13.9	
pH 7	54.6	56.0	56.9	
pH 8	75.2	74.4	65.2	
pH 10	89.5	94.2	86.2	
Water absorption, %	266	137	478	
Water adsorption, %	18.2	17.7	18.6	
Fat absorption, %	240	263	200	
Bulk density, ml/g	3.5	3.9	2.8	
Emulsifying capacity, ml oil/g sample				
1% dispersion	89.8	108.0	191.3	
2% dispersion	53.5	109.7	c	
Emulsion stability, ml[d]				
1% dispersion	44	27	14	
2% dispersion	42	3	–	
Whipping capacity, % vol. increase				
3% dispersion	554	628	19	687
5% dispersion	597	851	77	
Foam stability[e], 3% dispersion				
0 min	340	378	11	393
20 min	320	352	10	386
40 min	308	336	10	373
60 min	304	332	10	363
120 min	303	331	10	363
Viscosity, cps				
unheated	50	25	825	
heated	512–57,500	16,600–45,000	>16,600	
Color lightness, YCIE	65.4	57.6	69.1	

[a] Promine D from Central Soya Corporation
[b] Fresh egg white control in whipping and foaming tests
[c] Dispersion gave an emulsion too thick to determine titration end-point
[d] ml water separated after 35 hr
[e] ml foam remaining per given time

tional behaviour of the proteins (Kinsella, 1976). The pHs of the emulsions were essentially the same for those with and without protein additives. Formulation II wieners had significantly higher emulsion stabilities than the corresponding wieners of formulation I. This was expected since the second formulation had a higher percentage of lean beef and a lower percentage of back fat (Table 1). Lean beef has been reported (Sofos and Allen, 1977; Sofos et al., 1977) to give a more stable emulsion than other meat trimmings often used in wiener manufacture.

The emulsion stabilities of formulation I RC and SI supplemented wieners were significantly higher than the all-meat control. With formulation II, the RC emulsion was significantly more stable than the all-meat control. The SI emulsion, however, had the greatest stability confirming earlier results which showed SI to have slightly better emulsifying properties than RC (Table 2). Some rapeseed protein products have been shown to decrease the stability of wiener emulsions (Sosulski et al., 1977).

The RC and SI supplemented wieners did not differ significantly in composition (Table 3) since both RC and SI were added to the basic ingredients in almost the same amounts. The addition of RC and SI to the basic wiener mix resulted in a 2% increase in the protein content, as expected. A slightly higher protein content of all the formulation II than the formulation I wieners was probably due to the lower fat content of the formulation II meat block. Supplemented wieners of both formulations had higher water and ash but lower fat contents than the all-meat controls. The lower fat was mainly due to dilution of the fat by the protein additives which have low fat contents.

Higher percentage process shrinkage was observed in formulation I than formulation II wieners. As observed by Sofos and co-workers (1977), the increased concentration of lean beef in formulation II contributed to lower losses during cooking and smoking. The RC-supplemented wieners were not as firm as the all-meat control which may be due to the poor gelling properties of RC.

Color lightness as determined by reflectance spectrophotometry did not differ significantly among samples and between formulations. However, the mean color scores for RC as determined by the 12-member sensory panel was below acceptability levels compared to the mean score of the other two controls. All the samples were extremely easy to peel.

The effect of protein additives on the cooking and organoleptic qualities of wieners are presented in Table 4. The cooking yield of RC-supplemented wieners was less than the all meat control and SI-supplemented wieners. The fact that the RC-supplemented wieners did not absorb as much water upon cooking and also lost the highest percentage of nonfat components might have been responsible for the low yield. As previously shown in model systems, RC is relatively soluble in water and in the presence of excess water, had a low water absorption capacity (Table 2).
3), cooked RC-supplemented wieners exhibited the least firmness of all the samples. Mean scores for color, texture, flavor and overall acceptability of all RI-supplemented wieners were significantly lower than the all-meat and SI-supplemented wieners. The change to a better quality meat block and the addition of extra flavor and color ingredients did not have any significant effect on the acceptability of the wieners.

Evaluation of beef patties

The effects of extending beef patties with RC were determined and the results compared with those of all-beef and SI-extended patties. The composition, cooking yield and organoleptic properties of the beef patties are presented in Table 5.

In the raw state, the patties did not differ significantly in moisture and protein contents. The protein extended patties had less fat due primarily to dilution by the added protein products. When cooked, changes in composition

Table 3—Effect of protein additives on functional characteristics of wiener emulsions and processed wieners[a]

Characteristics	Formulation[b]	Protein additive None	RC	SI[c]
Wiener emulsion				
pH	I	6.9	6.8	6.9
	II	6.7	6.8	6.8
stability, %	I	82.3^{XA}	88.7^{YA}	89.9^{YA}
	II	86.0^{XB}	91.9^{YB}	94.2^{ZB}
Moisture, %	I	56.4^{X}	57.0^{Y}	57.8^{Y}
	II	56.3^{X}	57.7^{Y}	57.4^{Y}
Protein[d], %	I	13.7^{XA}	15.4^{YA}	15.7^{YA}
	II	14.4^{XB}	17.1^{YB}	16.5^{YB}
Fat[d], %	I	25.9^{X}	22.9^{Y}	23.4^{Y}
	Ii	25.2^{X}	23.5^{Y}	23.0^{Y}
Ash[d], %	I	3.2	3.6	3.4
	II	3.3	3.9	3.5
Shrinkage, %	I	9.9	9.1	10.2
	II	8.4	8.6	9.2
Firmness, mm	I	12.1^{XA}	14.5^{YA}	13.1^{ZA}
	II	13.3^{XB}	15.1^{YB}	14.0^{ZB}
Color lightness, YCIE	I	46.5	46.0	47.4
	II	45.6	46.1	47.8
Color score[e]	I	4.7^{X}	2.1^{Y}	5.3^{X}
	II	4.9^{X}	2.8	4.8^{X}
Peelability score[f]	I	7.0	7.0	7.0
	II	7.0	7.0	7.0

[a] Means with different superscripts (X-Z) within each row, and different superscripts (A-B) within each column are significantly different ($p \leq 0.05$).
[b] Described in Table 1
[c] Promine D from Central Soya Corporation
[d] Expressed on a 52% moisture basis
[e] 7 = like very much; 1 = dislike very much.
[f] 7 = extremely easy to peel; 1 = extremely difficult to peel.

Table 4—Effect of protein additives on cooking and organoleptic properties of wieners[a]

Quality	Formulation	Protein additive None	RC	SI[b]
Cooking yield, %	I	98.1^{XA}	95.3^{Y}	98.2^{X}
	II	99.2^{XB}	96.0^{Y}	98.4^{X}
Cooking loss, %				
total	I	4.0^{X}	5.3^{Y}	3.1^{Z}
	II	2.8^{X}	4.8^{Y}	3.0^{X}
nonfat	I	1.7^{XA}	3.2^{Y}	1.9^{X}
	II	1.3^{XB}	3.5^{Y}	1.9^{Z}
fat	I	2.3^{XA}	2.1^{XA}	1.2^{Y}
	II	1.5^{B}	1.3^{B}	1.1
Water absorption, %	I	2.1^{X}	0.6^{Y}	1.3^{Z}
	II	1.9^{X}	0.8^{Y}	1.4^{Z}
Firmness, mm	I	19.1^{XA}	22.0^{YA}	20.3^{Z}
	II	21.1^{XB}	22.9^{YB}	20.3^{Z}
Acceptability[c]				
color	I	4.9^{X}	3.4^{Y}	5.4^{X}
	II	5.1^{X}	3.7^{Y}	4.7^{X}
texture	I	5.5^{X}	4.1^{Y}	5.3^{X}
	II	5.4^{X}	3.8^{Y}	4.3^{Y}
flavor	I	5.5^{X}	3.6^{Y}	5.1^{X}
	II	5.3^{X}	3.3^{Y}	4.1^{Z}
overall	I	5.4^{X}	3.7^{Y}	5.1^{X}
	II	5.2^{X}	3.5^{Y}	4.3^{Z}

[a] Means with different superscripts (X-Z) within each row, and different superscripts (A-B) within each column are significantly different ($p \leq 0.05$).
[b] Promine D from Central Soya Corporation
[c] 7 = like very much; 1 = dislike very much.

could be attributed to losses from evaporation of volatile materials and release of fluid components as drip; volatile losses are the result of evaporation of water and other volatile components, whereas drip losses are the result of losses of fat which melts during the cooking process and losses of sarcoplasmic proteins and/or water (Smith et al., 1976). The cooked patties had similar moisture contents but the protein content of the extended patties were slightly higher than the control. The RC patties had a slightly higher fat content than the control and SI patties.

The protein extended patties retained more of the initial fat and moisture than the control after cooking. The higher fat absorption capacity of RC compared to SI (Table 2) probably resulted in the RC patties retaining the most fat. Both RC and SI have comparable water adsorption capacities (Table 2); hence the patties with these extenders had similar moisture retention values. The higher cooking yield of the extended patties could also be related to their higher retention of fat and moisture. Other investigators also found that the addition of soy protein extenders to meat patties resulted in higher cooking yields (Anderson and Lind, 1975; Bowers and Engler, 1975; Drake et al., 1975).

The mean shrinkage of the RC and SI extended patties were lower than the all-beef control. The changes in the diameter of the patties during cooking negatively correlated with cooking yield. The patties with the highest yield (RC patties) showed the smallest decrease in diameter. Thomas et al. (1978) reported similar observations in beef patties extended with milk coprecipitates.

While the appearance and texture of the RC patties were acceptable and did not differ significantly from the SI and all beef patties, the flavor was less acceptable. The undesirable flavor was obviously responsible for the lower acceptability of the RC patties. The flavor and acceptability of the patties did not improve even when served with catsup.

Evaluation of meringue

Since the RC has good whipping properties, its performance in meringue was evaluated in comparison with egg white. Results of the evaluation are summarized in Table 6.

The RC-meringue was of poorer quality than the egg white meringue. It did not retain much of its volume after baking and liquid dripped from it upon standing. The RC, obviously, did not have the same heat coagulability and capacity to retain air as egg white. The RC-meringue also had darker color and undesirable off-flavor. Mixing the RC with egg white, however, solved some of these problems. The meringue made from the mixture and the meringue with only egg white had similar characteristics except for slight differences in color and flavor. This implies that up to 50% of egg white in meringue may be substituted by a 9% RC dispersion if the color and flavor of RC can be improved.

CONCLUSIONS

THE RC PREPARED with 2% SHMP has very good nitrogen solubility, fat absorption, emulsification and whipping capacities but poor water absorption and gelling properties. Supplementation of wiener with 3.4% RC resulted in an increased emulsion stability, higher protein and ash, and lower fat, firmness and acceptability. There was no change in percent shrinkage, color lightness and peelability score. The cooking yield of RC wieners was less than the all-meat control due to lower water absorption and higher loss of nonfat components during cooking. Except for its higher degree of acceptability, the SI-supplemented wieners did not differ very much from the RC-supplemented wieners. Extending ground beef with RC in meat patties resulted in decreased fat content, higher cooking yield, greater retention of fat and moisture, less shrinkage, and a more tender product than the all-beef patties. Many of the effects of RC were similar to those of SI except for its effect on flavor of which SI was obviously better. Meringue made from 9% RC dispersions lacked the stability and other quality characteristics of those from egg white. When a 9% RC dispersion was mixed with an equal volume of egg

Table 5—Composition, cooking yield, and organoleptic properties of meat patties[a]

Quality	Protein extender		
	None	RC	SI[b]
Moisture, %			
Raw	56.7	57.2	57.1
Cooked	47.5	46.8	47.9
Fat, %			
Raw	15.6^{X}	14.1^{Y}	14.3^{Y}
Cooked	12.3^{X}	13.8^{Y}	12.4^{X}
Protein, %			
Raw	19.8	20.0	20.6
Cooked	25.0^{X}	26.4^{Y}	26.5^{Y}
Cooking yield, %	70.4^{X}	79.6^{Y}	77.7^{Y}
Retention, %			
Fat	55.5^{X}	78.0^{Y}	67.5^{Z}
Moisture	59.0^{X}	65.2^{Y}	65.3^{Y}
Shrinkage, %			
Diameter	23.8^{X}	20.6^{Y}	21.9^{Z}
Height (thickness)	16.7^{X}	15.4^{XY}	14.6^{Y}
Mean	20.2^{X}	18.0^{Y}	18.3^{Y}
Firmness, mm	6.2^{X}	7.6^{Y}	8.0^{Y}
Appearance	4.9	4.8	5.4
Texture	4.5	4.5	5.6
Flavor			
Without catsup	5.6^{X}	3.1^{Y}	5.8^{X}
With catsup	5.3^{X}	3.1^{Y}	5.7^{X}
Overall acceptability			
Without catsup	5.0^{X}	3.5^{Y}	5.4^{X}
With catsup	5.0^{X}	3.3^{Y}	5.4^{X}

[a] Mean within each row with no superscript (X-Z) in common differ significantly ($p \leq 0.05$).
[b] Promine D from Central Soya Corporation

Table 6—Objective and subjective evaluation of soft meringues

	EW[a]	RC-EW[b]	RC[c]
Wt raw meringue, g	74.0	75.2	75.5
Wt cooked meringue, g	64.9	66.7	67.1
Wt loss on baking, g	9.1	8.5	8.4
% Wt loss on baking	12.3	11.3	11.1
Loss in volume after baking	little	little	big
Wt liquid drip after baking, g	0	0	0
Wt liquid drip 2 hr after baking, g	0	0	3.5
% Wt liquid drip 2 hr after baking	0	0	5.2
Cooked meringue			
Color—crust	light brown	light brown	darker brown
interior	white	off-white	light greenish yellow
Sheen	shiny	intermediate	dull
Texture	firm spongy, moist	firm, spongy, moist	soft, watery
Flavor	natural	intermediate	off flavor, grassy, beany after taste

[a] Fresh egg white
[b] 1:1 Mixture of fresh egg white and 9% repeseed protein concentrate dispersion
[c] 9% rapeseed protein concentrate dispersion

white, the mixture produced a meringue of comparable quality to that of egg white, except for the slightly darker color and off-flavor. On the whole, most of the effects of RC on food systems are favorable but its color and flavor still need further improvement for better acceptability.

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higher fiber content. This simple milling and screening procedure may increase the food potential for CDGS and CDG, because fractions that differ widely in protein and dietary fiber contents can be easily obtained.

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Sulfur Amino Acid Stability. Hydrogen Peroxide Treatment of Casein, Egg White, and Soy Isolate

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ABSTRACT

Egg white solids (EWS), soy protein isolate (SPI), and casein were treated with differing levels of H_2O_2 at both 40 and 90°C. Total and reactive cysteine/cystine (Cys), total methionine, methionine sulfoxide and sulfone contents were determined for all samples. At 40°C methionine in all samples was readily converted to its sulfoxide, with little sulfone or cysteic acid being formed. At 90°C Cys was rapidly converted to the stable cysteic acid and methionine to its stable sulfone form. The production of the less stable methionine sulfoxide at 90°C was minimal. Of the three proteins studied, the methionine and Cys residues in EWS were least stable under oxidizing conditions, whereas those present in SPI and casein were more stable.

INTRODUCTION

SULFUR AMINO ACIDS, methionine and cystine/cysteine (Cys), can be converted into various oxidized forms, i.e., methionine sulfoxide, methionine sulfone, cysteine sulfuric acid, and cystein acid, by heat treatment and/or by oxidizing agents under mild heat treatments (Kunachowicz et al., 1976; Strange et al., 1980; Slump and Schreuder, 1973; Schnack and Klostermyer, 1980). The bioavailabilities of the various oxidized forms of methionine and Cys differ, depending on their degree of oxidation. Cysteine sulfuric acid, cysteic acid, and methionine sulfone are completely unavailable to the rat (Anderson et al., 1976), whereas L-methionine sulfoxide is partly utilized by the rat [60% as effective as L-methionine (Anderson et al., 1976)]. Protein bound methionine sulfoxide has also been reported to range from only slightly inferior to as available as protein bound methionine (Cuq et al., 1978; Sjoberg and Bostrom, 1977). The discrepancy between results obtained by the above researchers indicate further research is needed to confirm the bioavailability of methionine sulfoxide in the rat. The variation within the data reported may be due to different methods, as well as different strains and ages of the rat used for determining bioavailability.

Hydrogen peroxide, a strong oxidizing agent, is widely used in food processing, e.g., in the pasteurization of egg white (Cunningham, 1973), in the improvement of the functional properties of egg white in angel cakes (Cunningham and Cotterill, 1962), in the treatment of skim milks for sponge bread baking (Guy et al., 1968), in the treatment of rapeseed flour to reduce glucosinolate content (Anderson et al., 1975), and in the bleaching of a fish protein concentrate (Rasekh et al., 1972). Oxidizing agents, such as benzol peroxide, are used to bleach flour and improve flour quality.

Hydrogen peroxide treatment of food proteins has been reported to decrease the protein nutritional quality (Rasekh et al., 1972; Slump and Schreuder, 1973; Anderson et al., 1975). The oxidation mechanisms of the sulfur amino acids under various heat treatments and H_2O_2 concentrations have not yet been reported. It is the objective of this study to investigate the oxidation of the sulfur amino acids in egg white, casein, and soy protein isolate under various conditions of heat and H_2O_2 concentration.

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MATERIALS & METHODS

Samples

ANRC casein was obtained from Teklad Test Diets, Madison, WI. The spray-dried egg white solids was obtained from Henningsen Foods, Omaha, NE. Soy protein isolate (Supro 710) was obtained from Ralston Purina Co., St. Louis, MO.

Reagents

All chemicals used in this study were of reagent grade quality. Calibration standards for the amino acid determinations were purchased from Pierce Chemical Co., Rockford, IL, or obtained from Sigma Chemical Co., St. Louis, MO. Ellman's reagent] 5,5'dithiobis (2-nitrobenzoic acid)] (DTNB) (Ellman, 1959) used for the reactive Cys determination was obtained from Sigma Chemical Co.

Instrumentation

A Beckman Model 120C Amino Acid Analyzer was used for all amino acid analyses. All spectrophotometric measurements were performed on a Hitachi Perkin-Elmer Model 139 UV-Vis spectrophotometer.

Hydrogen peroxide treatment of egg white solids, ANRC casein, and soy isolate

Two sets of duplicate EWS samples (0.5g/5.0 ml H_2O) containing varying concentrations of H_2O_2 (0.3–4.0% v/v) were heated, with occasional stirring, for 2 hr at 40 or 90°C. After 2 hr, the 40°C samples were brought to 90°C and immediately cooled in order to coagulate the egg white solids. All samples were allowed to stand overnight at 25°C, then thoroughly washed with distilled H_2O to remove any residual H_2O_2. The samples were then freeze-dried.

Solutions containing 1g casein per 13–15 ml H_2O were adjusted to pH 6.7 before the addition of H_2O_2. Final H_2O_2 concentrations were adjusted to be in the range of 0.2–4% v/v. Duplicate sample sets were again prepared, and heated to 40 or 90°C for 2 hr. The 90°C sample was immediately cooled at the end of the 2 hr, and these samples were also allowed to stand overnight at 25°C. The pH of the sample was then adjusted to 4.6 to coagulate the casein. The coagulated samples were washed with distilled H_2O and then freeze-dried. Soy protein isolate was treated with H_2O_2 in the same way as that for the casein samples with the exception that H_2O_2-treated soy protein isolate sample was precipitated at pH 4.5.

Cysteine/cystine determinations

The determination of reactive Cys (presence of free SH group) in each protein used a modification of the procedure of Felker and Waines (1978). The modifications used are as follows: (1) sample weights were chosen so that after grinding with a mortar and pestle and dilution with water, a 0.2 ml aliquot of the sample solution contained 5–25 μg cysteine/cystine; (2) sample solubilization-denaturation was performed with 2.5 ml 8.6N urea-EDTA solution. All reagent concentrations and volumes were adjusted to account for the above listed changes. Additional modifications were: (1) for the reduction of cystine, 25 mg $NaBH_4$ was added to each tube containing sample; (2) the reduction time was lengthened to 2 hr and the temperature was raised to 40°C; (3) Ellman's reagent (DTNB) was prepared in 2M pH 7.5 Tris-CHl buffer to yield a more stable color and was added to the solutions immediately after mixing with acetone; (4) nitrogen purges were omitted; (5) absor-

bances of samples were read at 25 min after the addition of DTNB reagent; (6) a standard curve prepared with glutathione was used in lieu of calculating Cys concentrations from molar absorbtivity.

The total Cys content for a sample was determined as cysteic acid, from an acid hydrolysate of a performic acid oxidized sample (Moore, 1963). Cysteic acid was measured with the amino acid analyzer, which used a 56 cm Beckman AA-15 resin column and an elution buffer of pH 3.49, 0.21N Na citrate at a flow rate of 70 mg/hr.

Methionine determinations

The concentrations of methionine and its oxidized forms in each sample were measured with the amino acid analyzer. Total methionine, measured as methionine sulfone, was determined from an acid hydrolysate of a performic acid oxidized sample (Moore, 1963). Methionine sulfone, endogenous to the food protein was determined from an acid hydrolysate of each sample (Moore and Stein, 1963). A modification of the alkaline hydrolysis procedure of Hygli and Moore (1972), made by substituting thiodiglycol for potato starch (Oelshlegel et al., 1970), was used for the determination of methionine sulfoxide in the sample proteins. Note: a pH 2.2 0.21N Na citrate buffer was used for sample preparation of the alkaline hydrolysates. True methionine content for each sample was obtained by subtracting the methionine sulfoxide and endogenous methionine sulfone content (acid hydrolysate) from the total methionine content.

The measurement of methionine sulfone with the amino acid analyzer utilized the same column and conditions as those for the cysteic acid determinations. Column conditions for methionine sulfoxide separation were identical to those described for cysteic acid, with the exception that a pH 2.95, 0.21N Na citrate buffer was used for elution.

RESULTS & DISCUSSION

Cys oxidation

Fig. 1 shows the degree of oxidation of reactive Cys in the three protein samples after exposure to H_2O_2 at 40° and 90°C. Generally, the loss of reactive Cys increased with increasing H_2O_2 concentration, with the change in the extent of Cys oxidation occurring between 0.25 and 1.0% H_2O_2 for both temperatures. This dramatic change is very evident in the samples incubated at 90°C. The Cys in all samples exposed to 2% H_2O_2 and held at 90°C or greater concentration was nearly all oxidized into unreactive forms. The oxidation of Cys results in the formation of cysteic acid and cysteine sulfinic acid, both of which were not detected by the reactive Cys procedure used in this study. Within each sample, the loss of reactive Cys was much greater when heated at 90°C than when heated at 40°C. The decrease in the reactive Cys content in the EWS, SPI, and casein samples exposed to 0.25–0.5% H_2O_2 at 90°C was nearly three, five, and six times greater than that for the samples held at 40°C. A gradual increase in the rate of destruction of Cys with increased H_2O_2 concentrations was observed for the SPI and casein samples held at 40°C. The EWS samples held at 40°C exhibited greater degree of Cys destruction when compared to SPI and casein. The degree of Cys oxidation, when samples were held at 90°C and exposed to H_2O_2 levels between 0.25 and 1.0%, was not directly proportional to the original Cys concentration in each protein. The differences in Cys stability in these samples may be due to the effect that different protein structures have on the positioning and subsequent reactivity of these residues. Calculations utilizing the total Cys contents of both untreated and treated samples (H_2O_2, 90°C) show that total protein Cys levels decrease as H_2O_2 concentrations increased. This indicates that Cys is partially decomposed under these extreme oxidation conditions. The results from the Cys oxidation study indicate that exposure to low temperatures and H_2O_2 concentrations during the processing of foods (i.e., the use of low concentrations of H_2O_2 to pasteurize egg whites) would not be expected to cause a significant amount of Cys destruction.

Methionine oxidation

Methionine loss during H_2O_2 oxidation is similar for all three protein samples treated at both 40°C and 90°C (Fig. 2). Methionine is susceptible to oxidation, even at low concentrations (0.25%) of H_2O_2 and 40°C. Higher concentratins of H_2O_2 did not increase the total amount of methionine oxidized, but did increase the amount of methionine sulfone formed. Fig. 3a and b show the levels of methionine sulfoxide and sulfone formed during exposure of the three proteins to H_2O_2 at 40°C and 90°C. For all three samples treated at 40°C, methionine sulfoxide was the predominant product. With increasing concentrations of H_2O_2, proteins held at 90°C yielded methionine sulfoxide as a minor product, with methionine sulfone the major product. At 40°C, methionine sulfoxide production in all samples did not increase with increasing H_2O_2 concentrations.

At low concentrations of H_2O_2 (lower than 1%), no methionine sulfone was produced in any of three samples held at 40°C (Fig. 3b). Methionine sulfoxide was also observed to be the only oxidized methionine product in casein treated with very low concentrations of H_2O_2 at pH 8.0 and 50°C (Cuq et al., 1973). Methionine sulfone was produced in the EWS and casein samples when they were exposed to 2 and 4% H_2O_2, respectively, at 40°C. SPI did not produce any methionine sulfone when exposed to H_2O_2 levels as high as 4.0%, at 40°C. The production of

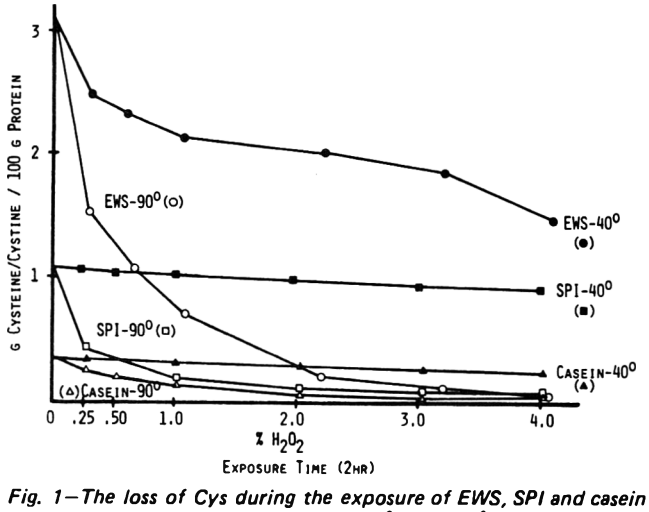

Fig. 1—The loss of Cys during the exposure of EWS, SPI and casein to increasing H_2O_2 concentrations at 40°C and 90°C.

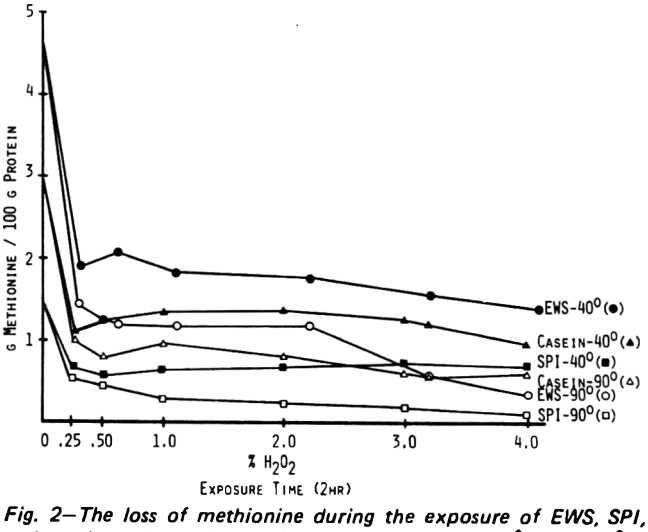

Fig. 2—The loss of methionine during the exposure of EWS, SPI, and casein to increasing H_2O_2 concentrations at 40°C and 90°C.

methionine sulfone in all three samples increased with increasing H_2O_2 concentration at 90°C. Only small amounts of methionine sulfone were produced when the proteins were held at 90°C and at low H_2O_2 concentrations. The increase in methionine sulfone at 90°C was at the expense of methionine sulfoxide. Methionine sulfoxide production decreased as the H_2O_2 concentration increased at 90°C.

There was no decomposition of methionine during H_2O_2 oxidation, as was noted earlier for Cys in EWS and SPI when exposed to high levels of H_2O_2 at 90°C. It should be noted that for all samples treated at 40°C with low H_2O_2 concentrations, oxidation of Cys did not parallel that of methionine. Under these mild conditions, there is a 60–70% conversion of methionine to methionine sulfoxide, while Cys oxidation occurs to only a slight degree. This finding is of importance for those instances where low concentrations of H_2O_2 would be used in a food as a bleaching agent or to serve as an aid in produce pasteurization. These results also indicate that further research is needed to confirm the bioavailability of methionine sulfoxide, since it is so easily formed by mild oxidizing conditions.

CONCLUSION

DIFFERENCES in protein structures appear to affect the ease with which their Cys and methionine residues may be oxidized. At a low temperature (40°C), Cys was oxidized only slightly when three protein samples were exposed to low concentrations of H_2O_2. At elevated temperature (90°C), a portion of the Cys residues in EWS and SPI was decomposed, while the majority was converted to cysteic acid. Methionine was easily oxidized to methionine sulfoxide at low H_2O_2 concentrations and temperatures. Higher concentrations of H_2O_2 and elevated temperatures (90°C) are necessary for the formation of methionine sulfone. These data, which show the harsh conditions needed for methionine sulfone production, help explain why in earlier studies, investigators have not found methionine sulfone in any food or isolated food protein.

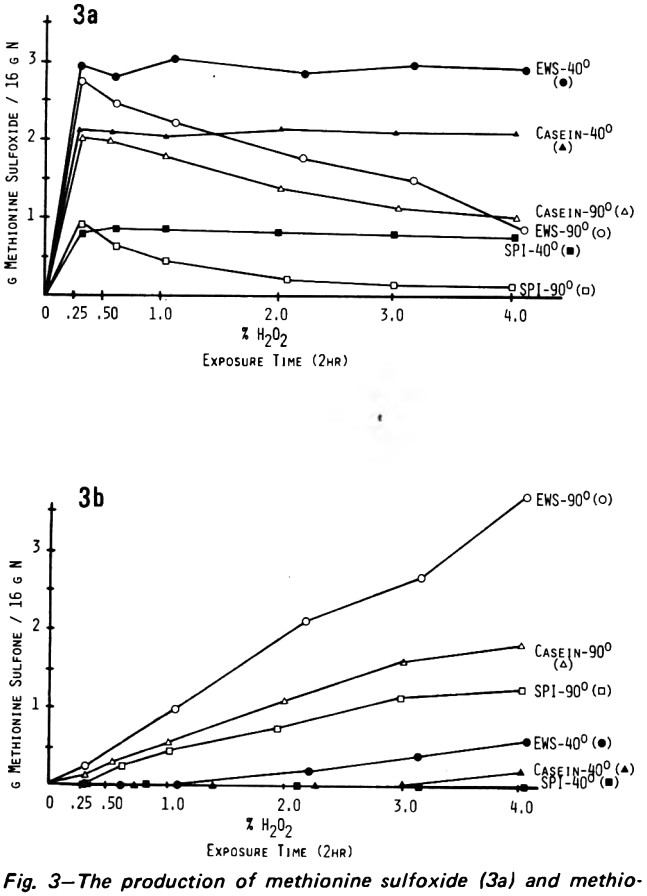

Fig. 3—The production of methionine sulfoxide (3a) and methionine sulfone (3b) in EWS, SPI and casein during expsoure to increasing concentrations of H_2O_2 at 40°C and 90°C.

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Nutritional Characteristics of Distiller's Spent Grain

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ABSTRACT

Five distiller's spent grain (DSG) samples were analyzed for their compositional and nutritional (protein efficiency ratio, PER) values. The protein content (N x 6.25) of these samples ranged from 26.9–34.9% (air-dry basis). True protein content, however, was substantially lower. Dietary fiber content of samples ranged from 29.1–35.8%. Samples contained about 3% nucleic acid. All samples contained appreciable amounts of potassium, magnesium, phosphorus, zinc, copper, iron, chromium, thiamin, riboflavin and niacin. The lysine content of DSG samples ranged from 2.50–3.74 g/100g protein. Except for one sample (PER, 1.7), the quality of protein in the samples was low (PER, 0.6 ± 0.1).

INTRODUCTION

DISTILLER'S SPENT GRAIN (DSG), a major by-product from ethanol production, has traditionally been used as an animal feed. Current interest in gasohol (gasoline and alcohol mixture as fuel source), and consequently increased availability of DSGs has, however, shifted the focus to possible uses of DSG in human food, both to conserve nutrient resources and to maximize DSG utilization.

The by-product of brewing (brewer's spent grain, BSG) has been investigated by some (Pomeranz et al., 1976; Prentice and D'Appolonia, 1977; Kissell and Prentice, 1979; and Finley and Hanamoto, 1980) as a possible adjunct for human food, but similar information on DSG is almost nonexistent. The purpose of this study was to develop, as an initial step, compositional and nutritional (protein quality) information on DSGs. Later studies would examine other nutritional aspects and food uses.

MATERIALS & METHODS

FIVE SAMPLES OF DSG (moisture: about 7%) were obtained from commercial sources (Table 1). Protein (Kjeldahl N), fat (ether extract), ash, moisture and fiber (crude) analyses on these samples (uniformly ground to 1 mm particle size) were conducted according to the standard AACC procedures (1962). Dietary fiber was determined by the recently approved method of AACC (1977). Total phosphorus was determined by the colorimetric method (40–55) of AACC (1962). All other minerals were determined by atomic absorption spectrophotometry using an IL (Instrumentation Laboratories, Inc.) Model 251 Spectrophotometer. Except that purchased standards were used and the ash was dissolved in (1 + 1) HCl, the method (40–70) was essentially that of AACC (1977). Standard AACC Methods (1962) were used to determine thiamin, riboflavin and niacin in DSG samples. Microbiological methods were used to determine B_6, folic acid and pantothenic acid contents. Vitamin B_6 was determined by the method of Atkin et al. (1943) modified by the replacement of potassium citrate with sodium citrate and the addition of niacin to the assay medium (25 mcg/tube). Folic acid was determined using the AOAC (1980) extraction procedure. The assay medium was that of Rabinowitz and Snell (1947) modified in that folic acid is omitted and 2 mcg per tube of pyridoxamine is added. Calcium pantothenate was determined after extraction by the AOAC (1980) procedure. The AOAC (1980) assay medium was modified in that Tween 80 was omitted. Nucleic acid content was determined by the method of Newell et al. (1975).

Amino acid analysis was carried out by ion-exchange chromatography using a Dionex D-300 Amino Acid Analyzer with ninhydrin detection and constant sodium normality buffers (Dionex Corp., Sunnyvale, CA). Samples were hydrolyzed as described earlier (Lorenz et al., 1980).

The standard AOAC (1980) method to determine the protein efficiency ratio (PER) was used to assess the quality of protein in DSG samples. Individually housed male weanling rats (Sprague-Dawley) were fed test diets (10 rats/diet) for a period of 4 wk. Each diet contained 10% Kjeldahl protein (N x 6.25).

RESULTS & DISCUSSION

THE MASH COMPOSITION of the DSG samples tested differed appreciably (Table 1). Except for sample D, which was all milo, the other samples contained variable amounts of corn, rye and barley (food grade and certified to be free of aflatoxins). To minimize sampling error, all samples were finely ground (1 mm) before proceeding with the analyses.

The corn-based DSG samples contained an average of 27.5% protein expressed on N x 6.25 basis while the protein in the all-milo sample was 34.9% (Table 2). The "true" protein content (based on recovered amino acid nitrogen) was, however, appreciably lower in all samples (Table 3). Not discounting the fact that the amino acid tryptophan was not determined and that cystine was only partially measured because of incomplete recoveries during protein hydrolysis, this suggests that the nonprotein nitrogenous (NPN) compounds (most likely from yeast) contributed substantially to the total N content. In BSGs, protein contents of 25% and over have been reported (Pomeranz et al, 1976; Prentice and D'Appolonia, 1977; and Kissell and Prentice, 1979).

The amino acid profile of DSG samples is shown in Table 3. The content of lysine (the first limiting amino acid in cereal grains) in these samples ranged from 2.50–3.74 g/100g protein or 0.64–0.95 g/100g sample (air dried). In distiller's protein concentrate (DPC), Scheller and Mohr (1975) reported a lysine value of 3.5 g/100g protein. Similar values for lysine in BSGs have been reported (Pomeranz et al., 1976; Prentice and D'Appolonia, 1977; Kissell and Prentice, 1979). The content of various other amino acids (Table 3) was also quite comparable to values reported for BSGs. Yeast most likely improves the amino acid profile

Table 1—Test samples of distiller's spent grain

Sample[a]	Source	Mash composition (%)			
		Corn	Rye	Barley	Milo
A	Distillery X	93.1	5.3	1.6	–
B	Distillery X	95.9	3.0	1.1	–
C	Distillery X	98.5	–	1.5	–
D	Distillery Y	–	–	–	100
E	Distillery Z	75.0	13.0	12.0	–

[a] Ground to particle size of 1 mm

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of spent grains. However, the extent of such an improvement, as well as the possible amino acid losses that might have occurred during processing, was not identified.

Except for Sample E, the quality of protein (PER) in DSG samples tested was less than satisfactory (Table 4) and thus was not reflective of the lysine content. This could be due to the presence of excessive NPN compounds in DSG samples causing an overestimation of the protein, and hence of lysine, content in the diet. Also, possible inhibitory effect of certain DSG components, e.g., tannins or other phenolics (Harborne and Van Sumere, 1975) on lysine availability cannot be discounted. PER of protein in distiller's protein concentrate has been reported (Scheller and Mohr, 1975) to be lower than the PER of the grain used in the mash (1.08 vs 1.44). In present studies, corrected PERs of DSGs were quite low indeed and did not (except for Sample E) exceed 0.65. PER on Sample E was appreciably higher than that reported for corn- and wheat-based products such as bread (Ranhotra et al., 1977) and was quite comparable to values reported for BSGs (Antonetti, 1979). The Kjeldahl (Table 2) and "true" (Table 3) protein values of DSG Sample E differed by a similar magnitude as the values for the other DSG samples. Thus interference with lysine utilization for the other samples most likely appears to be the cause of the poor PER values with Samples A-D.

Table 2—Proximate analysis and mineral and vitamin content of spent grain samples

	Sample[a]				
	A	B	C	D	E
Proximate components, %					
Protein (N x 6.25)	27.6	27.6	27.7	34.9	26.9
Fat (ether extract)	10.6	10.3	11.5	6.3	6.5
Ash	4.36	4.45	4.45	4.64	4.55
Fiber (crude)	7.9	7.4	7.5	8.5	7.0
Fiber (dietary)	35.6	35.8	34.1	29.1	32.4
Moisture	6.80	7.13	6.64	6.85	6.76
Minerals, %					
Sodium	0.06	0.13	0.06	0.12	0.03
Potassium	1.07	1.07	1.02	0.85	0.98
Calcium	0.068	0.071	0.076	0.083	0.049
Phosphorus	0.82	0.85	0.89	0.79	0.86
Magnesium	0.325	0.328	0.336	0.315	0.328
Minerals, ppm					
Chromium	4.62	4.48	4.50	4.74	4.43
Zinc	59.4	68.6	60.4	69.8	68.6
Copper	20.9	25.3	23.7	15.0	19.9
Iron	9.3	10.0	11.0	29.9	7.7
Vitamins, mg/100g					
Thiamin	0.19	0.19	0.18	0.60	0.61
Riboflavin	0.39	0.43	0.49	0.36	0.62
Niacin	6.66	6.57	6.22	10.37	7.17
Pyridoxine	1.20	0.97	0.90	0.75	1.05
Folic Acid	0.018	0.016	0.019	0.038	0.027
Pantothenic Acid	0.22	0.24	0.20	0.71	0.94

[a] As in Table 1

The crude fiber content of DSG samples ranged from 7.0–8.5% (Table 2). These values were considerably lower than those reported for BSGs (Pomeranz et al., 1976; Prentice and D'Appolonia, 1977). Compared to the crude fiber content, the dietary fiber content in DSG samples was about fourfold higher. Dietary fiber measures a number of additional (to those measured by the crude fiber method) components of which hemicellulose(s) is (are) the most significant. Because the nonstarch portions of grains are high in mineral content, the ash content of spent (starch depleted) grains was also high (4.36–4.64%).

The sodium content of DSG samples was low but the content of a number of other nutritionally significant minerals, particularly potassium, phosphorus (phytate phosphorus was not determined), magnesium, zinc, copper and chromium was high (Table 2). The values obtained for these elements were quite comparable to values normally associated with bran fractions of grains. Like minerals, the profile of various vitamins determined suggests that spent grains are good sources, particularly of thiamin, riboflavin and niacin. These vitamins were present in appreciably higher amounts than reported for BSG (Pomeranz et al., 1976). The fat (ether extractable) content of DSG samples ranged from 6.3 to 11.5%. However, all extracted residues were highly pigmented.

Not all of the starch appears to have been utilized during fermentation. This resulted in calculated values for "available carbohydrates" (mainly starches) of up to 25.3%. Because yeast contributes substantially to the nucleic acid

—Continued on page 1207

Table 3—Amino acid content of spent grain samples[a] (g/100g protein)

	Sample[b]				
Amino acid	A	B	C	D	E
Aspartic acid	7.88	9.31	7.89	7.82	8.10
Threonine	4.13	4.00	3.90	3.67	4.01
Serine	5.18	5.06	5.15	4.81	5.27
Glutamic acid	17.87	16.61	16.69	18.50	18.99
Proline	7.91	6.69	7.73	7.95	8.06
Glycine	4.75	4.55	4.47	3.94	4.65
Alanine	9.83	9.53	9.91	11.83	9.77
Half cystine	1.10	1.63	1.51	0.94	1.15
Valine	2.41	2.57	2.59	2.58	2.42
Methionine	3.62	4.50	4.58	2.87	3.36
Isoleucine	1.76	1.82	1.91	1.92	1.79
Leucine	11.25	10.38	10.76	11.47	10.22
Tyrosine	4.76	4.81	4.90	4.40	4.49
Phenylalanine	5.13	6.56	5.71	6.03	5.13
Histidine	3,92	3.77	4.01	3.30	3.87
Lysine	2.55	3.42	3.74	2.50	3.27
Arginine	3.38	2.69	2.67	2.98	3.38
Ammonia	2.53	2.11	1.88	2.51	2.07
Tryptophan	---------- not determined ----------				
Nitrogen, %	2.95	2.98	2.52	3.43	3.15
Protein, %	18.44	18.63	15.75	21.43	19.69

[a] Corrected to 100% recovery protein basis.
[b] Table 1.

Table 4—Protein efficiency ratio (PER) of spent grain samples

	Sample[a]					
	A	B	C	D	E	Casein
Diet intake, g	150 ± 16	149 ± 26	163 ± 14	143 ± 15	217 ± 33	263 ± 20
Weight gain, g	8 ± 2	9 ± 3	12 ± 3	9 ± 3	41 ± 8	74 ± 7
PER (measured)	0.56 ± 0.13	0.61 ± 0.14	0.73 ± 0.15	0.60 ± 0.18	1.87 ± 0.15	2.82 ± 0.09
PER (corrected)	0.50 ± 0.11	0.54 ± 0.13	0.65 ± 0.13	0.53 ± 0.16	1.66 ± 0.13	2.50 ± 0.08

[a] Table 1

Perception of Texture by Trained and Consumer Panelists

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ABSTRACT

Four experiments were conducted to assess the relationships between judgments of the perceived texture of foods by trained and consumer panelists. In Experiment 1, no differences were observed between trained texture profile panelists and naive consumers in a similarities scaling task. In Experiments 2 and 3, good linear correlations were observed between scalar judgments of texture, although a broader perceptual range was evidenced for trained panelists. In Experiment 4, psychophysical exponents of texture were found to be larger for trained than for consumer panelists, and judgments of acceptability also differed between the two groups. It was concluded that, through experience, trained texture profile panelists develop a broader perceptual range of textures, but that regression equations can be developed to relate these data to consumer data.

INTRODUCTION

SUCCESSFUL PRODUCT DEVELOPMENT in the food industry is dependent upon the proficiency of the organization's sensory evaluation group. The decisions made by this group are, in turn, dependent upon both the propitious selection of sensory panels for testing and the proficiency of these panels. The panels may be either affective (preference or acceptance) or analytical (discriminative or descriptive).

The two major factors that distinguish most sensory panels are the nature of the tasks that they perform and the nature and degree of training that they have received. Affective panels normally consist of naive and untrained consumers of the product, who evaluate the product for its hedonic qualities or its acceptability. Analytical panels, on the other hand, consist of "experienced," "trained" or "expert" panelists, who evaluate a product for some more specific aspects of its taste, odor, texture, etc.

Although the decision to use one type of panel versus another is determined by the nature of the question to be answered and the sensory/psychophysical method to be used, on occasion, the availability of a panel will become an important factor. For example, since the cost and personnel requirements to establish a wide variety of test panels is often prohibitive, especially for smaller laboratories, the situation may arise wherein a test requires a particular type of panel that is not available at the testing facility. In most cases this situation is correctly resolved by contracting the test to another laboratory. Unfortunately, under some circumstances, the panel(s) that is (are) available will be used to address the problem. When this happens, some training or re-orientation is usually undertaken to prepare the panel for the new task. However, the degree to which this training produces a panel that is truly adequate to the task is rarely examined.

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While several reports have discussed techniques for the screening, selection and training of members for various types of test panels, e.g., Martin (1973), Bressan and Behling (1977), Cross et al. (1978), Sawyer et al. (1962), Kirkpatrick et al. (1957), Swartz and Furia (1977), Zook and Wessman (1977), Girardot et al. (1952), Wittes and Turk (1968), Scholosberg et al. (1954) Civille and Szczesniak (1973), Hall et al. (1959), Gruber and Lindberg (1966), Stone et al. (1974), and Caul (1957), relatively fewer attempts have been made to quantitatively compare responses made by one type of panel to those made by another type of panel. The most significant of the early studies in this area were those conducted by Miller et al. (1955), Kiehl and Rhodes (1956), Simone et al. (1956), Peryam and Haynes (1957), Calvin and Sather (1959), Ellis (1963), and Pangborn and Dunkley (1964), in which it was generally found that laboratory consumer panel judgments of acceptability did not correspond well with consumer ratings of acceptability in the field. It was concluded that laboratory consumer panel judgments of acceptability agree in direction, but not in magnitude, with field consumer panel ratings. More recently, Kluter (1974) has reported on a study by Nichols et al. (1972) that showed rank-order correlations ranging from -0.8 to $+1.0$ between laboratory and field panel ratings of acceptability, depending upon the food product. Only the recent study by Moskowitz, et al. (1979a) has examined the relationship between judgments of specific sensory attributes by a laboratory consumer panel and by a trained analytical panel. In that study, data on certain relationships between consumer and trained texture profile panel judgments for rye breads were presented.

Knowledge of the relationship between consumer panel judgments and trained or expert panel judgments is important for several reasons. First is the fact that trained and expert panels are expensive to establish and maintain, while consumer panels require little formal training and members need not have highly specialized abilities. If reliable and predictive relationships can be established between judgments (either affective or analytical) made by these two types of panels, then consumer panels could be interchanged, under suitable conditions, with trained or expert panels, in order to achieve the most cost-effective use of personnel resources. Secondly, important time can be saved in the research and development cycle by employing a single panel to generate multiple types of sensory information, thereby eliminating the need to wait for results of one type of test before proceeding with another type. Thirdly, knowledge of such relationships can assist in the interpretation of discrepancies that arise between trained or expert panel data and consumer data, and help limit the extent to which data generated by a trained or expert panel poorly reflect the sensory experiences of actual consumers of the product. Lastly, from a theoretical perspective, comparison of judgments made by untrained consumer panels and trained or expert panels would make it possible to assess the experiential factors that are most important in determining sensory judgments, so that the limits of panel interchange can be delineated.

In the study of the textural attributes of food, two types of panels are most commonly employed. These are the trained profile panel, commonly modeled after the General Foods Texture Profile Panel (Brandt et al., 1963; Szczesniak et al., 1963) and the consumer panel, used frequently for obtaining hedonic measures of texture for marketing and product optimization purposes, and also used for "consumer texture profiling" (Szczesniak and Skinner, 1973; Szczesniak et al., 1975). In several recent studies conducted in our laboratory, both types of panels have been employed in the evaluation of food products, wherein both panels were instructed to perform exactly the same sensory/psychophysical tasks. These tasks included simple similarity scaling, in which only judgments of the similarity among samples was required, hedonic scaling, and scaling of specific textural attributes, using both category scaling and magnitude estimation. Comparison of the relationships between these consumer and trained panel judgments highlight the effects of training on the judgmental process of making textural evaluations and on the feasibility of using consumer panels to generate data normally obtained from trained panels, and vice versa.

EXPERIMENT 1

IN VIEW OF THE OBVIOUS difference between trained and consumer panels in their knowledge of descriptive sensory terminology, the first study was designed to assess the relationship between consumer and trained panel judgments in a task that does not require such knowledge. For this purpose, similarities scaling was chosen, because it is a simple psychophysical judgment that can be made without knowledge of specific terminology and because neither our laboratory consumers nor trained panelists had had prior experience in making this type of judgment.

Method

Panels. The first panel was a nine-member trained texture profile panel consisting of employees of the U.S. Army Natick R&D Laboratories. All members had been trained in the General Foods' Texture Profile Method, and all had served on the panel for a minimum of 1 yr. The panel training consisted of 1 wk of intensive discussion and demonstration of the basic techniques for making textural evaluations, exposure to each of the six standard scales of texture (Szczesniak et al., 1963), exposure to food items representing each of the geometrical characteristics of texture, construction of a series of increasingly complex texture profiles for a variety of food items, and training in the rendering of operational definitions of more complex texture attributes. During the time between initial training and the conduct of the test described herein, panel members met on an average of one – two times per week and were involved in the description and scaling of the textural attributes of meats, gelatins, food bars, and various other food products. The panel had never previously evaluated fish products.

The second panel was an untrained laboratory consumer panel consisting of 10 volunteer employees. None of these panelists had ever received training in making textural or other analytical sensory judgments, although many had previously participated in consumer acceptance testing of a variety of foods in the laboratory. This panel had also never evaluated fish products, performed a similarities scaling task nor used a line scaling technique.

Test samples. Six species of fish: Haddock *(Melanogrammus aeglefinus)*, Halibut *(Hippoglossus hippoglossus)*, Blackback Flounder *(Pseudopleuronectes americanus)*, White Hake *(Urophycis tenuis)*, Mackerel *(Scomber scombrus)*, and Pollock *(Pollachius virens)* were chosen as test samples. Fish fillets were selected as the test product because they had not been tested previously by either panel. All samples were purchased fresh locally, baked in foil to an internal temperature of 160°F and maintained at a constant temperature in a steam table for a period not exceeding 10 min prior to serving. Samples were presented to subjects in approximately 2-ounce servings.

Procedure. The experiment was conducted in the standard sensory testing booths of the Food Acceptance Laboratory at NLABS. Panelists were first presented with a sample of each of the six fish to acquaint them with the range of samples to be encountered. The panelists rinsed their mouth with distilled water between samples. Panelists judged the overall similarity of each fish paired once with itself and once with each of the other species, for a total of 21 pairs of samples. Responses were made by placing a slash-mark on a 30 cm line. The end points were labelled "similar" and "dissimilar." Sample pairs were presented in random order to all panelists.

Data analysis. The line-ratings of similarity were transformed to numerical values by measuring the distance from the end-point of the scale to the line marked by the subject. These proximity measures were then used to generate a "sensory map" of the fish, using multidimensional scaling procedures.

The similarity judgments for the consumer and trained panels were analyzed using ALSCAL–4 (Young and Lewyckyj, 1979). ALSCAL–4 is a metric multidimensional scaling program that uses similarity measures between pairs of stimuli and arranges the stimuli into a multidimensional space, in which the relative distances between stimulus points reflect the relative disimilarities between judged samples. The dimensionality of the space is determined by an iterative procedure in which the goodness of fit of any n-dimensional space to the data is assessed by a "Stress" value. Usually, as n increases, the stress (error) decreases, due to reduced constraint in positioning the stimuli, until a nonsignificant decrease in stress is achieved. The program also includes the capability of analyzing individual differences among subjects.

Results

The results of the application of ALSCAL–4 to the similarities data are presented in Fig. 1. The best-fitting solution was found with three dimensions and had a calculated stress of 0.15.

The three dimensional solution in Fig. 1 is intuitively appealing, because the perceptual space is easily interpretable from data collected subsequently in our laboratory on the sensory properties of fish (Kapsalis and Maller, 1981).

Dimension 1 is clearly related to the color of the cooked fish, and is best described as a light-dark dimension. Mackerel, a very dark-fleshed fish, appears at one end of this dimension, while halibut, white-hake and haddock, all very

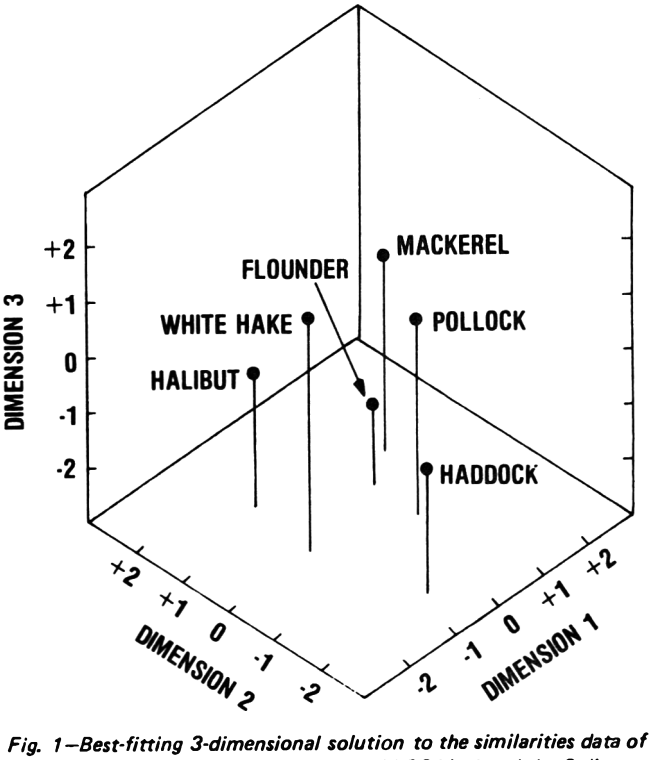

Fig. 1–Best-fitting 3-dimensional solution to the similarities data of Experiment 1. Data were analyzed by ALSCAL-4 and the 3-dimensional solution had a stress of 0.15.

white-fleshed fish, appear at the other end. Pollock and flounder, which are grayish in color, fall between these two extremes. This light-dark dimension has repeatedly been found to be one of the best discriminative variables in descriptive tests of fish conducted in our laboratory, and its emergence as a primary sensory dimension is in agreement with these data.

Dimension 2 in this space appears to be a textural dimension and is related to the perceived flakiness of the fish. Halibut, which appears as one extreme on this dimension, is a solid-muscle fish with little or no flakiness. Mackerel is also lacking in appreciable flakiness. Haddock, pollock and white hake, on the other end of this dimension, are characterized by a high degree of flakiness.

Dimension 3 appears to be related to the overall flavor intensity of the fish, with mackerel loading high on this dimension due to its high oil content and correspondingly strong fish-oil flavor, and flounder and haddock appearing at the other end, due to their mild flavor. The white hake and pollock are of surprisingly similar magnitude to the mackerel on this dimension. However, white hake has been described as possessing a characteristically strong "stale fish" or "earthy" flavor and pollock may sometimes possess an off-taste due to season of catch or feeding ground.

Fig. 2–4 show plots of the derived subject weights for each panelist on each pair of the three dimensions. These weights represent the relative degree to which each panelist used each perceptual dimension in making his judgement of overall similarity. The uncircled letters in each plot represent trained panelists, the circled letters represent consumer panelists. If either group weighted one or more dimensions differently from the other group, then the individuals within the groups would cluster in different segments of the plots. The fact that they do not indicates that the trained and consumer panelists did not differentially weight the importance of any of the three obtained dimensions. The important aspect of these data for the present discussion is that the trained texture profile panelists did not place any more weight on the textural dimension of flakiness (Dimension 2) than did the consumer panelists.

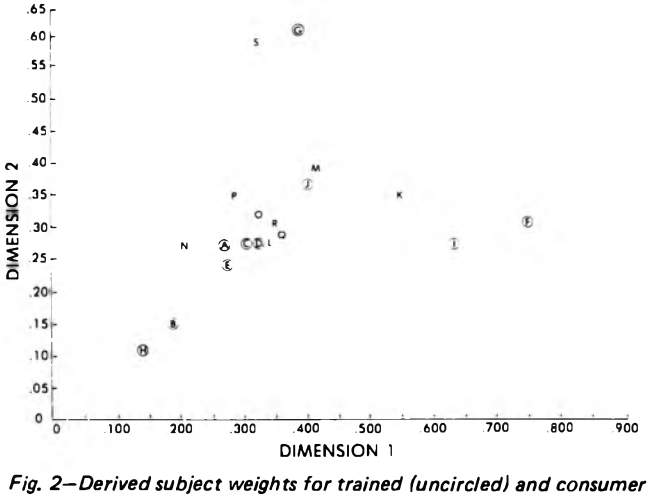

Fig. 2–Derived subject weights for trained (uncircled) and consumer (circled) panelists on Dimensions 1 and 2.

EXPERIMENT 2

THE SECOND STUDY was designed to assess the relationship between scalar judgements made by trained and consumer panelists on specific textural attributes. This task is considerably more complex than that of Experiment 1, requiring that the panelist be able to identify specific textural attributes in the product. Thus, prior experience in making such judgments may be more likely to affect panelist ratings.

Method

Panels. The first panel was a trained texture profile panel, similar to that used in Experiment 1, and comprised of six members. Although Experiment 2 was conducted approximately 18 months after Experiment 1, five of the six members were the same as in Experiment 1. In the 18 month interim, all panelists acquired extensive experience in judging the textural attributes of a wide variety of fish.

The second panel was a consumer panel similar to that described in Experiment 1, but was composed of a different random sample of 40 panelists for each test session. The selection of a different sample of consumer for each test session was made to minimize the experience that these panelists would acquire in judging the textural attributes of the test samples over the course of the experiment. All of the consumer panelists had prior experience in using category scales, but none had any known experience in judging specific textural attributes of fish or other seafood products.

Test samples. Eighteen species of fish: Whiting *(Merluccius bilinearis)*, Mackerel *(Scomber scombrus)*, White Hake *(Urophycis tenuis)*, Cusk *(Brosme brosme)*, Monkfish *(Lophius americanus)*, Pollock *(Pollachius virens)*, Tilefish *(Lopholatilus Chamealeonticeps)*, Wolffish *(Anarchichas lupus)*, Striped Bass *(Morone saxatilis)*, Blackback Flounder *(Pseudopleuronectes americanus)*, Weakfish *(Cynoscion regalsi)*, Grouper *(Mycertoperca microlepis)*, Haddock *(Melanogrammus aeglefinus)*, Halibut *(Hippoglussus hippoglossus)*,

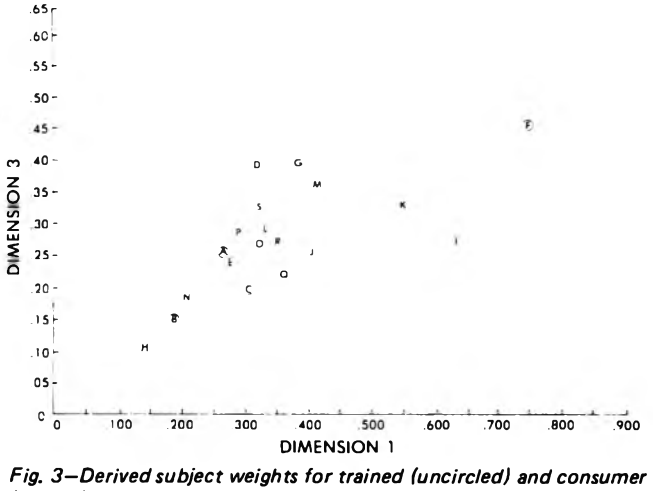

Fig. 3–Derived subject weights for trained (uncircled) and consumer (circled) panelists on Dimensions 1 and 3.

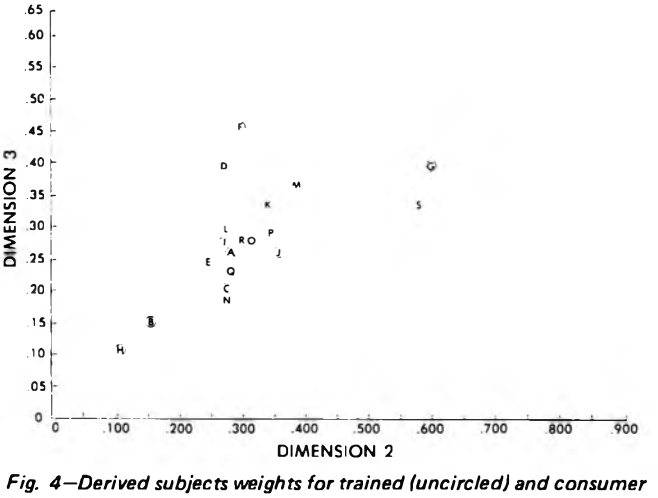

Fig. 4–Derived subjects weights for trained (uncircled) and consumer (circled) panelists on Dimensions 2 and 3.

Swordfish *(Xiphias gladius)*, Cod *(Gadus morhua)* – Scrod and market size, and Bluefish *(Pomatomus saltatrix)* served as test samples. All samples were purchased fresh locally as fillets, cut, sealed in individual plastic cooking pouches, and cooked in a retort to an internal temperature of 160°F. Samples were presented to panelists in approximately 2-ounce servings.

Procedure. Consumer tests were conducted in the same test booths as in Experiment 1 and all tests involved the presentation of a single fish sample. At the start of each session consumer panelists were given a printed response sheet with written instructions for the test. Panelists were asked to rate six separate textural attributes of the fish. These were the "hardness," "flakiness," "chewiness," "fibrousness," "moistness," and "oily mouthcoating" of the fish. In addition, each rated the visual attribute of "darkness" and six flavor attributes of the fish. Only the textural and visual attributes are discussed here, since flavor attributes were not evaluated by the texture profile panel. All attributes were rated on a 7-point category scale, with end-points labeled "slight" (1) and "strong" (7). A "none" category was also provided in the event that a panelist did not perceive any of a given attribute in the sample. Neither definitions of the attributes nor demonstration of specific techniques for judging the attributes were provided to the consumer panelists.

Trained panelists received the same samples as did the consumer panelists, rated them on the same textural and visual attributes and used the same 7-point scale. Neither group of panelists were informed about the species of fish being tested, and all panelists' judgments were made independently of one another.

Results

The mean ratings for each attribute and sample were calculated for each panel. In order to assess the relationship between judgments of the two panels, mean ratings for the trained panel were regressed against mean ratings for the consumer panel for each attribute. The slope of the obtained regression equation reflects the rate of growth of perceived magnitude for the trained panel as a function of the perceived magnitude for consumer panelists. Thus, a slope less than 1.0 indicates that for each unit increase in perceived magnitude by consumers, a smaller increase was perceived by trained panelists. A slope equal to 1.0 reflects equivalent sensory increases, and a slope greater than 1.0 indicates a more rapid growth of perceived magnitude for trained panelists.

Table 1 contains the obtained linear regression equations relating trained panel judgments to consumer panel judgments for each attribute, as well as the corresponding correlation coefficients and coefficients of determination for each.

Although the correlation coefficients are statistically significant for six of the seven attributes, indicating a significant degree of linearity between judgments made by the two panels, the slopes (b) of the regression equations for these attributes are all greater than 1.0, indicating that, for all significantly correlated textural attributes, a greater range of perceptual differences were perceived in the stimulus series by the trained panel than by the consumer panel. The relative magnitudes of these slopes also give information about the extent to which the range of perceptual differences are larger for trained versus consumer panelists, with larger slopes reflecting larger differences in ranges. The fact that the "darkness" attribute was the only significantly correlated attribute that had a slope close to 1.0, is evidence that the increased perceptual range of trained texture profile panelists is specific to textural attributes and does not extend to visual attributes. Although the slope for "oily mouthcoating" is less than 1.0, the non-significant correlation coefficient indicates that it should not be considered meaningful.

EXPERIMENT 3

WHILE THE DATA OBTAINED in Experiment 2 show differences in the sensory ratings assigned by trained and consumer judges for fish samples, it is not clear whether these effects on perceptual range are due to a general heightened awareness and/or sensitivity to these textural attributes or whether the effects are (1) specific to fish, since the texture profile panel had been evaluating fish samples for 18 months prior to the start of this experiment, (2) specific to the use of a 7-point category scaling procedure, which had also been used extensively by the trained panel, but not by consumers, or (3) simply due to differences between consumers and trained panelists in their definitions of, or techniques for evaluating, these attributes. To determine whether these factors contributed to the obtained results, the following experiment was conducted.

Method

Panels. The first panel was a trained texture profile panel similar to that used in Experiments 1 and 2. Eight members served in these tests. Five members participated in Experiments 1 and 2, three did not. The illness of one member during one series of sessions, reduced the panel size to seven on these occasions. All panelists had previous experience in using the method of magnitude estimation.

The second panel was a consumer panel similar to that described in Experiments 1 and 2, and comprised of 12 members. None of these panelists had previous experience in judging textural attributes of food, but all had previous experience in using the method of magnitude estimation.

Test samples. In order to determine if the effects observed in Experiment 2 were product-specific, a heterogeneous group of commodities was chosen for testing. Samples were chosen from the list of food items constituting the standard rating scales for mechanical texture attributes (Szczesniak et al., 1963). Some substitutions to these lists were made to compensate for current and local availability of items. The complete list of test items is shown in Table 2 for each standard scale.

Procedure. Six sessions were conducted in the same test booths as in Experiment 1. Each session was devoted to the testing of a single textural attribute. The six attributes were those of "hardness," "viscosity," "adhesiveness," "chewiness," "gumminess," and "fracturability." At the start of each session all panelists were given written instructions. In addition to containing a description of the task, the instructions contained an operational definition of the attribute to be judged during that session. The definitions for each attribute appear in Table 3. After reading the instructions and accompanying definition, each panelist was given a demonstration of the exact technique for judging the attribute. In addition, each panelist was observed while he/she made one or more trial evaluations using this technique.

After this orientation session, panelists were directed to the test booths to begin their evaluations. Panelists were allowed to keep the written instructions and definitions of the attributes with them in the booths and were encouraged to refer to them. The above procedures were all adopted to ensure, as best as possible, that consumers were defining and evaluating each textural attribute in the same manner as trained panelists.

All samples to be rated on the particular attribute were presented to panelists individually and in random order. After receiving the samples, panelists rated them using the method of modulus-free magnitude estimation. This scaling method was chosen because there were no differences between the two panels in their past

Table 1—Regression equations, Pearson product-moment correlation coefficients and coefficients of determination for the relationship between trained and consumer panel judgments of texture and appearance

	Regression equation[a]	Correlation coefficient	Coefficient of detm
Flakiness	T=2.42 C – 5.33	0.77**	0.59
Hardness	T=1.66 C – 3.44	0.75**	0.57
Chewiness	T=1.58 C – 2.14	0.84**	0.71
Fibrousness	T=1.52 C – 0.06	0.72**	0.52
Moisture	T=0.79 C – 0.62	0.53	0.28
Oily Mouthcoating	T=1.59 C – 0.87	0.75**	0.57
Darkness	T=1.04 C + 0.76	0.91**	0.82

[a] T = Trained Panel Ratings; C = Consumer Panel Ratings
** $p<0.01$

Table 2—Food items comprising the test samples used in Experiment 3

Product	Brand, type or preparation	Manufacturer	Sample size	Temp
Hardness				
Cream Cheese	Philadelphia	Kraft Foods	½" cube	45–55°F
Egg White	hard-cooked 5-min.		½" cube	room
Frankfurters	large, uncooked	Hebrew Nat'l	¼" slice	50–65°F
Cheese	yellow, American, pasteurized process	Kraft Foods	½" cube	50–65°F
Olives	Spanish, stuffed	Durkee Famous Foods	1 olive, cut placed back to back	room
Peanuts	cocktail type in vacuum tin	Planters Peanuts	1 nut	room
Carrots	uncooked, fresh		½" slice	room
Peanut Brittle	candy part	Kraft Foods		room
Hard Candy	Charms	Charm Co.	1 piece	room
Viscosity				
Water	distilled		½ tsp	45–55°F
Light cream	Sealtest	Sealtest Foods	½ tsp	45–55°F
Heavy cream	Sealtest	Sealtest Foods	½ tsp	45–55°F
Evaporated milk		Carnation Co.	½ tsp	45–55°F
Maple Syrup	Vermont Maid	R.J. Reynolds Foods	½ tsp	45–55°F
Chocolate Syrup		Hershey Chocolate Corp.	½ tsp	45–55°F
Mixture: 1½ cup condensed milk & 1 tbl heavy cream		Borden Foods Sealtest Foods	½ tsp	45–55°F
Condensed milk		Borden Foods	½ tsp	45–55°F
Adhesiveness				
Hydrogenated vegetable oil	Crisco	Proctor & Gamble Co.	½ tsp	45–55°F
American Cheese		Kraft Foods	½" cube	45–55°F
Cream Cheese	Philadelphia	Kraft Foods	½" cube	45–55°F
Marshmallow topping	Fluff	Durkee-Mower	½ tsp	45–55°F
Peanut Butter	Skippy, smooth	Best Foods	½ tsp	45–55°F
Chewiness				
Rye bread	fresh, center cut	Arnold's Baking Co.	½" cube	room
Frankfurter	large, uncooked skinless	Hebrew National	½" slice	50–70°F
Cherry Red candy	Switzer Licorice	Beatrice Foods Co.	1 piece	room
Black Crows candy		Mason Candy Co.	1 piece	room
Caramel candy		Kraft Co.	1 piece	room
Tootsie rolls	midget size	Sweets Co. of America	1 piece	room
Gumminess				
40% flour paste	Gold Medal	General Mills	1 tbs.	room
45% flour paste	Gold Medal	General Mills	1 tbs.	room
50% flour paste	Gold Medal	General Mills	1 tbs.	room
55% flour paste	Gold Medal	General Mills	1 tbs.	room
60% flour	Gold Medal	General Mills	1 tbs.	room

(continued)

Table 2—Continued

Product	Brand, type or preparation	Manufacturer	Sample size	Temp.
Fracturability				
Corn muffin	Finast	First Nat'l Stores	½" cube	room
Eggs Jumbos		Stella D'Oro Biscuit Co.	½" cube	room
Graham crackers	Nabisco	National Biscuit Co.	½" square	room
Melba Toast		Devonsheer Melba Corp.	½" square	room
Rye Crisp	Finn Crisp	Vaasa Mills Ltd	½" square	room
Ginger snaps	Nabisco	National Biscuit Co.	½" square	room
Peanut brittle	Candy part	Kraft Foods	½" square	room

experience with this scaling technique. Each sample was judged once, after which the panelist expectorated, rinsed with distilled water, and awaited the next sample. A 90-sec interstimulus interval was maintained.

Data analysis. The magnitude estimation data were normalized (Stevens, 1971), and geometric means for trained and consumer panelists were calculated for each test sample. The means obtained from the trained panel for each item were regressed against the consumer panel means for these items in the same manner as described in Experiment 2.

Results

Table 4 shows the obtained linear regression equations relating trained panel judgments to consumer panel judgments for each attribute, as well as the corresponding correlation coefficients and coefficients of determination for each. All attributes were significantly correlated between panels. The high coefficients of determination observed in this experiment, as contrasted with Experiment 2, is likely due to the much wider range of each textural attribute represented in the test stimuli of this experiment. The limited range of each textural attribute in the fish samples of Experiment 2, probably resulted in statistical restriction of range. As was found in Experiment 2, the slopes (b) of the equations in Table 4 are all greater than 1.0. Thus, for all tested attributes, a greater range of perceptual differences were noted in the stimulus series by the trained panel than by the consumer panel. The fact that members of both panels had equivalent experience with the method of magnitude estimation supports the conclusion that these results are not due to differences in their experience with the scale type. Furthermore, since judgments of each attribute were made on a heterogeneous series of food products, the results cannot be attributed to differences in experience with a particular food product. Lastly, since both consumers and trained panelists used the same definitions and techniques for evaluating these attributes, the differences cannot be due to differences in the sensory attributes being evaluated. Rather, the results appear to be due to differences between consumer and trained panelists in either their awareness of, or sensitivity to, textural attributes, resulting from prior experience in judging these attributes in food.

Table 3—Definitions of textural attributes used in Experiment 3

Hardness	The perceived force required to compress a substance between the molar teeth.
Chewiness	The total perceived work required to masticate a sample to reduce it to a consistency suitable for swallowing.
Viscosity	The perceived force required to draw a liquid from a spoon over the tongue.
Gumminess	The perceived denseness that persists throughout mastication; the perceived energy required to reduce a semi-solid food to a state ready for swallowing.
Adhesiveness	The perceived force required to remove material that adheres to the mouth (generally the palate) during normal eating.
Fracturability	The perceived force with which a sample crumbles, cracks or shatters when a constant vertical force is applied to it.

Table 4—Regression equations, Pearson product-moment correlation coefficients and coefficients of determination for the relationship between trained and consumer panel judgements of each texture attribute

	Regression equation	Correlation coefficient	Coefficient of detm
Hardness	T=2.45 C − 14.51	0.99**	0.99
Chewiness	T=2.45 C − 3.14	0.99**	0.99
Fracturability	T=1.07 C − 0.36	0.98**	0.97
Gumminess	T=1.43 C − 7.36	0.98**	0.97
Viscosity	T=1.32 C − 1.12	0.97**	0.95
Adhesiveness	T=2.20 C − 12.85	0.93**	0.87

[a] T = Trained panel judgment; C = Consumer panel judgment
** $p<0.01$

EXPERIMENT 4

THE FOURTH EXPERIMENT was designed to further investigate the relationships between trained and consumer panel judgments of texture by specifically comparing the growth of perceived magnitude of textural attributes as a function of objective measures of the test samples. These data were collected as part of a more comprehensive study of the texture of bread, which has been reported elsewhere (Moskowitz et al., 1979a, b). From these data it was also possible to assess the relationship between consumer and trained panel judgments of acceptability.

Method

Panels. The first panel was the same trained texture profile panel described in Experiment 3, consisting of seven members for this series of tests. The second panel was a 28-member consumer panel, similar to that described in Experiment 1 – 3, with most having had experience in using the method of magnitude estimation. However, to ensure that all consumer panelists understood this scaling method, an orientation session (see below) preceeded the test sessions.

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Test samples. Twelve rye breads were made by varying the percent rye flour (12, 19.5, 27, 42%) and the percent sucrose (0, 3, 6%) in a complete factorial design. These variations produced concomitant variations in the wheat flour level. All the breads also contained 1.5% shortening, 1% table salt, 38.7% water, and 0.8% yeast. The breads, in the form of 18-oz loaves, were baked in a rotary oven at 425°F for 30 minutes and were stored frozen (0°C) until used. Immediately prior to testing, the breads were removed from storage and thawed to room temperature. Samples were then tested with an Instron Universal Testing Machine to provide measures of their rheological properties (modulus of elasticity, hysteresis loss, strain energy, and stress at 60% strain). Density and moisture content were also determined.

Procedure. All consumers were given a 30-min orientation session on the day before the test to refresh them with the method of magnitude estimation and to give them practice in using this method to rate non-food stimuli. During the test session, the consumers rated each of the 12 samples of bread on each of ten textural attributes defined by the expert panel: "denseness," "firmness," "cohesiveness," "ease of particle removal," "roughness," "moistness," "cohesiveness during chewing," "adhesiveness," and "graininess." The definitions for each attribute appear in Table 5. In addition, both panels rated the breads for overall liking. Magnitude estimates were collected for each product on all attributes from both panels normalized to reduce interpanelist variability, panelist-by-panelist (Moskowitz, 1977), then averaged.

Table 5—Definitions of textural attributes for bread used in Experiment 4. Attributes are grouped into three categories: those which are evaluated initially and which involve surface characteristics; those evaluated upon first bite, using the incisor teeth; and those evaluated during mastication with the molar teeth

Surface

Ease of particle removal — The perceived ease with which particles (grains) can be removed from the surface of the sample, when sliding the tongue over the cut surface.

Roughness — The perceived roughness of the surface of the sample, when sliding the tongue over the cut surface.

First Bite

Firmness — The perceived force required to bite through the sample.

Cohesiveness — The perceived degree to which the sample holds together upon biting.

Denseness — The perceived degree to which the particles of bread are packed closely together.

Mastication

Moistness — The perceived degree of moisture in the sample.

Graininess — The number of grainy particles perceived in the mouth during initial stages of chewing.

Chewiness — The perceived effort required to prepare the sample to a state ready for swallowing.

Cohesiveness during chewing — The degree to which the sample holds together as a single mass during chewing.

Adhesiveness — The perceived degree to which the sample sticks to the teeth during chewing.

Results

The multivariate relationships between ingredient levels and rheological measures, between ingredient levels and trained panel ratings of specific textural attributes, between rheological measures and trained panel ratings, and between consumer ratings of liking and both ingredient levels and rheological measures have been reported elsewhere (Moskowitz, et al., 1979). Of interest here are the relationships between trained and consumer panel judgments of specific textural attributes and of liking. Because rheological measures were made on all test samples, a direct assessment and comparison of the growth of perceived magnitude as a function of stimulus intensity was made. Similarly, the relationships between liking and the rheological measures for both panels were also examined.

Intercorrelation matrices. Table 6 shows the intercorrelation matrices among sensory attributes for the trained and consumer panels. The patterns of correlations are similar for both panels. In particular the surface characteristics of "ease of particle removal" and "roughness" are positively correlated with one another and negatively correlated with all other attributes. "Firmness," "cohesiveness," "denseness," and "adhesiveness" are all highly positively correlated, while "moistness" is only correlated with the destructive attributes of "chewiness" and "cohesiveness during chewing." Of the total of 45 correlations, 19 were significant for the trained panel and 24 were significant for the consumer panel, suggesting somewhat more independence of responding for trained panelists.

Psychophysical functions. Although many of the correlations between texture attributes were high for both panels, such a situation could co-exist with a large absolute difference between panels in intensity ratings or in their rate of growth. Figures 5–12 show trained and consumer panel judgments of firmness, cohesiveness, denseness, and adhesiveness as a function of the modulus of elasticity and density of the breads, plotted in full logarithmic coordinates. These particular sensory and instrumental measures were chosen because of their presumed relationships with one another. Sensory attributes involving only surface characteristics of the breads or involving evaluation of destructive changes in the breads during mastication were not deemed theoretically appropriate for this analysis.

The equations describing the relationships in Figures 5–12 are power functions of the form $S = KI^n$, where S is the perceived magnitude of the textural attribute, I is the physical magnitude of the rheological property, n is the exponent of the function and k is a constant of proportionality. The value of n is of special interest, because it is an index of the rate of growth of perceived magnitude as a

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Table 6—Pearson product-moment correlation coefficients among all pairs of sensory texture attributes. The coefficients on the left of each cell are for the trained panel data. The coefficients on the right of each cell are for the consumer data

	Ease of particle removal	Roughness	Firmness	Cohesiveness	Denseness	Adhesiveness	Moistness	Chewiness	Cohesiveness during chewing
Roughness	0.97*/ 0.71*								
Firmness	−0.95*/−0.84*	−0.89*/−0.51							
Cohesiveness	−0.92*/−0.87*	−0.91*/−0.61	0.91*/ 0.95*						
Denseness	−0.96*/−0.88*	−0.94*/−0.50	0.95*/ 0.99*	0.92*/ 0.96*					
Adhesiveness	−0.73*/−0.77*	−0.72*/−0.54	0.72*/ 0.90*	0.74*/ 0.92*	0.84*/ 0.89*				
Moistness	−0.41 /−0.28	−0.46 /−0.54	0.35 / 0.40	0.30 / 0.47	0.51 / 0.36	0.57 / 0.66			
Chewiness	−0.68 /−0.82*	−0.60 /−0.64	0.69 / 0.92*	0.59 / 0.93*	0.78*/ 0.90*	0.71*/ 0.96*	0.77*/ 0.67		
Cohesiveness During Chewing	−0.51 /−0.66	−0.57 /−0.75*	0.30 / 0.71*	0.46 / 0.77*	0.57 / 0.66	0.66 / 0.79*	0.74*/ 0.74*	0.64 / 0.87*	
Graininess	0.14 / 0.66	0.55 / 0.65	−0.14 /−0.60	−0.20 /−0.60	−0.22 /−0.57	−0.22 /−0.64	−0.50 /−0.65	−0.44 /−0.77*	−0.45 /−0.79*

* $P<0.01$

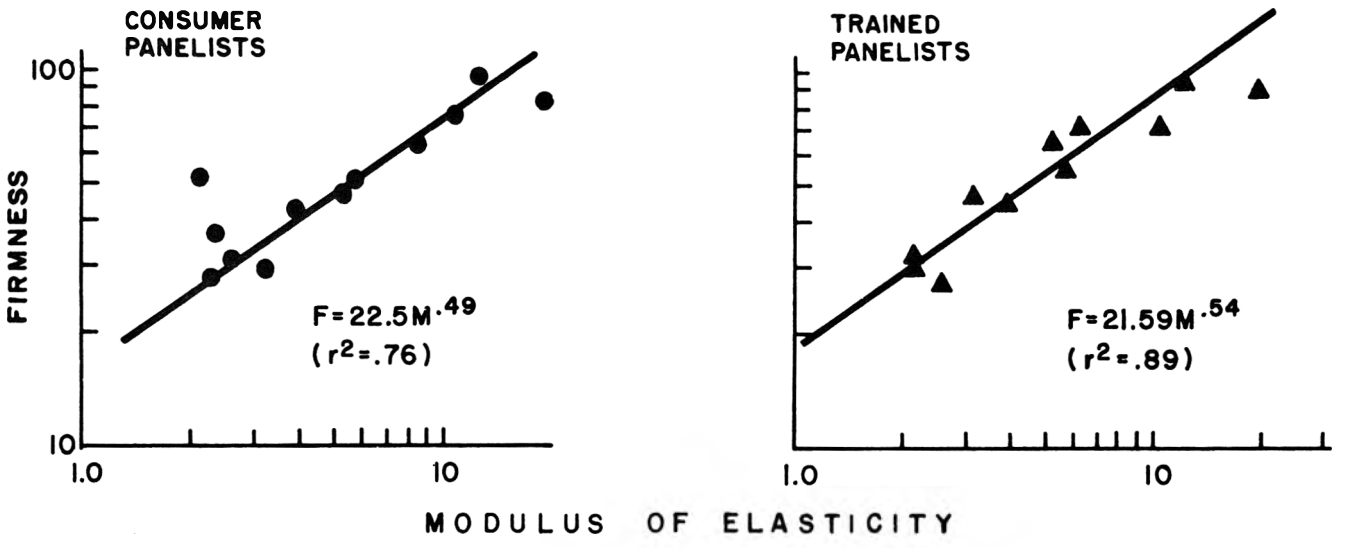

Fig. 5—Consumer (left) and trained (right) panel ratings of firmness as a function of the modulus of elasticity for the bread data of Experiment 4.

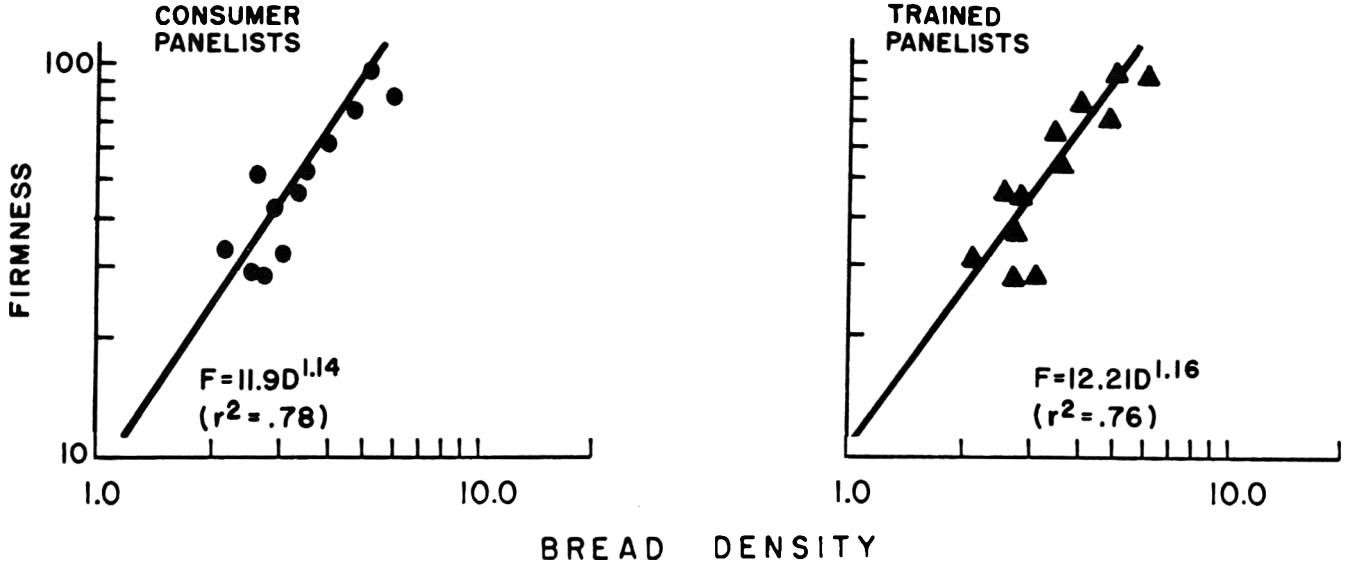

Fig. 6—Consumer (left) and trained (right) panel ratings of firmness as a function of bread density for the bread data of Experiment 4.

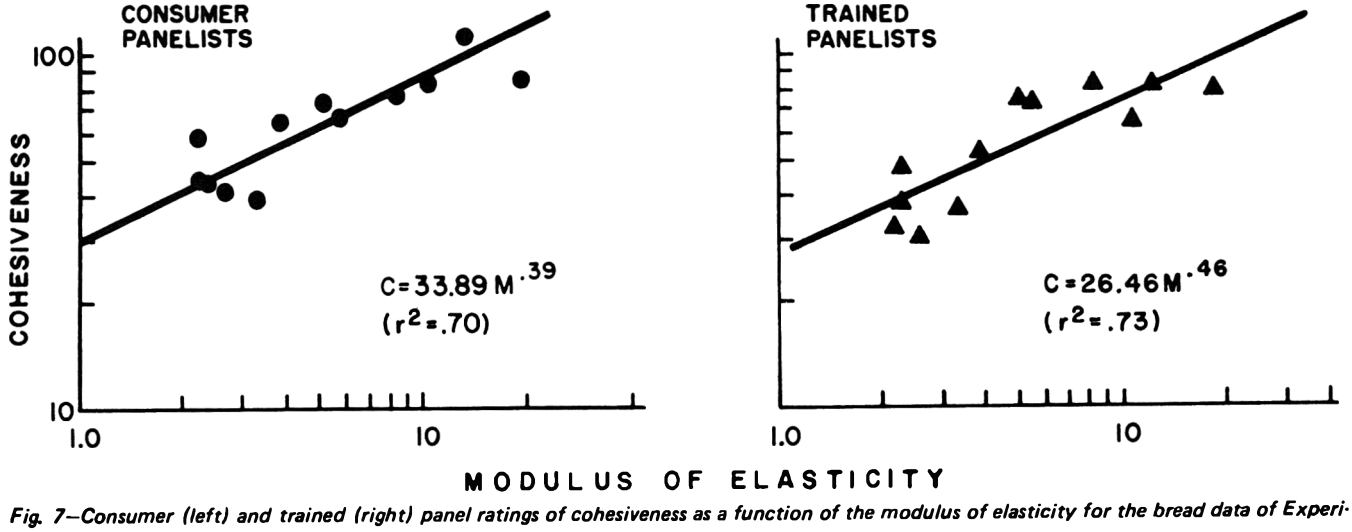

Fig. 7—Consumer (left) and trained (right) panel ratings of cohesiveness as a function of the modulus of elasticity for the bread data of Experiment 4.

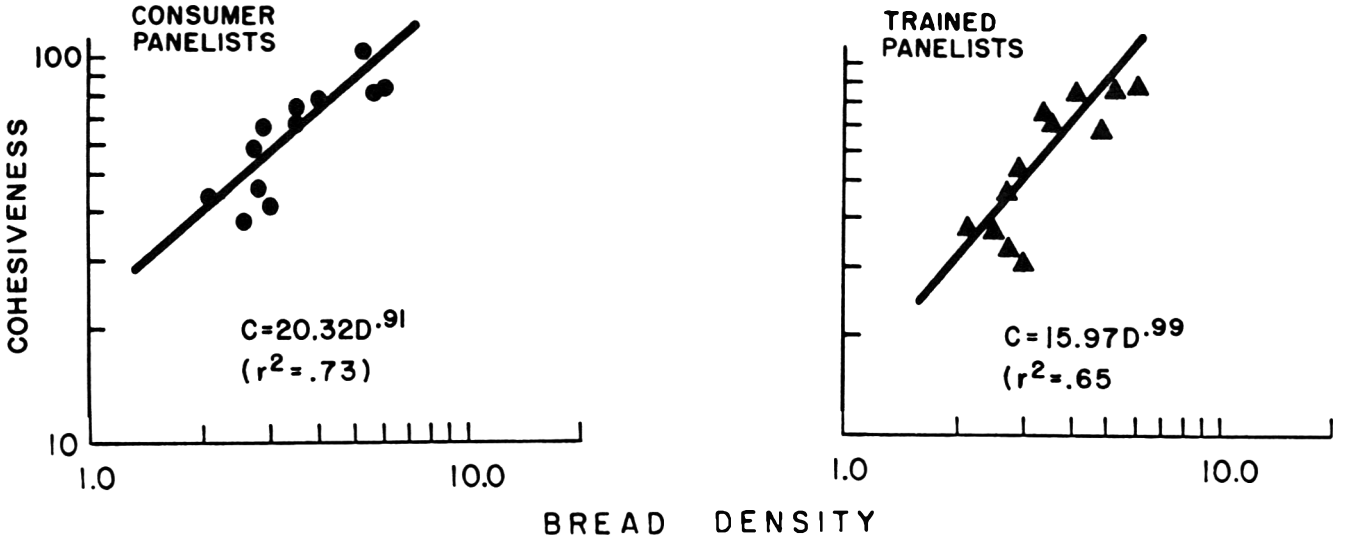

Fig. 8—Consumer (left) and trained (right) panel ratings of cohesiveness as a function of bread density for the bread data of Experiment 4.

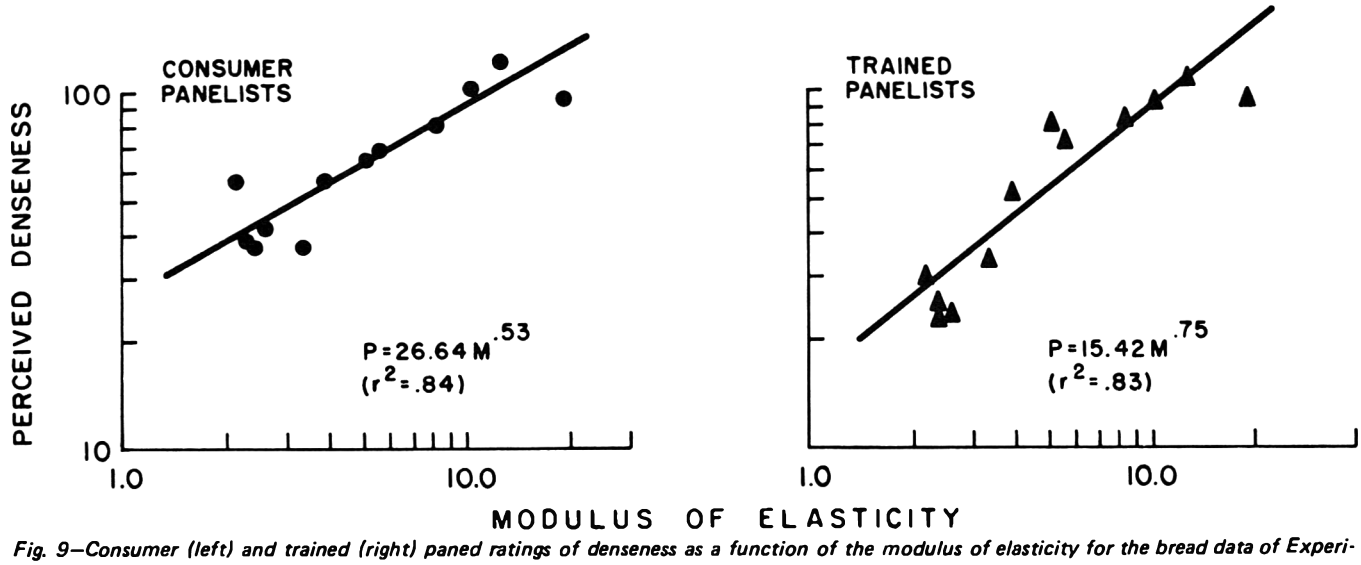

Fig. 9—Consumer (left) and trained (right) paned ratings of denseness as a function of the modulus of elasticity for the bread data of Experiment 4.

Fig. 10—Consumer (left) and trained (right) panel ratings of denseness as a function of bread density for the bread data of Experiment 4.

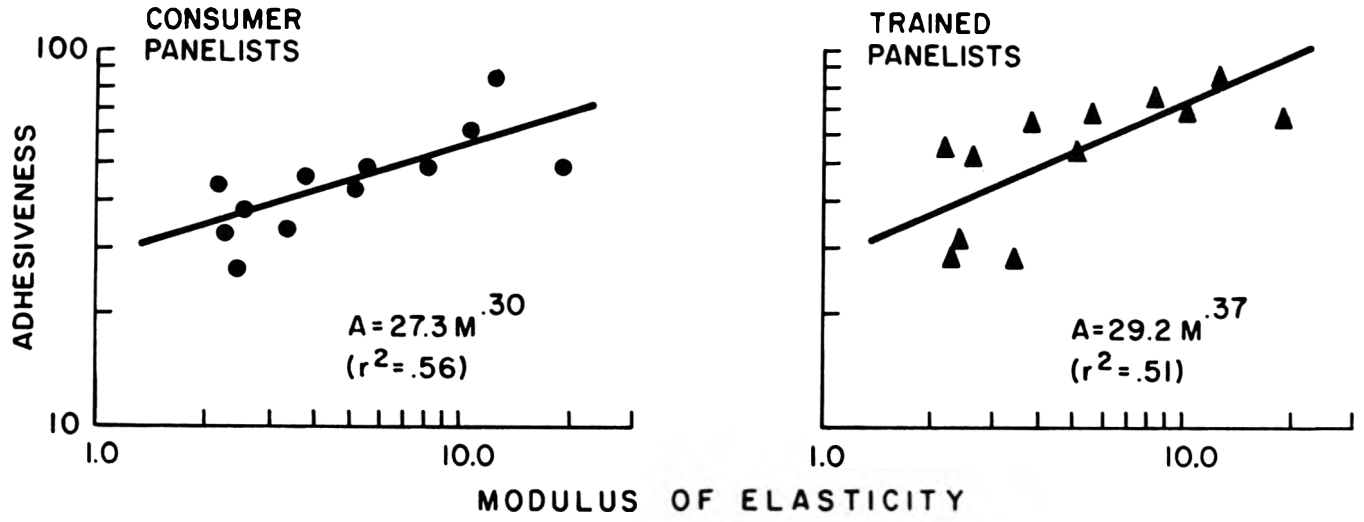

Fig. 11—Consumer (left) and trained (right) panel ratings of adhesiveness as a function of the modulus of ealsticity for the bread data of Experiment 4.

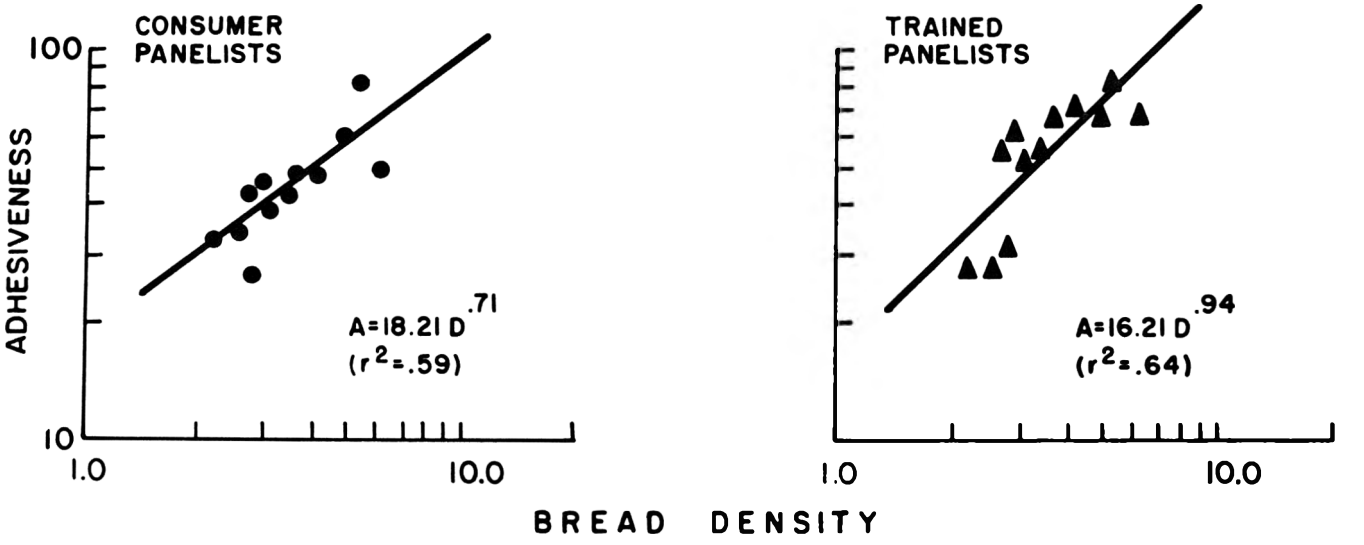

Fig. 12—Consumer (left) and trained (right) panel ratings of adhesiveness as a function of bread density for the bread data of Experiment 4.

function of increasing physical magnitude. In all cases, n is greater when the perceived magnitude is regressed against bread density than when it is regressed against the modulus of elasticity. This difference is due to the more restricted range of densities among the breads, as compared to the greater range of moduli of elasticity. The Pearson correlation coefficient between these two instrumental measures was high ($r=0.97$, $p<0.01$). Of greater interest, however, is the greater value of n noted for trained panelists. While the value of n is often considered to be a constant, reflecting physiological response properties of the receptor system, differences in the value of n have been reported under a variety of circumstances (Engen, 1956; Engen and Levy, 1958; Jones and Woskow, 1966; Poulton, 1968, Beck and Shaw; 1965; Pradhan and Hoffman, 1963). In the present study the larger value of n for trained panelists indicates a greater dynamic range of perceived intensities in this group of panelists than for consumers. This result parallels the results found in Experiments 2 and 3, where the slopes of the regression equations relating trained and consumer panel judgments reflected a greater range of perceived intensities for the trained panelists.

Pleasantness functions. Fig. 13 and 14 show the relationships of consumer and trained panel judgments of liking/disliking with the same objective measures above. Linear regression equations relating these measures show a decline in acceptability with increasing modulus of elasticity and/or density for both groups. However, the rate of decline, as reflected in the slope of the equation is greater for the trained panelists. In addition, the coefficients of determination (r^2) for the equations show that the acceptability of the bread is more closely associated with the modulus of elasticity than with the density of the bread. Direct linear regression of trained panel judgments of liking against consumer panel judgments of liking resulted in the regression equation $T_L = 1.66\ C_L - 35.08$ ($r^2 = 0.74$), with the slope of 1.66 reflecting a faster rate of change of liking with physical change in samples.

DISCUSSION

TAKEN TOGETHER, the results of these experiments show clear differences between consumer and trained panel judgments of the intensity of specific textural attributes and of liking, although no differences were observed between ratings of the two panels on a similarities scaling task.

The failure to find differences between ratings of the two panels on the similarities task was unexpected. If panelists are extracting specific features or attributes from the total percept in order to make estimates of stimulus similarity, it would be expected that the trained panelists would place

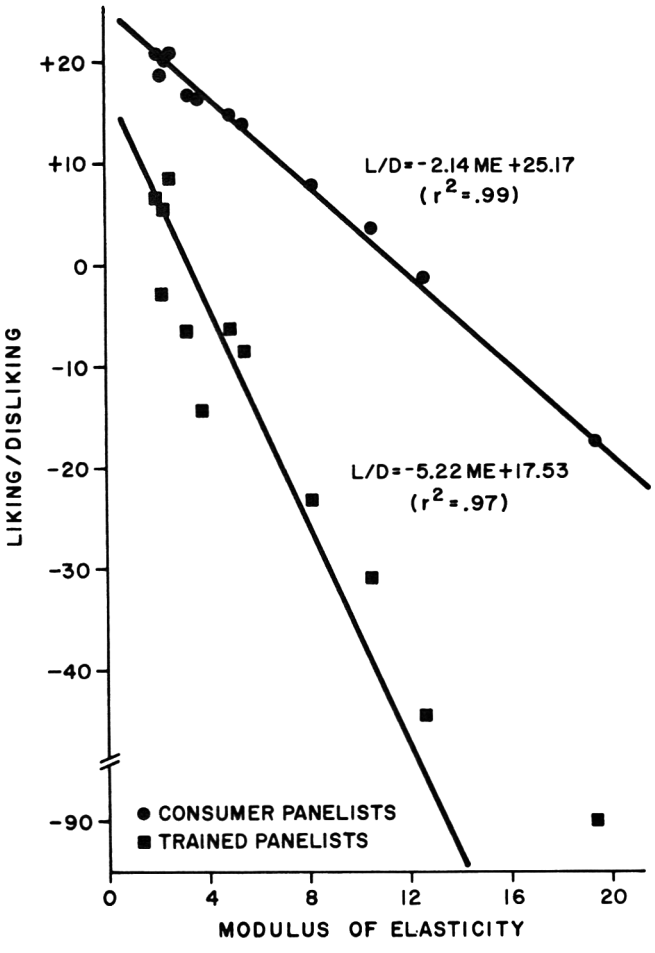

Fig. 13—Consumer and trained panel ratings of liking/disliking as a function of the modulus of elasticity for the bread data of Experiment 4.

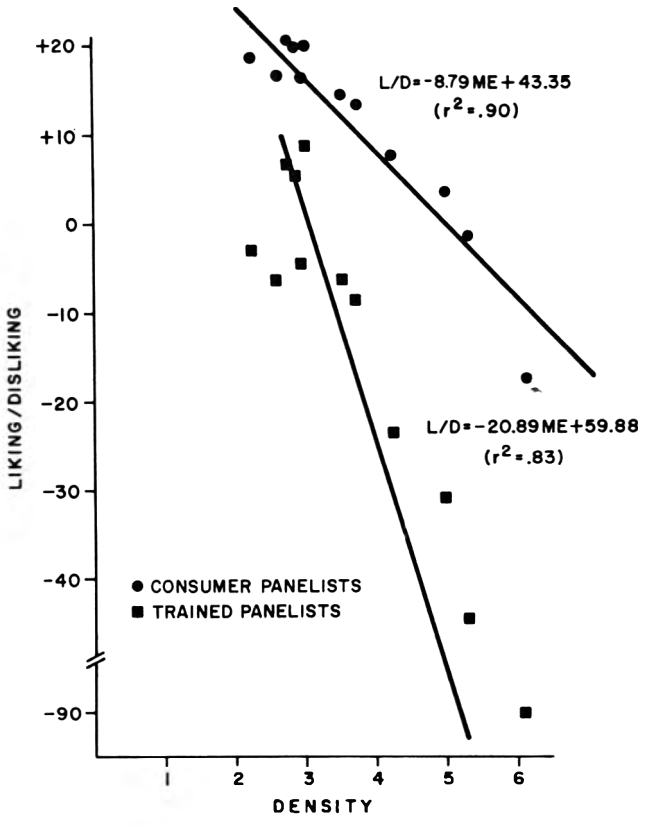

Fig. 14—Consumer and trained panel ratings of liking/disliking as a function of bread density for the bread data of Experiment 4.

more weight on the textural dimension of flakiness than consumers. This was not the case. However, the data from Experiments 2, 3 and 4 are clear in showing that the range of perceptual magnitudes for a variety of textural attributes is augmented in panelists who have undergone texture profile training. One possible explanation of the failure to observe such differences in the similarities scaling task is that the panelists are not operating in an analytic or "feature extracting" mode when judging similarities. That is, they are not cognitively aware of, and judging, each attribute independently. Thus, the major advantages of analytic training—commonality of attribute definition and commonality in procedure of evaluation of a single attribute—is lost. In the case of flakiness of fish, for example, judgment of this attribute by a trained panelist, will be guided by a specific pre-established definition of flakiness, such as "the perceived force required to produce separation between adjoining flakes within the muscle", and a specific method of evaluation, such as, "by manipulation of the muscle between tongue and palate." However, in a similarities task, the panelist neither considers each textural dimension of the sample sequentially nor considers the specific definition or method of evaluation for each. As a result, the analytic aspect is totally absent, and differences between trained panelists and consumers are not observed. Whether attribute definitions and methods of evaluation become more ingrained in trained panelists after many years of experience, so that training effects might appear in similarity judgments after ten or 15 yr of continued practice is a possibility that deserves further examination with a long-established panel.

The difference between consumer and trained panel judgments of specific textural dimensions in these experiments appear to be the result of an increased perceptual range for textural attributes resulting from training and experience. This notion is supported by the regression analyses of Experiment 2 and 3, in which the slope of the regression lines relating trained to consumer panel judgments were greater than 1.0 for all judged attributes. This notion was further supported by the results of Experiment 4, in which it was found that the exponents of the psychophysical functions relating perceived magnitude to underlying rheological measures were greater for trained panelists on all examined textural attributes.

These results parallel the results of numerous studies conducted over the years in a wide variety of senosry modalities, which have examined the effects of experience on perception. These studies, many of which have been catalogued and discussed in the text by Gibson (1969), have demonstrated an increased ability to discriminate among sensory stimuli following repeated experience with a specific stimulus or attribute domain. Examples of such perceptual learning in the food senses range from the ability of experienced wine-tasters to differentiate wines made from grapes of different vintage to the demonstration that taste thresholds for compounds characteristic of the four basic taste qualities can be significantly reduced by training (Pangborn, 1959). In the case of the present experiments, the experience that the trained panel had received improved their ability to discriminate among stimuli varying along given textural dimensions. This improved discriminative ability was manifested in an overall greater perceptual range of intensities for the stimulus series examined in these experiments. In addition, the present studies demonstrate the effect that such perceptual learning can have on judgments of liking for a product.

The observed difference between consumer and trained panel judgments of liking for bread in Experiment 4 was

somewhat unexpected, since scaling of liking involves simultaneous assessment of all the sensory characteristics of a product, in a manner similar to that for judging similarities. Thus, differential training on one set of attributes, such as texture, was not expected to affect overall judgments of liking to any significant degree. Nevertheless, it was found that liking decreased much more quickly as a function of increasing modulus of elasticity and bread density for trained panelists than for consumer panelists. These results are consistent with the notion that trained panelists perceive a greater range of textural intensities than consumer panelists and that this exaggerates the perceptual effect of changes in the modulus of elasticity and bread density, causing exaggerated effects on associated liking. Thus, the differences in acceptability between panelists provide an independent source of validating information for the differences observed in response magnitudes of the underlying texture attributes.

Overall, these experiments demonstrate that trained and consumer panel judgments of texture differ quantitatively, due primarily to an expanded perceptual range (increased discriminative ability) on the part of the trained panelists. The fact that linear regression equations between judgments of the two types of panels account for up to 99% of the variability among judgments (Table 4) means that predictive relationships that take into account differences in perceptual ranges between the two groups, are possible. Data need to be obtained on a wider sample of food products and textures before the efficacy of panel-interchange can be fully assessed.

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Effect of Trace Mineral Fortification on the Storage Stability of Ascorbic Acid in a Dehydrated Model Food System

D. B. DENNISON and J. R. KIRK

ABSTRACT

The catalytic influence of the transition metals, iron, copper and zinc, on the storage stability of ascorbic acid in a dehydrated model food system was studied as a function of water activity (a_w). No catalysis by the added metals was observed at 0.10 and 0.40 a_w. This is interpreted as a lack of metal ion mobility and/or insolubility at low a_w, thus hindering the participation of the metals as catalysts. At 0.65 a_w, which is in the capillary region on the adsorption isotherm, a 2–4 fold increase in the rate of ascorbic acid destruction was observed in the presence of the trace minerals. The increased rate of ascorbic acid degradation at a_w 0.65 is believed to be the result of greater mobility of metal ions.

INTRODUCTION

THE CATALYTIC PROPERTIES of transition metals on the oxidation of ascorbic acid, notably copper and iron, have been previously discussed in the literature. Weissberger and LuValle (1944) reported that only the monoionic ascorbic acid species was susceptible to Cu(II) catalysis. More recently, studies by Khan and Martell (1967) showed the oxidation of ascorbic acid in solution to be linearly dependent on the concentration of Cu(II) and Fe(III) ions. Ogata et al. (1968) found that the ability of Cu(II) ion to catalyze the destruction of ascorbic acid in aqueous solutions was dependent on the anion of the salt in the order $CuCl_2$, $Cu(NO_3)_2$ and $CuSO_4$. Shtamm and Shurlator (1974) and Jameson and Blackburn (1975) have also reported the catalytic properties of Cu(II) ions in the degradation of ascorbic acid in solution.

The mechanistic interpretation of the metal catalyzed oxidation of ascorbic acid in aqueous solutions is varied. Khan and Martell (1967) postulated an ascorbate-metal-oxygen complex involving a one electron transfer to oxygen. Jameson and Blackburn (1975) proposed the formation of a metal-metal dinuclear ascorbate-oxygen intermediate with a two electron transfer to oxygen.

The purpose of this study was to determine if the presence of selected trace minerals common to mineral fortification of dehydrated foods, including ready-to-eat breakfast cereals, would catalyze the destruction of ascorbic acid in a dehydrated model food system equilibrated to water activities which were below and above the BET monomolecular moisture content.

EXPERIMENTAL

A DEHYDRATED model food system (pH 6.8) designed to simulate a ready-to-eat breakfast cereal with the composition shown in Table 1 was prepared as outlined by Kirk et al. (1977). The homogenized model system slurry (ca. 40% total solids) was fortified with U.S.P. reduced ascorbic acid at 25% NAS/NRC RDA (11.25 mg ascorbic acid) per 100g on a dry weight basis. The model system slurry was then subdivided and fortified with ferrous sulfate, ferrous chloride, reduced iron, zinc sulfate, zinc chloride, zinc oxide, cuprous sulfate and cuprous chloride. Mineral fortification levels were at 10 and 25% of the NAS/NRC RDA per 100g dry weight of the model system for iron and zinc. Fortification of Cu was 1.0 mg and 2.5 mg per 100g of model food system, dry basis. All salts were of reagent grade and used without further purification. Each lot of the model system was freeze-dried and equilibrated on the adsorption isotherm to 0.10, 0.40 and 0.65 a_w at 30°C according to the method described by Kirk et al. (1977). Approximately 15g of the equilibrated samples were sealed in 303 x 406 enameled metal cans and stored isothermally at 30°C.

Total and reduced ascorbic acid were determined according to the continuous flow o-phenylenediamine microfluorometric procedure of Kirk and Ting (1975). Ascorbic acid determinations were performed in triplicate at constant intervals ranging from 48 hr to 168 hr depending on a_w. Samples having an a_w of 0.65 were monitored through two half-lives of ascorbic acid, while samples having an a_w of 0.10 and 0.40 were not monitored through more than one half-life.

The reaction rate constants and the activation energies were calculated by linear regression analysis and by a computer program, KINFIT (Dye and Nicely, 1971). The KINFIT program differs from the usual least squares technique in that numerical integration procedures are used to provide a fit to the desired differential equation. This method of calculation assists in accounting for errors in vitamin assays and small variations in storage times and is specifically written for chemical reactions. Thermodynamic activation parameters for destruction of ascorbic acid were calculated based on the theory of absolute reaction rates (Eyring, 1935). Ascorbic acid levels for zero time storage were determined after each aliquot of the dehydrated model food system was equilibrated to the desired water activity.

RESULTS & DISCUSSION

THE INFLUENCE of trace mineral fortification on the storage stability of ascorbic acid in a low moisture model food system was studied as a function of water activity at a constant storage temperature of 30°C. Experimental data describing the degradation of reduced ascorbic acid (RAA) in the model system fortified with $FeSO_4$ and $FeCl_2$ at 10% RDA, equilibrated and stored at 0.10, 0.40 and 0.65 a_w are presented in Fig. 1. Linear regression analysis confirmed that the loss of RAA could be satisfactorily described by first-order kinetics, however, samples stored at 0.10 and 0.40 a_w were not held through more than one half-life. Correlation coefficients were > 0.93, the standard deviations were $<10\%$ of the rate constants. Other rate functions did not provide a better fit. The anticipated dependence of ascorbic acid degradation on the water activity as reported

Table 1—Composition of model food system

Component	%[d]
Protein[a]	10.2
Fat	1.0
Carbohydrate[b]	76.6
Reducing sugar[c]	5.1
Sucrose	5.1
Salt	2.0

[a] Soy protein—Promine D. Central Soya.
[b] Food Grade Powdered Starch—A.E. Staley, Inc. and Corn Syrup Solids 15 D.E., American Maize.
[c] Supplied by the corn syrup solids.
[d] Calculated on dry weight basis.

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by Lee and Labuza (1975), Kirk et al. (1977) and Dennison and Kirk (1978) was clearly evident.

The experimentally determined first-order rate constants and half-lives for RAA and TAA degradation are presented in Tables 2 and 3, respectively. No significant difference at the 0.05 level in TAA and RAA destruction rates were observed at the three water activities studied. These rate constants are consistent with those previously reported by Kirk et al. (1977).

It is suggested from the data for TAA and RAA losses (Tables 2 and 3) that there is no catalytic activity associated with any of the metals studied at 0.10 a_w. A water activity of 0.10 is considerably below the BET monomolecular moisture content (a_w = 0.24) of this model system (Bach, 1974). Therefore, the solubility and mobility of the metal ions would be quite limited.

No increase in the rate of RAA and TAA degradation as a result of metal ion catalysis was observed at 0.10 a_w or at 0.4 a_w. A slight catalytic effect was noted in the model system containing 2.5 mg $CuCl_2$ and $CuSO_4$ per 100g of model system (dry basis) at 0.40 a_w. The Cu(II) ion may possess somewhat greater mobility at 0.40 a_w than the

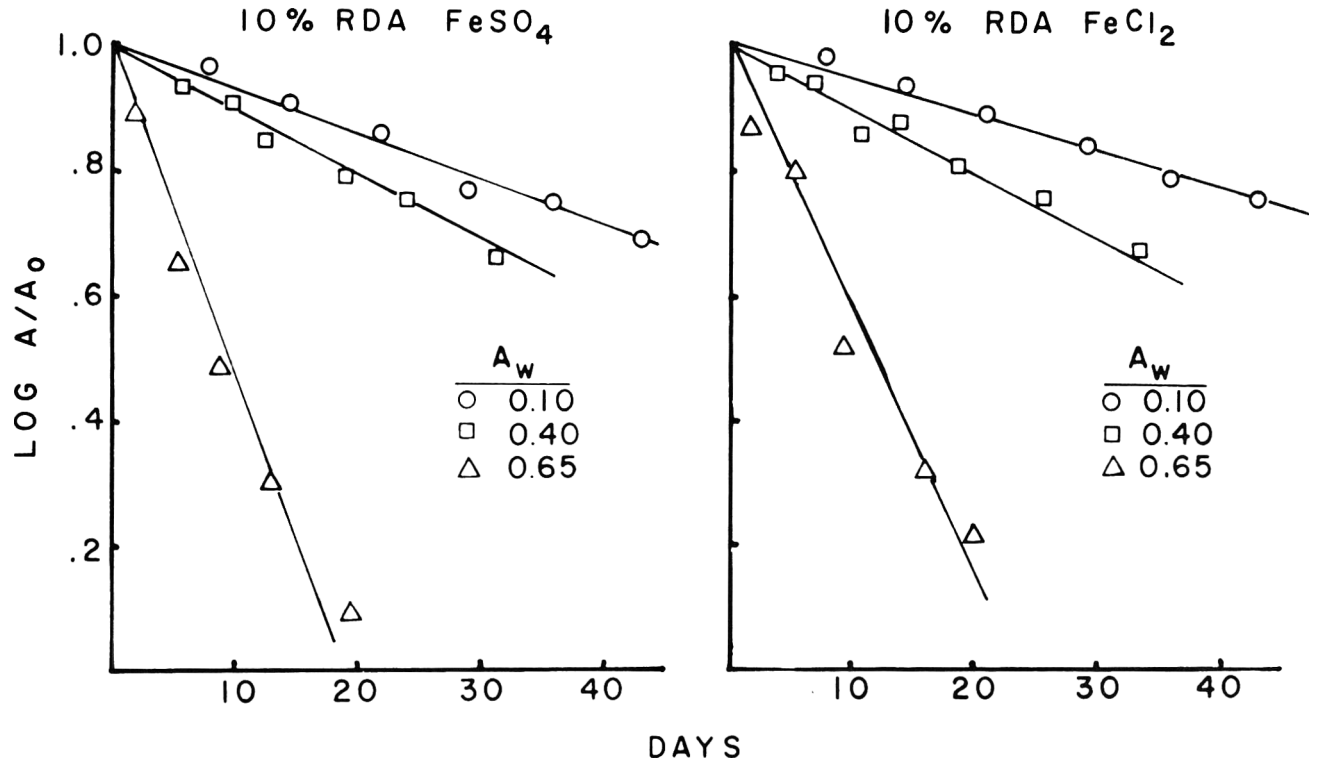

Fig. 1—Fraction of RAA remaining vs time for model system fortified with 10% RDA $FeSO_4$ and $FeCl_2$ and equilibrated at selected water activities and stored at 30°C.

Table 2—Rate constants and half-lives for RAA loss as a function of mineral fortification and water activity in a dehydrated model food system stored at 30°C in 303 cans

Mineral supplement	% RDA	0.10 a_w		0.40 a_w		0.65 a_w	
		k[a]	$t_{1/2}$	k[a]	$t_{1/2}$	k[a]	$t_{1/2}$
None	–	2.04	33	3.45	20	5.01	14
$FeSO_4$	10	1.74	40	2.47	28	11.48	6
	25	2.16	32	2.44	28	11.73	6
$FeCl_2$	10	1.70	41	2.97	23	10.07	7
	25	1.72	40	2.69	26	9.87	7
Fe	10	1.32	52	2.64	26	9.38	7
	25	1.65	42	2.20	31	17.51	4
$ZnCl_2$	10	1.96	35	2.55	27	17.06	4
	25	2.13	33	2.47	28	23.19	3
$ZnSO_4$	10	1.87	37	2.93	24	18.13	4
	25	1.77	39	3.02	23	18.65	4
ZnO	10	1.74	40	2.52	28	3.37	21
	25	1.63	43	2.46	28	3.90	17
$CuCl_2$	1.0[c]	1.28	54	3.01	23	5.94	12
	2.5[c]	1.75	40	5.93	12	12.01	5
$CuSO_4$	1.0[c]	1.40	50	4.00	17	4.72	15
	2.5[c]	1.80	39	5.92	12	9.18	8

[a] First-order rate constant, x 10^{-2} $days^{-1}$.
[b] Half-life, days.
[c] Mg Cu/100g model system d.b.

Table 3—Rate constants and half-lives for TAA loss as a function of mineral fortification and water activity in a dehydrated model food system stored at 30°C in 303 cans

Mineral supplement	% RDA	0.10 a_w		0.40 a_w		0.65 a_w	
		k[a]	$t_{1/2}$	k[a]	$t_{1/2}$	k[a]	$t_{1/2}$
None	—	1.92	36	3.50	20	5.09	14
$FeSO_4$	10	1.31	53	2.20	31	11.22	6
	25	1.81	38	2.11	33	11.62	6
$FeCl_2$	10	1.33	52	2.57	27	10.48	7
	25	1.59	44	2.94	24	9.41	7
Fe	10	1.31	53	2.69	26	8.65	8
	25	1.53	45	2.25	31	17.28	4
$ZnCl_2$	10	1.40	50	2.38	29	17.25	4
	25	1.62	43	2.47	28	25.65	3
$ZnSO_4$	10	1.44	48	2.91	24	18.60	4
	25	1.75	40	2.30	30	17.53	4
ZnO	10	1.34	52	2.25	31	3.19	22
	25	1.63	43	2.50	28	2.91	18
$CuCl_2$	1.0[c]	1.36	51	3.86	18	5.51	13
	2.5[c]	1.38	50	4.50	15	11.67	6
$CuSO_4$	1.0[c]	1.17	59	5.25	13	5.00	14
	2.5[c]	1.52	46	6.37	11	9.00	8

[a] First-order rate constant, x 10^{-2} days^{-1}.
[b] Half-life, days.
[c] Mg Cu/100g model system d.b.

other minerals studied, thus facilitating its participation in the degradation of ascorbic acid.

At 0.65 a_w, which is in the capillary region on the adsorption isotherm (Bach, 1974), a 2-4 fold increase in the degradation rate of TAA and RAA is noted for each of the added trace minerals as compared to the control model system. The only exception was zinc oxide, which did not exhibit a catalytic effect and the cuprous salts at a level of 1 mg per 100g of model system (dry basis). Catalysis by copper and iron was expected based on previously published data concerning oxidation of ascorbic acid in aqueous solutions (Khan and Martell, 1967) and the mobility of solutes in dehydrated model systems equilibrated to a_w in their capillary region (Duckworth and Kelley, 1973; Lee and Labuza, 1975). Ferrous iron is probably oxidized to Fe (III) by molecular oxygen before catalyzing the oxidation of ascorbic acid. The catalytic nature of elemental iron is surprising because it should be insoluble. One could speculate that Fe° is oxidized to Fe (III) in the presence of O_2 and water, but this has not been shown and is beyond the scope of this study.

It is difficult to discern the precise effect of mineral concentration on the reaction due to the limited number of data points, but ascorbic acid degradation appears to increase with increasing copper and iron levels. This concentration dependence is in accord with the results of Khan and Martell (1967) and Ogata et al. (1968). No catalytic properties could be associated with the anion of the salt as suggested by Mapson (1945) and Ogata et al. (1968).

The observation that metal ions in the model system did not show a significant effect on ascorbic acid stability at a_w's below 0.65 (Tables 2 and 3) may indicate that the complete hydration of the metal is required for mobility. This may only be possible in the free water of the capillary region of the isotherm (0.65 for this system). Below this region, the metal ions may have one or more charged species from the product matrix as substituted ligands which could effectively immobilize or hinder their migration.

The absence of a catalytic effect associated with ZnO in the degradation of TAA and RAA in the model system may be explained in terms of its characteristic insolubility in aqueous solution. The catalysis exhibited by zine chloride and zinc sulfate was unexpected because Zn (II) is reportedly inactive as a catalyst of ascorbic acid oxidation due to the lack of a stable lower valence state (Khan and Martell, 1967). However, Long and McDevit (1952) showed that the activity of oxygen increased linearly with increasing electrolyte concentration and was dependent on both the cation and anion present. This phenomenon may be responsible for the rate enhancment observed for ascorbic acid degradation in the model food system with added $ZnCl_2$ and $ZnSO_4$.

Data describing the rate of ascorbic acid degradation in the dehydrated food system equivalent to 0.65 a_w are interpreted as reflecting two types of behavior: (1) catalysis by copper and iron ions as suggested by Khan and Martell (1967) and (2) rate enhancement due to the increased activity of oxygen on the addition of soluble zinc electrolytes.

In conclusion, data from this research indicate that trace mineral fortification of a low moisture food would appear to have a negligible effect on ascorbic acid stability if the water activity of the food system is below the capillary region.

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A Simplified Model for Freezing Time Calculations in Foods

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ABSTRACT

A model is proposed for freezing time calculations which combines Plank's equation with the unsteady heat transfer solutions for the cooling of a slab of constant properties through the addition of precooling, change of phase and tempering periods. The change of thermal properties with the ice content is taken into account by proposing average values for the different periods. No adjustable parameters are used in developing the model. Results are compared for the case of beef freezing with those obtained numerically by using a heat transfer model with simultaneous change of phase and with experimental measurements showing good agreement.

INTRODUCTION

THE CALCULATION of freezing times for foods, involves a complex problem of heat transfer with simultaneous phase change, variable thermal properties and, in many cases, anisotropy problems.

These calculations can be made using various models of behavior, ranging from those that can be solved analytically and involve relatively simple equations, to those which are numerical and must be solved by computer.

When using analytical models, the calculation is generally based on Plank's equation (1941) or on one of its many modified forms adopted with the intention of eliminating part of its practical limitations, since it was developed for one dimensional pseudo-steady state heat transfer in the frozen product, constant properties and initial temperature equal to that of the phase change which is assumed as constant.

Plank himself made corrections to his original model in order to take into account various initial and final temperatures (Plank, 1963). The proposed equation for slab geometry (Fig. 1) is shown in Table 1 where T_r is the final temperature at the center of the piece to be frozen and n is a function of the Biot number ($\bar{B}i_c = hL/\bar{k}_c$) supplied by Plank and shown in Table 2.

Since, in the case of foods, properties are functions of the ice content depending in turn on the temperature (Riedel, 1951; 1956; 1957) the temperatures at which thermal properties should be evaluated as recommended by Plank (1963) are included in Table 1.

The model proposed by Nagaoka et al. (1955) also involves a modification of Plank's original equation and is shown in Table 1. The properties are proposed to be calculated in a similar way to that of Plank except ΔH_r which must take into account the enthalpy change between T_i and T_r.

Cleland and Earle (1977) proposed another modification of Plank's original formula in accordance with the equation shown in Table 1. The specific heat $\bar{C}_{p_r}$ and the density ρ_r should be evaluated at T_r according with authors' recommendations. The enthalpy change to be considered is between T_e and T_r. Their work was exclusively for $T_r = -10°C$, although they state that their formula is applicable to other T_r using the corresponding values of ΔH_r.

With regard to numerical methods, several have been published recently which produce fairly exact results by including the variation of thermal properties with temperature. In general, the differences among these methods are due to the type of functions used to express those dependencies, either through physical models or through regression of experimental data.

The start point in all cases was the microscopic balance of thermal energy (Eq 1) which was solved by using numerical methods and adopting in general slab geometry.

Thus, Bonacina and Comini (1971) solve the differential equation by using a three-level implicit finite difference method with the properties evaluated at intermediate points to those calculated.

This model was applied to predict the freezing of gel simulating the food, satisfactory agreement with experimental results having been obtained.

Cleland and Earle (1977); (1979a, b), used the same method for predicting freezing times in slabs, cylinders, spheres and rectangular bricks of food materials.

Heldman (1974), used a Crank-Nicholson method while Joshi and Tao (1974) applied an explicit method ensuring stability through the use of small time increments. In both cases the thermal properties were obtained from equations developed from published data on beef. The thermal conductivity was assumed to be isotropic and even though temperature distributions for different times were calcu-

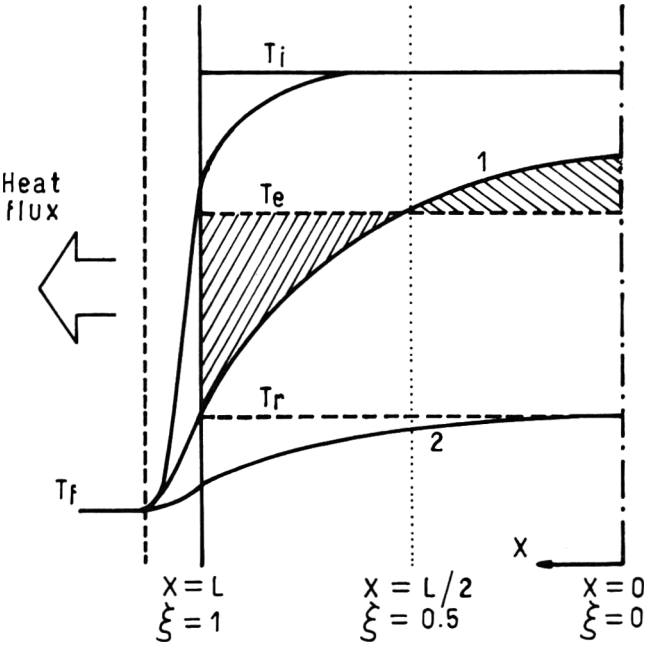

Fig. 1—Sketch of temperature profiles at the end of pre-cooling (Curve 1) and tempering periods (Curve 2).

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lated no values of freezing times covering a wide range of parameters were reported.

However, although numerical models are more exact, they are difficult to use in practice. In the first place, although they provide numerical results, it is hardly possible to trace curves covering the ranges of all the parameters concerned. The other alternative involves possession of the numerical program and the appropriate computer, something rarely feasible for the user of this type of calculation. If we add to the above the fact that calculation times are relatively long, owing to the type of equations involved, it is not strange that approximate models continue to be used, which, although less exact, permit rapid and simple calculation of the freezing times in terms of actual operating conditions.

The present paper develops a simple calculation model which combines Plank's original equation with heat transfer solutions without change of phase, by the addition of latent and sensible heat transfer times, respectively.

The results are compared, in the case of meat freezing, with those obtained numerically based on a heat transfer model with simultaneous change of phase (Mascheroni and Calvelo, 1980), with those calculated using several existing analytical models and with freezing times measured in laboratory experiences which simulate industrial processing.

THEORY

IN ORDER TO COMPARE the various equations proposed in the bibliography as well as those developed in the present study under a wide range of operating conditions it is necessary to have a reference model that is accepted as correct.

For this purpose, a previously developed heat transfer model with simultaneous change of phase was used to simulate beef freezing (Mascheroni and Calvelo, 1980). This numerical model agreed satisfactorily with experimental temperature profiles obtained under conditions similar to those of industrial processing.

The general characteristics of this model are described below.

Heat transfer model with simultaneous change of phase

Considering one dimensional heat flux in a slab of beef of thickness 2L refrigerated on both sides (Fig. 1), the microscopic energy balance becomes:

$$\rho(\omega)\,\hat{C}_p(\omega)\frac{\partial T}{\partial t}=\frac{\partial}{\partial x}\,k(\omega)\frac{\partial T}{\partial x} \qquad (1)$$

where it will be seen that density ρ, specific heat $\hat{C}_p$ and thermal conductivity k are considered to be a function of the frozen water fraction ω.

The initial and boundary conditions which simulate the operation in plate freezers are:

$$t \leq 0 \qquad T = T_i \qquad 0 \leqslant x \leqslant L \qquad (2)$$

$$t > 0 \qquad -k\frac{\partial T}{\partial x} = h\,(T - T_f) \qquad x = L \qquad (3)$$

$$t > 0 \qquad \frac{\partial T}{\partial x} = 0 \qquad x = 0 \qquad (4)$$

where T_i is the initial temperature, T_f is the temperature of the cooling agent, and h is the heat transfer coefficient in the interphase between the beef and the refrigerated plate. Fig. 1 presents a sketch of the system.

In order to solve Eq (1) it is necessary to know the functions of ρ, $\hat{C}_p$, and k in terms of ω as well as the relationship between ω and T.

Table 1—Simplified models for freezing times calculation

Author	Calculating equation	Auxiliary functions
Plank (1963)	$t_{c_r} = \frac{\bar{\rho}\,L^2\,\Delta\bar{H}}{(T_e - T_f)\bar{k}_c}\left(\frac{1}{\bar{Bi}_c}+\frac{1}{2}\right)\left[1 + 0.0053\,(T_i - T_e)\right] + t'_r$ $\bar{Bi}_c = hL/\bar{k}_c$	$t'_r = \frac{1.866\,L^2\,n\,\bar{\rho}\,\bar{C}p}{\bar{k}_c}\left[\log\left(\frac{T_e - T_f}{T_r - T_f}\right) - 0.0913\right]\left[\frac{1}{\bar{Bi}_c}+\frac{1}{2}\right]$ $\bar{\rho} = 1$ $\Delta\bar{H}$ = Change of enthalpy between T_e and 261°K $\bar{C}_p = \frac{\text{Change of enthalpy between 269°K and } T_r}{(269 - T_r)}$ $\bar{k}_c$ = Thermal conductivity evaluated at 261 K n = Function given in Table 2
Nagaoka et al. (1955)	$t_{c_r} = \frac{\bar{\rho}\,L^2\,\Delta H_r}{(T_e - T_f)\bar{k}_c}\left[\frac{1}{\bar{Bi}_c}+\frac{1}{2}\right]\left[1 + 0.008\,(T_i - T_e)\right]$	ΔH_r = Change of enthalpy between T_i and T_r
Cleland and Earle (1977)	$t_{c_r} = \frac{\rho_m\,L^2\,\Delta H'_r}{(T_e - T_f)\,k'_c}\left[\frac{P}{2Bi'_c}+R\right]$ $Bi'_c = hL/k'_c$	$P = 2.0288 + 0.8072\,P_K + Ste\,(1.2896\,P_K + \frac{0.021}{Bi'_c} + 0.2724)$ $R = 0.6736 + Ste\,(1.096\,P_K - 0.054)$ $P_K = \rho_o\hat{C}_{p_o}(T_i - T_e)/\Delta H'_r\rho_m$; $Ste = \rho_r\hat{C}_{p_r}(T_e - T_f)/\Delta H'_r\rho_m$ $\hat{C}p_r$ = Specific heat evaluated at T_r k'_c = Thermal conductivity of the frozen product evaluated at −30°C (Cleland and Earle 1976) $\rho_m = (\rho_o + \rho_r)/2$ $\Delta H'_r$ = Change of enthalpy between T_e and T_r
This work	$t_{c_r} = t_p + t_{c_e} + t_r$ $t_{c_e} = \frac{\rho_o\,Y_o\,\lambda^o{}_f\,\omega_m\,L^2}{(T_e - T_f)k_c}\left[\frac{1}{Bi_c}+\frac{1}{2}\right]$ $Bi_c = hL/k_c$	Fig. 2 and 3 $\eta_p = (T_e - T_f)/(T_i - T_f)$; $\eta_r = (T_r - T_f)/(T_e - T_f)$ $\bar{k} = (k_o + k_c)/2$; $\bar{\alpha} = (\alpha_o + \alpha_c)/2$ k_c = Thermal conductivity evaluated at $T_c = (T_e + T_f)/2$ α_c = Thermal diffusivity evaluated at $T_c = (T_e + T_f)/2$

The functionality of ω with T was obtained from a cryoscopic descent model developed in a previous study (Mascheroni and Calvelo, 1978) which condensed experimental data on beef obtained by Riedel (1957) in the following equation:

$$\omega = E - FT/(T_o - T) \quad (5)$$

where E and F are constants depending on the initial water content of meat (for 74% E = 0.9309 and F = 3.466 x 10^{-3}), T is the absolute temperature and T_o is the freezing temperature of pure water (273.16°K).

The density of partially frozen meat was calculated assuming it to be constituted by ice, water, and dry tissue as follows:

$$\rho(\omega) = \rho_o / [1 + \omega\gamma(r - 1)] = \rho_c \quad (6)$$

where $r = \rho_a/\rho_h$ and $\gamma = Y_o \rho_o/\rho_a$.

In these equations, ρ_o is the density of unfrozen meat, Y_o is the water content on a wet basis, ρ_h is the density of ice and ρ_a is the water density.

Obviously, the latent heat of solidification is included in the apparent specific heat $\hat{C}_p$ of Eq (1). The different contributions can be evaluated by an enthalpy balance to give:

$$\hat{C}_p(\omega) = \hat{C}_{po} - \omega Y_o \Delta\hat{C}_p - Y_o \left[\lambda_f^o + \Delta\hat{C}_p (T - T_o)\right] d\omega/dT \quad (7)$$

where $\Delta\hat{C}_p = \hat{C}_{p_a} - \hat{C}_{p_h}$; $\hat{C}_{p_o}$ is the specific heat of unfrozen meat and $\hat{C}_{p_h}$ is that of ice, which, like that of water, $\hat{C}_{p_a}$, was assumed to be constant.

The first two terms of Eq (7) correspond to the change in the specific heat of beef when the water is converted into ice, while the last two represent the crystallization heat involved during freezing.

Contrary to the previously analyzed properties, the prediction of thermal conductivity in partially frozen meat calls for the adoption of a certain arrangement of ice in the meat tissue.

The model adopted for the calculation was developed in a previous study (Mascheroni et al., 1977) and involves a certain arrangement of ice and partially dehydrated fibers.

The water contained in the fibers is assumed to be dispersed in a continuous matrix forming spheres distributed according to the Maxwell-Euken model (Kingery, 1960).

The ice is outside the fibers, surrounding them, thus generating a different thermal conductivity perpendicular or parallel to the fibers.

When the temperature drops, the quantity of ice outside the fibers increases, while at the same time the water dispersed within them diminishes. Consequently, the model changes its thermal conductivity in accordance with those changes.

In the case of freezing parallel to the fibers, the thermal conductivity k_L will be:

$$k_L = k_h\lambda + (1 - \lambda)\left[k_h \lambda^2 + k_t (1 - \lambda)^2 + \frac{4\lambda(1 - \lambda)}{1/k_t + 1/k_h}\right] \quad (8)$$

where

$$\lambda = 1 - \sqrt{1 - \omega Y_o \rho_c/\rho_h} \quad (9)$$

$$k_t = k_m \frac{1 - (1 - \alpha_k k_a/k_m)\beta}{1 + (\alpha_k - 1)\beta} \quad (10)$$

$$\alpha_k = 3 k_m/(2 k_m + k_a) \quad (11)$$

and

$$\beta = (1 - \omega)\gamma/(1 - \omega\gamma) \quad (12)$$

When the heat flux is perpendicular to the beef fibers, the thermal conductivity can be predicted by using the following equation:

$$k_p = \frac{k_h k_t (1 - \lambda)}{\lambda k_t + k_h (1 - \lambda)} + k_h \lambda \quad (13)$$

In Eq (8) to (13), k_h, k_a and k_t are the thermal conductivities of ice, water and fibers, respectively, and λ the surface ice fraction. The thermal conductivity of the matrix inside the fibers k_m which depends on the direction of the heat flow was evaluated from that of unfrozen meat as k_{mL} = 0.3540 W/m°K and k_{mp} = 0.2419 W/m°K.

The solution of Eq (1) to (13) was obtained numerically using an explicit method of finite differences and an IBM/360 computer.

As will be observed from the equations involved, the number of parameters is large, although they can be divided into those required by the particular system (in this case, beef) and those connected with the operating conditions used during freezing.

Once the system is adopted (for example, beef with 73.36% of water content) the parameters of the first group are all defined as shown in Table 3.

Consequently, from the second group of parameters it follows that the temperature of the system will be:

$$T = T(x, t, T_i, T_f, Bi_o) \quad (14)$$

where $Bi_o = hL/k_o$, k_o being the thermal conductivity of unfrozen meat and h the heat transfer coefficient introduced in the boundary condition (3).

The analysis of the thermal histories for different positions was made in a previous study (Mascheroni and Calvelo, 1980). However, the analysis of freezing times calls for their prior definition. In general, different definitions have been adopted, but those most often used are the following:

$t_{c_{10}}$, defined as the time required for a piece of meat to reduce its temperature from the initial T_i, assumed to be uniform, until the thermal center reaches −10°C. This definition has been adopted by several authors (I.I.R., 1972; Cleland and Earle, 1977).

$t_{c_{18}}$, defined as the time for the temperature of the thermal center of the meat to drop from a uniform initial temperature T_i to −18°C. This definition of freezing time is more in accordance with some international regulations on frozen foods. (Italy, 1971; Buenos Aires, 1968).

The position and temperature in Eq (14) having been defined, it follows that any of the freezing times will maintain the following dependence:

$$t_{c_r} = t_{c_r}(T_i, T_f, Bi_o) \quad (15)$$

Values of $t_{c_{18}}$ as predicted by the described numerical model are presented in the Results and Discussion section.

Simplified analytical model

As mentioned before, the use of numerical models such as the above is complicated unless the calculation programme and the computer facilities are available. That is why the use of simplified models leading to analytical solutions has been preferred in most cases, often even at the cost of exactitude.

The analytical model proposed in the present paper consists in the addition of periods for pre-cooling (t_p), change of phase (t_{c_e}) and tempering (t_r) as follows:

$$t_{c_r} = t_p + t_{c_e} + t_r \quad (16)$$

The model was developed for a slab geometry which shows symmetry in x = 0 (Fig. 1), In the same figure the dimensionless coordinate $\xi = x/L$ has also been included.

The pre-cooling period t_p is defined as the time that elapses between the beginning of cooling with the whole system at T_i until T_e is reached for x = L/2 (ξ = 0.5) (curve 1 in Fig. 1). This coordinate was chosen so as to approximately compensate the excess heat removed from points $\xi > 0.5$ with that still to be removed from $\xi < 0.5$ (dashed areas in Fig. 1).

The change of phase period involves an initial condition given by the system at uniform temperature T_e (temperature for beginning of freezing) and a final condition with the system frozen and a temperature T_e at the center of the product.

For the tempering period, it is assumed that the system starts frozen with an initial temperature T_e and is cooled until the temperature T_r (−10°C or −18°C) is attained at the center (curve 2, Fig. 1).

To calculate the pre-cooling and tempering periods, the solution of Eq (1) for heat transfer without change of phase for an infinite slab was used, with a constant initial temperature T_i and constant external temperature T_f (Carslaw and Jaeger, 1959):

Table 2—Auxiliary function in Plank's simplified model

$\overline{Bi}_c$	0.25	0.5	1.0	2.0	4.0	10.0	20.0	∞
n	1.210	1.188	1.156	1.112	1.065	1.020	1.008	1.000

$$\eta = \frac{T - T_f}{T_i - T_f} = 2 \sum_{n=1}^{\infty} e^{-\lambda_n^2 \tau} \frac{\sin \lambda_n \cos(\lambda_n \xi)}{\lambda_n + \sin \lambda_n \cos \lambda_n} \quad (17)$$

where λ_n is the root n of the equation:

$$\lambda_n \tan \lambda_n = Bi \quad (18)$$

and $\tau = \alpha t/L^2$ is the dimensionless time, α being the thermal diffusivity ($\alpha = k/\rho C_p$).

Fig. 2 which represents Eq (17) for $\xi = 0.5$ allows for calculation of the precooling time t_p. For obtaining t_p, $\eta_p = (T_e - T_f)/(T_i - T_f)$ must be calculated and a value of $\bar{\alpha} = (\alpha_o + \alpha_c)/2$ has to be used, where α_o and α_c are the thermal diffusivity of the unfrozen food (considered independent of T_i) and that of the frozen product at an average temperature $T_c = (T_e + T_f)/2$.

The choice of an average thermal diffusivity $\bar{\alpha}$ is made because during this period the system behaves with the properties of the unfrozen product in the central region and those of the frozen food with temperatures varying from T_e to values approximating T_f near the border. Similarly, in calculating the Biot number an average thermal conductivity $\bar{k} = (k_o + k_c)/2$ is used.

In calculating tempering time, t_r, Fig. 3 is used, which is the representation of Eq (17), but now for $\xi = 0$. The value to be used is $\eta_r = (T_r - T_f)/(T_e - T_f)$ where T_r is the temperature to be attained at the thermal center of the product when freezing is finished (−10°C, −18°C or any other desired).

The properties values to be used in Fig. 3 are those for the frozen product evaluated at the average temperature $T_c = (T_e + T_f)/2$. Thus, in Fig. 3 it will be $Bi_c = hL/k_c$ and $\tau_r = \alpha_c t_r/L^2$.

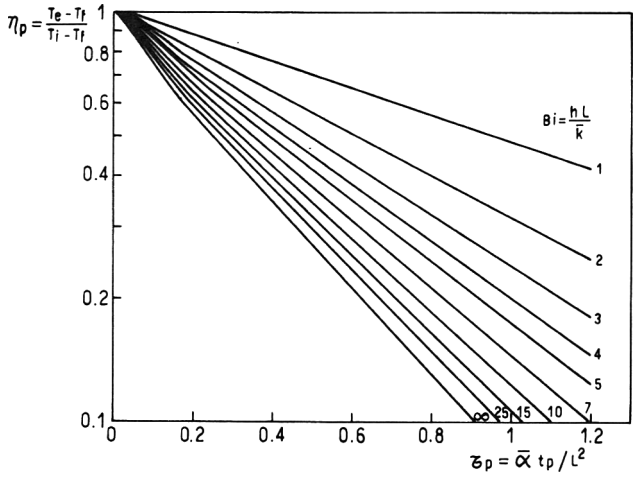

Fig. 2—Curves for pre-cooling time calculation.

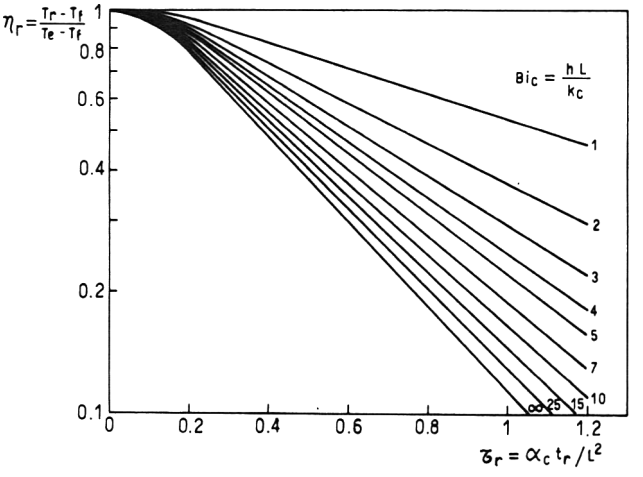

Fig. 3—Curves for tempering time calculation.

For the change of phase period the original Plank's equation is written as:

$$t_{c_e} = \frac{\rho_o Y_o \lambda^o_f \omega_m L^2}{(T_e - T_f) k_c} \left[\frac{1}{Bi_c} + \frac{1}{2} \right] \quad (19)$$

where ρ_o and Y_o are the density and water content of the unfrozen food, λ^o_f is the heat of solidification of water at 0°C, $\omega_m = (\omega_f + \omega_r)/2$ is the average between the ice fractions at the temperatures T_f and T_r and $Bi_c = hL/k_c$ (k_c being the thermal conductivity evaluated at $T_c = (T_e + T_f)/2$).

Freezing times predictions by this simplified method were compared with other existing models and with experimental values in the Results and Discussion section.

EXPERIMENTAL

IN ORDER TO CHECK experimentally the predictions of the proposed models, beef freezing times were measured in laboratory experiments which simulate industrial processing in plate freezers.

Experiments were carried out on semimembranosus muscle stored at 4°C for 2 or 3 days after quartering.

Samples of meat with cylindrical shape were cut with fibers perpendicular or longitudinal to the axial direction. The cylinders (6 cm length) were mounted in an acrylic sample-holder as shown in Fig. 4. The bases of the cylinder were in contact with metallic slab-shaped exchangers, inside which thermostatized methanol was circulated. Methanol temperature was controlled within ± 0.05°C. The lateral surface of the cylindrical sample holder was isolated with polystyrene foam.

Between the heat exchangers and the meat sample, acrylic slabs were placed to simulate thermal interfacial resistances, whose values could be modified in different experiments changing the thickness of the acrylic slabs.

Temperatures at different positions along the cylinder were measured using copper-constantan thermocouples with a diameter of 0.2 mm connected to a data logger.

Different initial temperatures were attained by storing the mounted experiments in controlled temperature rooms for one night.

The heat transfer coefficients h simulated by the acrylic slabs placed between the beef and the heat exchangers were calculated from the equation:

$$h = \int_o^{t_{cr}} \frac{k(T)}{(T - T_f)} \left. \frac{\partial T}{\partial x} \right|_{x=L} dt \quad (20)$$

where the temperature T and the temperature gradient ($\partial T/\partial x$) at the border of the piece of meat (x = L) were measured experimentally.

The integral over the whole freezing time was used to account for poor thermal contacts detected during the initial stage of the experiments.

The operating conditions used in the experiments covered the following range: T_i from 11°C to 22°C; T_f from −27° to −45°C;

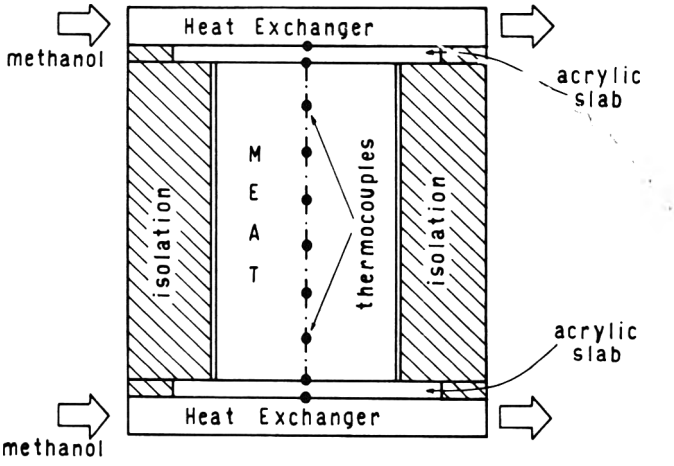

Fig. 4—Sample holder for freezing experiments.

h from 72 to 205 W/m^2 K and Bi_o from 4.6 to 13.3. The beef cylinder had a length 2L = 6 cm. Parameters for beef fed to the computer program are included in Table 3.

RESULTS & DISCUSSION

FIG. 5 SHOWS the freezing times to attain −18°C at the center ($t_{c_{18}}$) predicted by the simplified model proposed in this paper in terms of the values calculated with the numerical model, particularly in the parameters range commonly used in plate freezing (coolant temperatures between −25°C and −45°C and heat transfer coefficients from 80 to 400 W/m^2 °C). The results were calculated for freezing parallel as well as perpendicular to the fibers.

It will be observed that the values predicted by the simplified model show satisfactory agreement for low freezing times, with a tendency to provide smaller values for longer freezing times. In no case are the differences above 8.6%. The average of the absolute percent errors is 3.6%. Similar results are obtained for $t_{c_{10}}$.

In the same figure, experimental freezing times measured as described in the Experimental section, are included for freezing with both heat flow perpendicular and parallel to the meat fibers. As can be seen the agreement is also satisfactory with differences less than 10%.

No special tendency was detected for data with freezing parallel or perpendicular to the meat fibers when comparing with the numerical model as well as when checking against experimental results.

Fig. 6 and 7 show values of $t_{c_{18}}$ predicted by the simplified model proposed in comparison with the values obtained by other simplified methods existing in literature. Properties for beef freezing perpendicular to the fibers were used in all cases. In the same figures the results calculated by the described numerical model are also included.

Fig. 6 shows the influence of the initial temperature on the freezing times as predicted by the different models, while Fig. 7 analyzes the effect of the cooling medium temperature T_f.

As can be seen in Fig. 6 and taking as reference the numerical model, all the equations differ in less than 10% with the exception of Nagaoka et al. (1955) model which is within the mentioned difference only for $T_i < 16$°C.

Fig. 7 shows a very similar behavior in the predictions of the different models with results also differing in less than 10% respect to the numerical calculation.

As can be seen from these figures, all the models have good accuracy in predicting freezing times and the selection criterion has to be established on the basis of a simpler calculation or of a better physical meaning in the development of the model.

The simplified model proposed in this paper claims both advantages. For instance, it allows for a comparison of the relative contributions of the pre-cooling, change of phase and tempering periods on the total processing time, as shown in Fig. 8 where the ratio R_t between the pre-cooling, change of phase or tempering time and the total freezing time is plotted against the initial temperature T_i. Thus, change of phase times are in most cases above 70% of the total freezing time while tempering time is higher than pre-cooling time probably due to the lower driving forces attained in these period.

Calculations also show a negligible effect of the cooling medium temperature T_f and of the heat transfer coefficient h on the ratio R_t. For this reason Fig. 8 is valid for $-25° \leq T_f \leq -45$°C and $35 \leq h \leq 250$ W/m^2 °K with an error less than 5%.

Table 3—Parameters for beef fed to the computer program

E = 0.92864		k_h =	$e_1 + e_2 T$
F = 0.003591		k_a =	$f_1 + f_2 T$
ρ_o = 1053 kg/m^3		e_1 =	4.749 W/m °K
ρ_a = 1000 kg/m^3		e_2 =	-0.929×10^{-2} W/m °K^2
ρ_h = 917 kg/m^3		f_1 =	-0.6751×10^{-2} W/m °K
T_o = 273.16 °K		f_2 =	2.051×10^{-3} W/m °K^2
Y_o = 0.7336		k_{mL} =	0.3540 W/m °K
$\bar{C}_{p_o}$ = 3.475 kJ/kg °K		k_{mp} =	0.2419 W/m °K
$\Delta\bar{C}_p$ = 2.135 kJ/kg °K		k_{oL} =	0.5057 W/m °K
$\lambda°f$ = 334.4 kJ/kg		k_{op} =	0.4635 W/m °K

CONCLUSIONS

(1) An approximate model is presented which allows for simple and fast calculation of foods freezing times in plate freezers.

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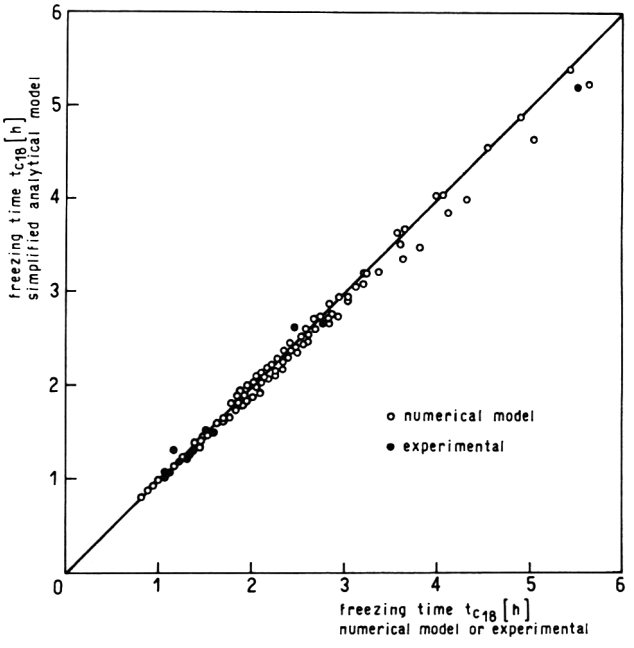

Fig. 5—Beef freezing times calculated with the simplified model in comparison with those experimentally or numerically evaluated.

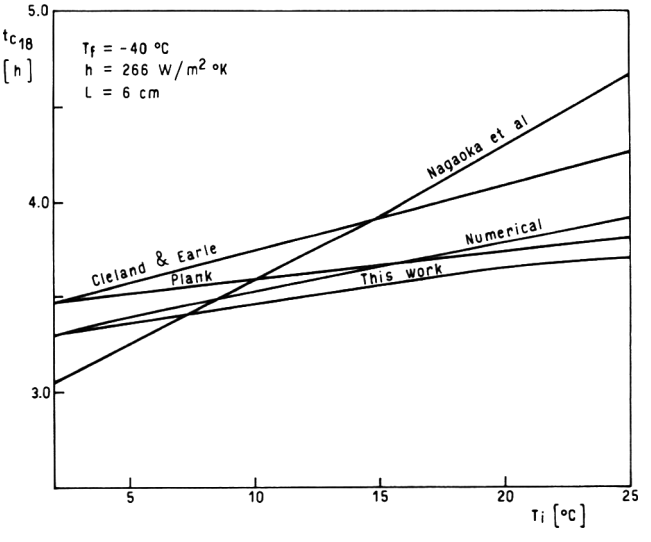

Fig. 6—Beef freezing times t_{c18} as a function of the initial temperatures T_i as predicted by different models.

(2) The model is developed on a heat transfer basis rather than on empirical corrections and involves the addition of pre-cooling, phase change and tempering times. No adjustable constants have been used and once the calculation procedure was specified, the results were directly compared with those accepted as a reference.

(3) Pre-cooling and tempering times were obtained by using heat transfer equations without change of phase while Plank's equation was applied for calculating the change of phase time.

(4) The model allows calculations for different initial temperatures and various final temperatures at the thermal centre.

(5) Comparison of calculated beef freezing times with experiments and with those predicted by a numerical model developed elsewhere shows an error of less than 8.6% in a broad range of operating conditions. (The average of the absolute percent errors being 3.6%).

(6) The main contribution to the total freezing time corresponds to the phase change period. (70% approximately). Moreover tempering is longer than precooling.

(7) Comparison of the proposed simplified model with other equations existing in literature shows a similar accuracy. However, the development on a heat transfer basis allows greater physical meaning when calculations are performed and future generalizations to other freezing situations different from those existing in plate freezers (two or three-dimensional heat transfer, other geometries, etc.)

(8) While comparisons between the simplified and the numerical models were made for beef, taking into account that the proposed model is based on heat transfer considerations, a good agreement for other types of foods can be expected.

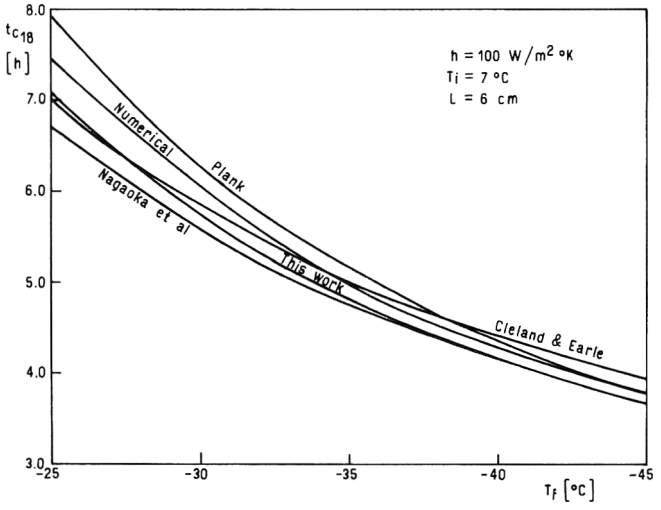

Fig. 7—Beef freezing times $t_{c_{18}}$ as a function of the cooling medium temperature T_f as predicted by different models.

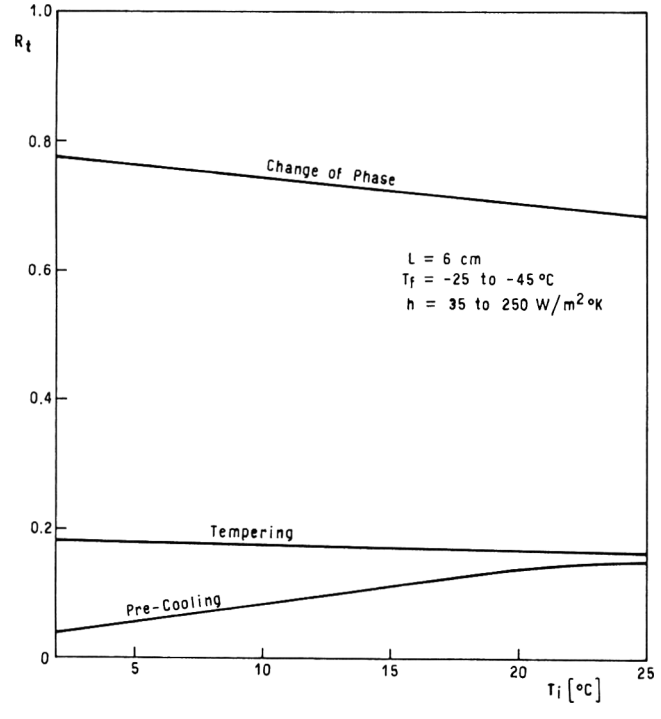

Fig. 8—Relative contributions to the total freezing time.

LIST OF SYMBOLS

Bi	Biot number BI = hL/k
$\overline{Bi}_c$	Biot number for Plank model $\overline{Bi}_c = hL/\overline{k}_c$
Bi'_c	Biot number for Cleland and Earle model $Bi'_c = hL/k'_c$
Bi_c	Biot number for the simplified model $Bi_c = hL/k_c$
Bi_o	Biot number for the numerical model $Bi_o = hL/k_o$
$\hat{C}_p$	Apparent specific heat (kJ/kgK)
$\overline{C}_p$	Specific heat for Plank model (change of enthalpy between 269°K and T_r)/(269 − T_r)
$\hat{C}_{p_r}$	Specific heat of frozen food evaluated at T_r
E	Parameter defined in Eq (5)
F	Parameter defined in Eq (5)
h	Heat transfer coefficient (W/m² K)
ΔH_r	Change of enthalpy between T_i and T_r
$\Delta H'_r$	Change of enthalpy between T_e and T_r
$\Delta\overline{H}$	Change of enthalpy between T_e and 261°K
k	Thermal conductivity (W/m K)
$\overline{k}$	Average thermal conductivity $\overline{k} = (k_o + k_c)/2$
k_c	Thermal conductivity of the frozen product evaluated at T_c
$\overline{k}_c$	Thermal conductivity evaluated at 261°K
k'_c	Thermal conductivity of the frozen product evaluated at 243°K
k_L	Thermal conductivity of beef parallel to the fibers
k_{mL}	Thermal conductivity of matrix (parallel to the fibers)
k_{mp}	Thermal conductivity of matrix (perpendicular to the fibers)
k_p	Thermal conductivity of beef perpendicular to the fibers
k_t	Variable defined in Eq (10)
L	Half thickness of the food slab (m)
n	Function presented in Table 2
P	Coefficient in Cleland and Earle model (Table 1)
P_k	Plank number; $P_K = \hat{C}_{p_o}\,\rho_o\,(T_i - T_e)/\Delta H'_r\,\rho_m$
r	Parameter in Eq (6); $r = \rho_a/\rho_h$
R	Coefficient in Cleland and Earle model (Table 1)
Ste	Stefan number, $Ste = \rho_r\,\hat{C}_{p_r}\,(T_e - T_f)/\Delta H'_r\,\rho_m$
t	Time (s)
t_{ce}	Change of phase time
t_{cr}	Freezing time to attain the temperature T_r at the center
t_p	Pre-cooling time
t_r	Tempering time
t'_r	Variable explicited in Table 1 (Plank model)
T	Temperature (°K)
T_c	Average temperature $T_c = (T_e + T_f)/2$
T_e	Temperature for beginning of freezing
T_f	Coolant temperature
T_i	Initial temperature
T_o	Freezing temperature of pure water (273.16°K)

T_r Final temperature at the center of the frozen product
Y_o Food water content on wet basis
x Spacial coordinate (m)

Greek symbols

α Thermal diffusivity (m^2/s)
$\bar{\alpha}$ Average thermal diffusivity $\bar{\alpha} = (\alpha_o + \alpha_c)$
α_c Thermal diffusivity of frozen product evaluated at T_c
α_K Parameter defined in Eq (11)
α_o Thermal diffusivity of unfrozen food
β Parameter defined in Eq (12)
γ Parameter in Eq (6); $\gamma = Y_o \rho_o/\rho_a$
η Dimensionless temperature; $\eta = (T - T_f)/(T_i - T_f)$
η_r Dimensionless temperature in Fig. 3; $\eta_r = (T_r - T_f)/(T_e - T_f)$
η_p Dimensionless temperature in Fig. 2; $\eta_p = (T_e - T_f)/(T_i - T_f)$
λ Variable defined in Eq (9)
λ_f^o Heat of crystallization of water at T_o (J/kg)
λ_n Root in Eq (17)
ξ Dimensionless coordinate $\xi = x/L$
ρ Density (kg/m^3)
$\bar{\rho}$ Density in Plank and Nagaoka equations
ρ_c Density of partially frozen beef
ρ_r Density of the frozen product evaluated at T_r
τ Dimensionless time; $\tau = \alpha t/L^2$
τ_r Dimensionless tempering time; $\tau_r = \alpha_c t_r/L^2$
τ_p Dimensionless pre-cooling time; $\tau_p = \bar{\alpha} t_p/L^2$
ω Ice content (weight of ice/initial weight of water)
ω_f Ice content evaluated at temperature T_f
ω_m Average ice content; $\omega_m = (\omega_f + \omega_r)/2$
ω_r Ice content evaluated at temperature T_r

Subscripts

a Water
h Ice
o Unfrozen

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content of spent grains, all DSG samples tested tended to be high in nucleic acid (3.1–3.4%).

Additional studies are needed not only to generate more complete compositional information on DSGs but also to examine the physiological availability and physiological effects of nutrients (and nonnutrients) present in DSGs. These efforts should proceed concurrently with food application studies.

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Sorption/Desorption of VCM in PVC Selected Food Simulants Systems

M. KONTOMINAS, J. MILTZ, and S. G. GILBERT

ABSTRACT

Sorption/desorption studies of VCM in the systems PVC/VCM/water, PVC/VCM/corn oil an PVC/VCM/heptane were carried out. At low VCM concentrations, classical curves are obtained, where the sorption isotherms are located below their desorption counterparts. Above a certain VCM concentration a crossover exists and an inversion of the curves occurs. It was suggested that the intersection between the curves represents the point where all the active sites in the polymeric matrix are occupied by monomer molecules. The negative values of the total Gibb's free energy and the energy of mixing in the polymer were found to increase with the decrease in monomer concentration pointing out to the possibility that at low enough monomer concentrations no migration of VCM into the contained food from a PVC package may occur.

INTRODUCTION

Polyvinyl chloride (PVC) is one of the most important food packaging materials. However, this polymer usually contains low molecular weight compounds like plasticizers, stabilizers, processing aids and residual monomer that may desorb from the polymer and migrate into the contained food. Since the discovery that Vinyl Chloride Monomer (VCM) is a potential carcinogen and due to the possible effect of the different migrants on the sensory quality and the safety aspects of the contained food, desorption and migration have received a growing attention and much effort and resources have been devoted in recent years to study this subject. Figge (1972), Daniels and Proctor (1975), Gilbert (1976), Chudy and Crosby (1977), Morano et al. (1977).

Sorption/desorption phenomena are generally related to the relative solubility and equilibrium distribution of the migrant in the polymer and food (or food simulant) phases. The thermodynamic system is, therefore, related to the difference in the free energy of mixing of this migrant in the two phases while the kinetics is related to its diffusion and reactions in the polymer/migrant/food system.

Previous work by Morano et al. (1977) and Kashtock (1977) has shown that the partition coefficient for VCM in PVC/contacting phase systems, as determined by classical sorption studies, were concentration dependent, especially at very low monomer concentrations.

This behavior was attributed to sorption on active sites present in the polymer. At very low monomer concentrations the monomer molecules are sorbed onto the most active sites. With the increase in monomer concentration, less powerful sites are occupied resulting in a concentration dependent desorption process (Gilbert, 1976; Gilbert et al., 1978, 1980).

The total standard partial molar Gibbs' free energy, $\Delta\bar{G}^{\circ}_{T}$, is related to the partition coefficient, K_p (defined as the ratio between the migrant concentration in the polymer and in the contacting phase, respectively) through the expression:

$$\Delta\bar{G}^{\circ}_{T} = RT \ln K_p \quad (1)$$

The total free molar energy is also given by:

$$\Delta\bar{G}^{\circ}_{T} = \Delta\bar{G}^{\circ}_{i} + \Delta\bar{G}^{\circ}_{ex} = RT \text{ in } \gamma_i P_i \quad (2)$$

where $\Delta\bar{G}^{\circ}_{i}$ is the ideal molar free energy (which is also the molar free energy of vaporization); $\Delta\bar{G}^{\circ}_{ex}$ is the excess molar free energy (or the free energy of mixing); γ_i is the activity coefficient; and P°_{i} is the saturation vapor pressure of the pure solute (migrant). Eq (2) can therefore be written in the form:

$$\Delta\bar{G}^{\circ}_{i} = RT \ln P^{\circ}_{i} \quad (3)$$

$$\Delta\bar{G}^{\circ}_{ex} = RT \text{ in } \gamma_i \quad (4)$$

Combining Eq (1) (2) and (3) results in:

$$\Delta\bar{G}^{\circ}_{ex} = RT (\ln K_p - \ln P^{\circ}_{i}) \quad (5)$$

In the present work the sorption/desorption studies were extended to PVC/water, PVC/corn oil and PVC/heptane systems to examine the active site hypothesis of migration and the energies involved.

EXPERIMENTAL

Materials

A commercial powdered unplasticized PVC resin (VC47, BE-1) of particle size in the range 0.15–0.25 μm (supplied by Borden Chemical Co., North Andover, MA) was used in the present study.

The VCM was a 99.9% pure gas from Matheson Gas Products (East Rutherford, NJ).

Food grade corn oil packaged in glass was purchased locally.

Methods

PVC resin was stripped of residual monomer according to the method outlined by Morano et al. (1977). A preweighed amount of PVC powder (6 ± 0.1g), was transferred to a 26 ml opthalmic vial. The vial was partially filled with distilled water, corn oil or heptane and capped with a butyl rubber septum and aluminum crimp cap. A known amount of VCM solution in the appropriate liquid and additional pure liquid were then injected into the vial through the septum to completely fill the vial. After shaking for at least 2 hr to ensure equilibrium sorption (preliminary experiments have shown that equilibrium is reached after 1 hr), aliquots of 1 ml were taken from each vial from VCM concentration determination.

For desorption studies, the needles of one empty 5 ml syringe and one 5 ml syringe containing the appropriate fresh solvent were inserted into the vial through the septum. The fresh liquid was introduced into the vial at the region just above the settled resin and simultaneously at 5 ml aliquot was withdrawn into the empty syringe from the top of the liquid phase. This aliquot was assayed for VCM content to determine the exact amount removed thus correcting for any possible mixing during sampling. When the new equilibrium was reached, another 1 ml aliquot was withdrawn for analysis of the increase in VCM concentration as a result of desorption of monomer from the polymer. Analysis of the VCM in the liquid phase was carried out by an indirect headspace method similar to the one published by the FDA (Breder et al., 1976). A 1 ml aliquot of the liquid was transferred to a 26 ml empty septum sealed

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aluminum crimp-capped vial which was then kept at 100°C for 2 hr. After this period, 0.5 ml of the headspace was removed from the heated vial by a gas-tight syringe and rapidly injected into a gas chromatograph. The GC was a Hewlett Packard 5750 model with a dual flame ionization detector and a 6 x ¼" i.d. aluminum column packaged with 10% SE-30 coated Anakrom ABS 90–100 mesh. The operating conditions were: Temperatures (°C): column, 70; detector, 225; injection port, 65. Nitrogen at a flow rate of 60 ml/min was used as the carrier gas.

For standardization, headspace serial dilutions by an airtight syringe were made from the 99.9% pure gas into vials. VCM solutions in distilled water, corn oil, and heptane were prepared by their saturation from the 99.9% pure gas in a gas scrubbing bottle. Dilutions were then made by aliquot transfer to cover the concentration ranges of 20–340 μg/ml for water and 50–1000 μg/ml of VCM for oil and heptane.

RESULTS & DISCUSSION

IN FIG. 1 AND 2 the sorption/desorption isotherms of VCM in PVC/water and PVC/corn oil systems are plotted, respectively. The isotherms are represented as equilibrium concentration of VCM in the polymer versus its equilibrium concentration in the contacting phase. It can be seen that at low VCM concentrations, classical isotherms are obtained in which the sorption isotherm is located below the desorption one in both systems. At high VCM concentrations, an inversion occurs and the sorption isotherms are located above their desorption counterparts. The intersection between the sorption and the desorption curves occurs in the two systems at comparable VCM concentrations in the polymer (16 ppm W/W in the PVC/oil versus 18 ppm (W/W) in the PVC/water system). However, the corresponding VCM concentration in the oil is approximately one order of magnitude higher than that in the water. This result is reasonable in terms of the much greater solubility of VCM in oil as compared to water.

The observed hysteresis and crossover can be explained by the active site model proposed by Gilbert et al. (1980). According to this model three thermodynamically and kinetically different populations of migrant exist in the polymer matrix. Thus, the total migrant concentration (C_T) is the sum of a concentration C** that is completely bound (immobilized-nondiffusible) on the most active sites in the polymer matrix, of a species of concentration C* that is partially bound to active sites but, is diffusible and of a freely diffusible species of concentration C_D. As long as the concentration in the system is in the range where only completely and partially bound species exist in the polymer, sorption is thermodynamically favorable compared to desorption since the sorption process involves negative enthalpies and positive entropies. Desorption in this case requires an input of additional energy or a higher concentration gradient. Thus, the sorption curve is located below the desorption one due to the preference of the migrant molecules to be attached to active sites. At higher migrant concentrations, where all the active sites in the polymer are occupied and the remaining migrant molecules are freely diffusible, an inversion occurs thus placing the desorption curve below the sorption one. Sorption at concentrations higher than necessary to occupy all the active sites may require structural changes in the polymer to accomodate additional migrant molecules. Thus, swelling for instance of the polymer may expose additional sites for sorption, but requires energy input in proportion to the strains imposed on the matrix. As a result, the monomer swollen and strained polymer matrix will favor desorption at equal external monomer levels or reach equilibrium at lower concentrations than desired for sorption. The point of intersection between the two isotherms can therefore be interpreted as the situation in which all the active sites (strong and weak) are occupied by the completely and partially bound species.

Since the critical value for hysteresis crossover is the saturation of the active sites in the polymer, it is therefore clear that this is a property of the matrix structure and not of the external solution. Thus, the rather similar levels of 16 ppm for oil and 18 ppm for water for the crossover concentrations in the polymer are related to the concentrations required to cover the active sites in the specific matrix used in the present studies. These assumptions are supported by direct volumetric measurements of the degree of swelling as a function of external monomer concentration to be reported in a forthcoming publication. It should be stressed that while in the PVC/VCM/water system a small but detectable amount of swelling occurred, in the PVC/VCM/oil system the amount of swelling was significantly higher.

In Fig. 3 the sorption isotherms are shown for the PVC/VCM/heptane system. In contrast to oil, heptane is a very well defined liquid. It can be seen that also in this sytem a crossover of the sorption/desorption curves was found thus excluding the possibility of dealing with an artifact phenomenon. The VCM concentration in the PVC at the isotherms crossover point is in this case, however, much higher than the corresponding values in the previously mentioned systems while the VCM in the heptane is of the same order of magnitude as that in the oil. Volumetric measurements have shown that a considerable swelling of the PVC in heptane occured reinforcing our assumption that when

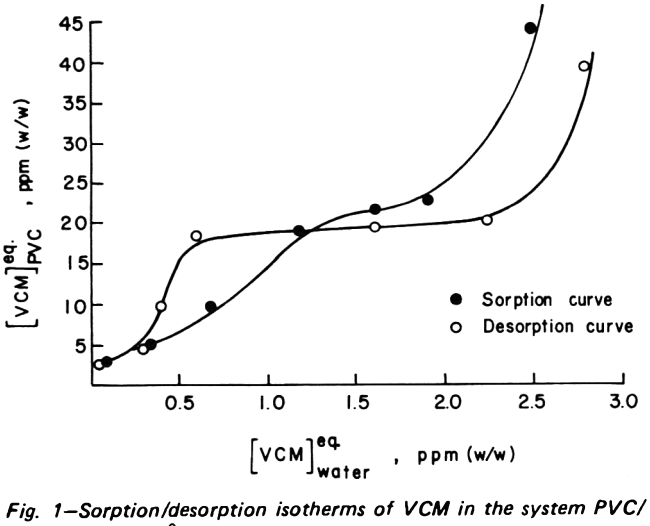

Fig. 1—Sorption/desorption isotherms of VCM in the system PVC/VCM/water at 22° C.

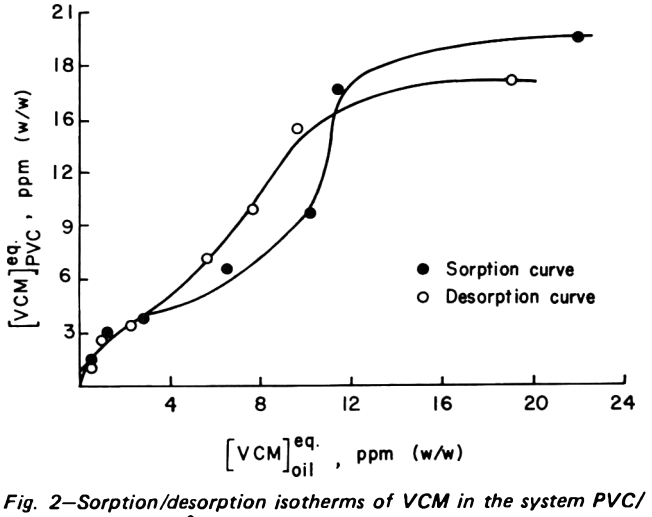

Fig. 2—Sorption/desorption isotherms of VCM in the system PVC/VCM/corn oil at 22° C.

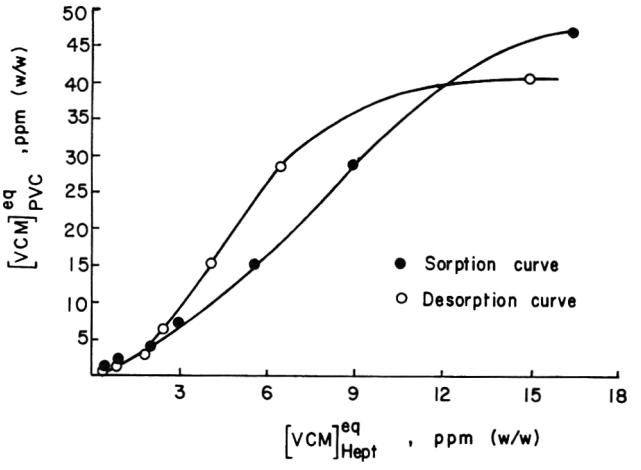

Fig. 3—Sorption/desorption isotherms of VCM in the system PVC/VCM/heptane at 22°C.

swelling of the matrix occurs, additional sites can be exposed allowing higher migrant concentrations to be present in the polymer at the point of isotherms crossover.

In Tables 1, 2 and 3 the Gibb's total standard partial molar free energies and the free energies of mixing as calculated from Eq (1) and (5), respectively, are given for the systems PVC/VCM/water, PVC/VCM/oil and PVC/VCM/heptane, respectively. The energies were calculated from the values taken from the corresponding isotherms for the lower region of VCM concentrations. It can be seen from Tables 1 and 2 that for the PVC/VCM/water and PVC/VCM/oil systems the negative values of the total free energy as well as of the energy of mixing increase with the decrease in monomer concentration indicating a more favorable adsorption. As could have been expected, the negative values in the PVC/VCM/water system are considerably higher than those in the PVC/VCM/oil system due to the lower solubility of VCM in water (as compared to oil) resulting in a more favorable VCM adsorption on the polymer.

It is clear that at a zero external monomer concentration, no additional adsorption on the polymer can take place. Desorption, however, proceeds from a finite initial concentration in the polymeric matrix. If a portion of this initial concentration is very strongly bound to the matrix, then even if the external concentration is zero, migration into the contacting phase does not necessarily have to occur from thermodynamic considerations. The increasing negative energies with the decrease in monomer concentration found in the present study point out towards such a possibility.

These results support the previously mentioned "active site model" and are in accordance with similar data for acrylonitrile-styrene copolymer reported by Orr et al. (1981a, b).

From Table 3 it can be seen that the energies are also negative, showing a favorable adsorption of VCM on PVC in the PVC/VCM/heptane system. However, the negative values of the energy are fairly constant and do not increase with the decrease in monomer concentration. This difference can be related to the amount of swelling in the PVC/VCM/heptane system. As in normal applications a polymer will not be used to package and store a product that causes its swelling, this result is not in contradiction to our general view of monomer migration from a polymeric package into the contained food.

Table 1—Thermodynamic data for sorption at 22°C in the PVC/VCM/water system

$(VCM)^{eq}_{water}$ (ppm)	$(VCM)^{eq}_{PVC}$ (ppm)	K_p	1n K_p	$\Delta\bar{G}^{\circ}_T$ (cal/mole)	$\Delta\bar{G}^{\circ}_{ex}$ (cal/mole)
0.1	3.0	30.0	3.401	−1994	−2508
0.2	3.9	19.5	2.970	−1741	−2255
0.3	5.0	16.7	2.815	−1650	−2164
0.4	6.0	15.0	2.708	−1587	−2101
0.5	6.8	13.6	2.610	−1529	−2044
0.6	7.9	13.2	2.580	−1512	−2026
0.7	8.9	12.7	2.542	−1490	−2004
0.8	10.8	13.5	2.602	−1525	−2039

Table 2—Thermodynamic data for sorption at 22°C in the PVC/VCM/oil system

$(VCM)^{eq}_{oil}$ (ppm)	$(VCM)^{eq}_{PVC}$ (ppm)	K_p	1n K_p	$\Delta\bar{G}^{\circ}_T$ (cal/mole)	$\Delta\bar{G}^{\circ}_{ex}$ (cal/mole)
0.5	1.76	3.52	1.258	−738	−1252
1.0	2.36	2.36	0.859	−503	−1017
1.5	2.79	1.86	0.621	−364	−878
2.0	3.36	1.68	0.519	−304	−818
2.5	3.60	1.44	0.365	−214	−728
3.0	3.96	1.32	0.278	−163	−677
3.5	4.14	1.18	0.166	−97	−611
4.0	4.45	1.11	0.104	−61	−575

Table 3—Thermodynamic data for sorption at 22°C in the PVC/VCM/heptane system

$(VCM)^{eq}_{Hep}$ (ppm)	$(VCM)^{eq}_{PVC}$ (ppm)	K_p	1n K_p	$\Delta\bar{G}^{\circ}_T$ (cal/mole)	$\Delta\bar{G}^{\circ}_{ex}$ (cal/mole)
1.0	2.6	2.60	0.955	−560	−1074
2.0	4.5	2.55	0.936	−549	−1062
3.0	7.5	2.50	0.916	−537	−1051
4.0	10.7	2.67	0.982	−576	−1090
5.0	13.6	2.72	1.000	−586	−1100
6.0	17.3	2.88	1.058	−620	−1134

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Mathematical Models for Nonsymmetric Freezing of Beef

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ABSTRACT

A microscopic balance with simultaneous change of phase together with equations for predicting the thermal properties as a function of the ice content and a cryoscopic descent model are used to simulate the nonsymmetric freezing of a beef slab. The equations are solved numerically to obtain temperature profiles as well as freezing times. Comparison with experimental results shows good agreement. A variation of the thermal center position throughout the freezing process is detected and assumptions to predict its position in the different periods of freezing are supplied. On the basis of these assumptions a simplified model for calculating processing times in plate freezers is proposed, showing good agreement with experimental freezing times and with predictions obtained from the numerical model.

INTRODUCTION

THE HEAT TRANSFER with simultaneous change of phase that takes place during the freezing of food has been the subject of several studies giving rise to analytical models, Plank (1941), (1963); Nagaoka et al. (1955); Cleland and Earle (1977); Mascheroni and Calvelo (1982), as well as numerical models, Bakal (1970); Bonacina and Comini (1971); Heldman (1974); Joshi and Tao (1974); Mascheroni and Calvelo (1980).

In general, and for greater simplicity, slab geometry was adopted, which satisfactorily simulates processing in plate freezers. In all cases the boundary conditions imposed on the governing equations pertained to symmetric freezing.

However, in industrial use, symmetric conditions can rarely be achieved with plate freezers unless special care is adopted in connection with the operation of the equipment. Thus, it is a common occurrence to find important temperature differences between one plate and another, as well as the existence of poor thermal contact between the food and the refrigerated plates.

At the same time, and as a means of minimizing labor, in some cases (freezing of meat, liver, etc.) processing in corrugated cardboard boxes is chosen. This also generates nonsymmetric freezing conditions, since the box provides a better thermal contact at the bottom (leaving an air space at the top which involves considerable thermal resistance).

In the case of heat conduction in slabs without change of phase and constant properties with nonsymmetric boundary conditions there exists an analytical solution of the differential equation (Carslaw and Jaeger, 1959). The corresponding roots were evaluated and tabulated in a subsequent study by Uno and Hayakawa (1979). However, in the case of freezing, where apart from conduction, the nonlinearity of the change of phase and variable properties must be added, the solution mentioned is not valid; hence, questions concerning the position of the thermal center and its dependence on time, should be answered in order to develop simplified models for freezing time calculations.

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In the present study, on the basis of a heat transfer model with simultaneous change of phase developed previously for beef freezing (Mascheroni and Calvelo, 1980), a numerical solution is obtained for the case of nonsymmetric boundary conditions, analyzing the position of the thermal center and its dependence with processing time. The results are compared with laboratory experiences of nonsymmetric freezing of beef and, on the basis of the aforementioned analysis, a simplified model is proposed for calculating processing times.

THEORY

Nonsymmetric freezing numerical model

Considering a unidirectional heat flow in a beef slab of thickness 2L = b, nonsymmetrically cooled from both faces, the microscopic energy balance results:

$$\rho(\omega)\,\bar{C}_p(\omega)\frac{\partial T}{\partial t} = \frac{\partial}{\partial x}\,k(\omega)\frac{\partial T}{\partial x} \qquad (1)$$

where the density $\rho(\omega)$, the specific heat $\bar{C}_p(\omega)$ and the thermal conductivity $k(\omega)$ are functions of the ice content of partially frozen beef ω which in turn can be related to the temperature by means of a cryoscopic descent model (Mascheroni and Calvelo, 1978).

The initial and boundary conditions are:

$$t \leq 0 \qquad T = T_i \qquad 0 \leq x \leq b \qquad (2)$$

$$t > 0 \qquad k\frac{\partial T}{\partial x} = h_1\,(T - T_{f_1}) \qquad x = 0 \qquad (3)$$

$$t > 0 \qquad -k\frac{\partial T}{\partial x} = h_2\,(T - T_{f_2}) \qquad x = b \qquad (4)$$

where T_i is the initial temperature, T_{f_1} and T_{f_2} are the temperatures of the cooling media in both sides of the slab, h_1 and h_2 being the heat transfer coefficients on the respective sides of the beef.

Functions $\rho(\omega)$, $\bar{C}_p(\omega)$, $k(\omega)$ and $\omega(T)$ necessary to solve Eq (1) with boundary conditions (2), (3) and (4) have been described in previous studies (Mascheroni et al., 1977; Mascheroni and Calvelo, 1980, 1982).

Eq. (1) to (4) were solved numerically using an explicit method of finite differences and an IBM/360 computer. Heat removal during freezing was assumed to be perpendicular to the meat fibers.

Obviously, the temperature in the system will be given by:

$$T = T\,(\xi, \tau, T_i, T_{f_1}, T_{f_2}, Bi_1 \text{ and } Bi_2) \qquad (5)$$

where $\xi = x/b$; $\tau = \alpha_o\, t/b^2$; $Bi_1 = h_1 b/k_o$ and $Bi_2 = h_2 b/k_o$, k_o and α_o being the thermal conductivity and diffusivity of the unfrozen meat, respectively.

Fig. 1 shows temperature profiles at different times predicted by means of the numerical model for nonsymmetric freezing of a beef slab. Asymmetry was generated in this case maintaining the same temperature of the cooling medium ($T_{f_1} = T_{f_2}$) but accepting different thermal resistances on each face ($Bi_1 \neq Bi_2$).

It will be seen that for short periods of time (0.17h) the thermal center has not yet been established, although greater heat penetration is observed on the left side as a result of greater heat removal ($Bi_1 = 7.0 > Bi_2 = 2.2$). For longer periods (0.5h) the thermal center is established in a position quite near to $\xi = 0.5$, admitting a slow displacement toward the right as time progresses.

–Continued on next page

When the central area approaches the temperature of the beginning of the phase change (−1.1°C) a flattening of the temperature profiles can be observed, making the position of the thermal center undetermined. For longer periods of time (8h) the location of the thermal center is reestablished, and it continues moving slowly toward the right until freezing is completed.

The position of the thermal center in the case of heat transfer without change of phase and constant thermal properties has been analyzed by Uno and Hayakawa (1979), who detected that this position is stationary and that it is determined by Biot numbers (Bi_1 and Bi_2) on both sides of the slab. However, the position of the thermal center (ξ) predicted by the model of Uno and Hayakawa using Biot numbers with properties of unfrozen meat (Bi_1 = 7.0, Bi_2 = 2.2) is ξ = 0.60, while for Fig. 1 with change of phase and variable thermal properties for short times it is ξ = 0.52. This more symmetrical position of the thermal center in the case with phase change can be interpreted in Fig. 2, which for greater simplicity analyzes a system where asymmetry is generated with different temperatures of the cooling media ($T_{f_1} \neq T_{f_2}$) and where the change of phase is produced at a single temperature T_e.

Thus, in Fig. 2a corresponding to short periods of time it will be observed that on both faces there is a frozen area giving rise to two freezing fronts moving toward the center of the slab. Obviously, as a result of the greater heat removal on face 1 the left front penetrates more. However, the position of the thermal center is almost symmetrical since the sensible heat removed from the unfrozen region is dissipated toward two heat sinks at the same temperature T_e on both freezing fronts.

The diagram also explains the slow displacement of the thermal center toward the right (Fig. 2b) due to the uneven growth of the freezing fronts.

The flattening effect of the profiles observed in Fig. 1 is schematically shown in Fig. 2c where, in accordance with the thermal diffusivity of the unfrozen region, it could happen that sensible heat would be eliminated in that zone before the two freezing fronts join. This effect has been reported in aqueous freezing systems (Meryman, 1966; Mascheroni and Calvelo, 1980), and is responsible for the flat profiles observed in Fig. 1 in the neighborhood of −1°C.

Fig 2d shows the moment in which both freezing fronts join, reestablishing the position of the thermal center.

In a system such as that shown diagrammatically in Fig. 2 with a constant freezing point at T_e the position of the thermal center should remain constant once both fronts join since there is no further change of phase. In the case of meat, since the change of phase occurs over a wide range of temperatures, the meeting of the fronts for beginning of freezing (at −1.1°C) does not necessarily involve the end of the change of phase, and that is the reason why the thermal center in Fig. 1 continues moving to the right, although more slowly now. At sufficiently low temperatures the thermal center attains a stationary position coinciding with that foreseen by Uno and Hayakawa (1979) for the case without change of phase although, for the calculation, the Biot numbers evaluated in terms of the thermal conductivity of frozen beef must be used.

This numerical model presents satisfactory information for predicting processing times under nonsymmetric freezing conditions. However, notwithstanding its accuracy, numerical models are scarcely used in industrial practice primarily because, although numerical results were provided, it is difficult to publish curves covering the ranges of all the parameters involved. The other alternative implies availability of a computation program and the corresponding computer, which is rarely feasible to users of this type of information. Consequently, there is a tendency to use approximate models which, although less accurate, make it possible to perform fast and simple calculations of freezing times in terms of the particular operating conditions. On the basis of the conclusions obtained with the described numerical model and using an analytical one developed by Mascheroni and Calvelo (1982) for freezing time calculations under symmetric conditions, a simple model to predict processing times for nonsymmetric freezing is proposed below.

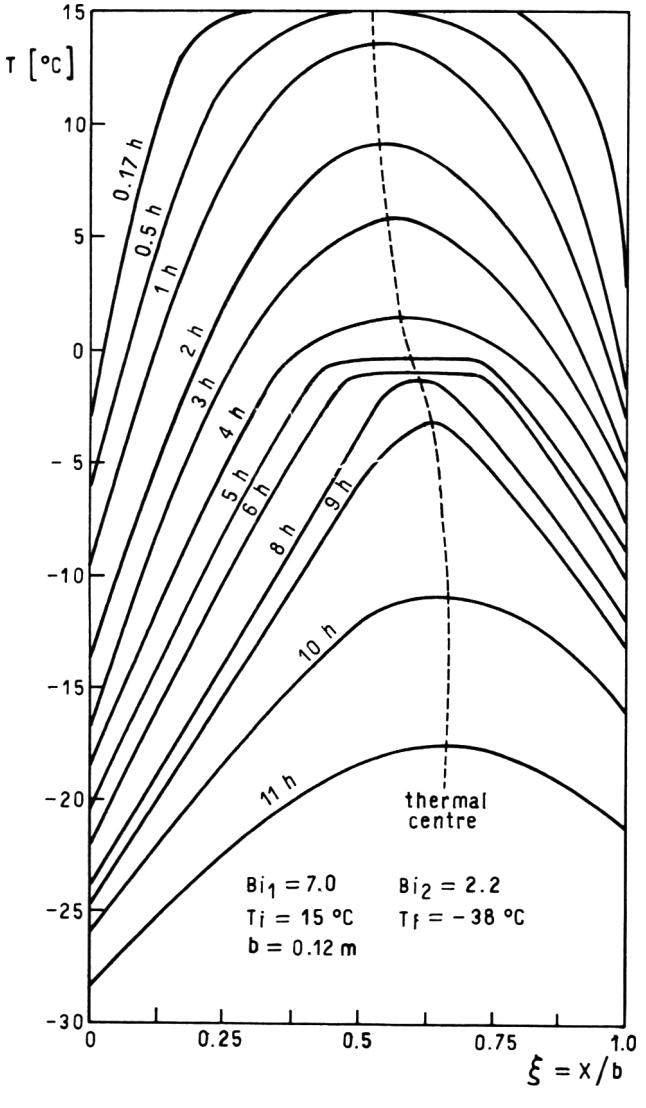

Fig. 1—Temperature profiles calculated with the numerical model for nonsymmetric freezing of a beef slab.

Simplified model for calculating freezing times under nonsymmetric conditions

The proposed simplified model is similar to another previously developed for the case of symmetric freezing (Mascheroni and Calvelo 1982).

This model calculates freezing time by the addition of precooling, change of phase and tempering periods according to:

$$t_{cr} = t_p + t_{ce} + t_r \tag{6}$$

For calculating the precooling and tempering periods the solution of the heat transfer equation without change of phase for a symmetric homogeneous slab of 2L thickness without end effects, with a constant initial temperature T_i, and a constant external temperature T_f is used. by admitting the possible existence of an interfacial resistance through a heat transfer coefficient h, the corresponding solution is:

$$\eta = \frac{T - T_f}{T_i - T_f} = \sum_{n=1}^{\infty} e^{-\lambda_n^2 \cdot \tau} \frac{\sin \lambda_n \cos(\lambda_n \delta)}{\lambda_n + \sin \lambda_n \cos \lambda_n} \tag{7}$$

where $\tau = \alpha t/L^2$ is the dimensionless time, α the thermal diffusivity and λ_n the roots of the equation:

$$\lambda_n \tan \lambda_n = h\,L/k \tag{8}$$

In Eq. (7) a dimensionless coordinate δ = y/L is used, where y is defined such that y = 0 at the thermal center of the homogeneous slab.

For the change of phase period Plank's equation (1963), valid for change of phase at constant temperature T_e, pseudo steady state heat transfer in the frozen product and constant properties is used.

For calculating these times in the nonsymmetrical case, the location of the thermal center in each of the periods must first be defined.

Estimating the position of the thermal center

As has been seen from the behavior of the numerical model, the position of the thermal center varies slowly in the precooling and tempering periods, while during the change of phase it changes rapidly (Fig. 1).

Furthermore, it can also be seen from the analysis that during the precooling period the thermal center coincides approximately with the geometrical center. For this reason, in calculating that period it will be assumed that we have a half-slab of thickness $L_p = b/2$.

The tempering period ends when the thermal center reaches temperature T_r and its position, as mentioned earlier, is the same as for a system without change of phase but with the properties of the frozen product.

This position, in a case where the asymmmetry stems from different heat transfer coefficients, can be predicted using the solution obtained by Uno and Hayakawa (1979) for heat transfer without change of phase. Fig. 3 shows the position of the dimensionless thermal center L_T/b in terms of the Biot numbers in both interphases. Obviously, these parameters should include the properties of the frozen product, good results having been obtained when evaluated at temperature $T_m = (T_e + T_r)/2$.

Fig. 3 is constructed for $Bi_1 > Bi_2$ and as it can be observed, the higher is the slab thickness, b (higher Bi_1 and Bi_2), the more symmetrical is the position of the thermal center.

When the asymmetry stems from different temperatures of the cooling media the dimensionless position of the thermal center can be determined from the chart in Fig. 4 wherein, entering with parameter H as a function of the Bi number, the value of L_r/b can be obtained in ordinates. Parameter H arises when equations (3) and (4) are written in dimensionless form and is given by:

$$H = (T_i - T_{f_2})/(T_i - T_{f_1})\ Bi \qquad (9)$$

where it is required that $T_{f_1} < T_{f_2}$.

If the causes of asymmetry are combined, i.e., stemming simultaneously from different cooling media temperatures and different Biot numbers, the use of the following equation in estimating the final position of the thermal center has given good results:

$$L_T/b = (L_r/b)_t \pm \left[(L_T/b)_h - 0.5\right] \qquad (10)$$

where $(L_r/b)_t$ is the dimensionless position of the thermal center estimated in the graph of Fig. 4 calculating the Biot number according to $Bi = h_1 b/k_t$, where k_t is the thermal conductivity at the lowest cooling medium temperature and $(L_r/b)_h$ is the dimensionless position of the thermal center estimated by using Fig. 3.

The double sign of Eq (10) indicates that its terms must be added when both causes of asymmetry contribute to the displacement of the thermal center in the same direction (T_{f_1} and Bi_1 correspond to the same side) and deduced when those causes tend to compensate each other (T_{f_1} and Bi_1 in different sides).

For the change of phase period, as seen before, the position of the thermal center changes rapidly and in a manner difficult to detect, since often the flattening of the profiles in the central part hampers its correct individualization. However, satisfactory results have been obtained using for that period a mean thermal center position L_m between the geometrical center and the thermal center at the end of the tempering period ($L_m = (L_p + L_r)/2$).

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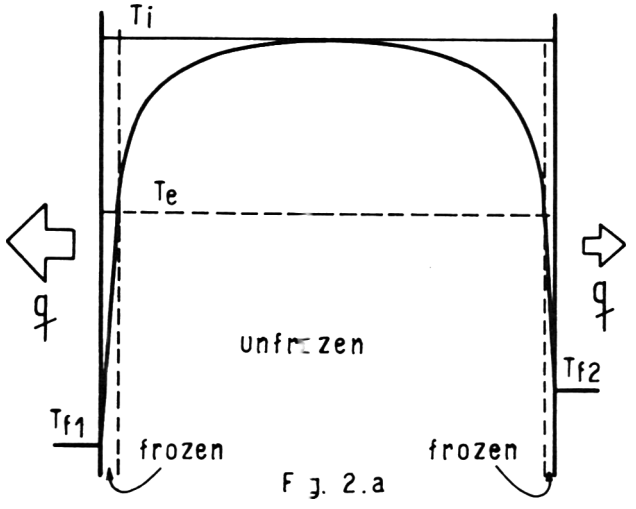

Fig. 2.a

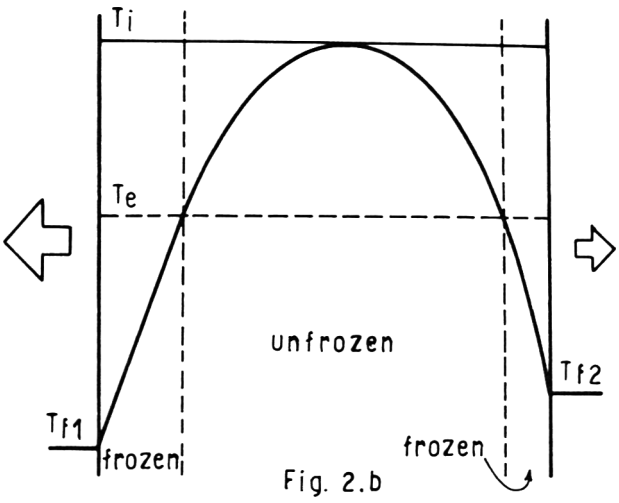

Fig. 2.b

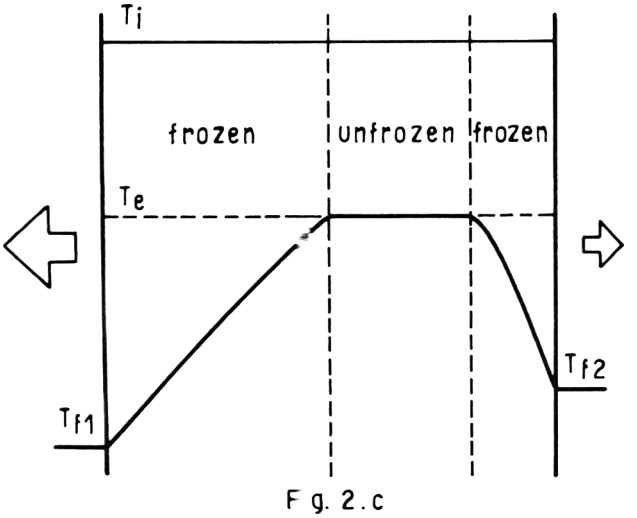

Fig. 2.c

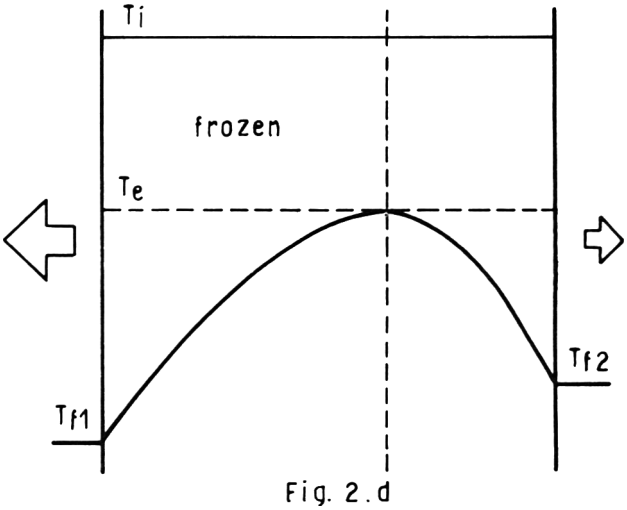

Fig. 2.d

Fig. 2—Schematic picture of nonsymmetric freezing at different stages.

Calculating precooling time

Precooling time t_p is defined (Mascheroni and Calvelo, 1982) as the time lapse between the beginning of chilling with the whole system at T_i until for $x = b/4$ ($\xi = 0.25$) the temperature for beginning of freezing (T_e) is reached. This coordinate was chosen because in this way it approximately offsets the excess heat removed for points of $\xi < 0.25$, with what still remains to be removed for $\xi > 0.25$ (Fig. 5).

To obtain t_p the graph of Fig. 6 is used, which represents Eq (8) for $\delta = 0.5$. In that graph it is $\eta_p = (T_e - T_f)/(T_i - T_f)$. For calculating $Bi_p = h\ L_p/k_p$, as explained before, $L_p = b/2$ was used. Thermal conductivity k_p is the average between that of the unfrozen product (k_o) and (k_m) corresponding to temperature $T_m = (T_e + T_f)/2$. Better results were obtained when performing calculations with conditions of the greater heat removal side.

Consequently with the value of τ_p obtained from Fig. 6 it is possible to calculate $t_p = \tau_p\ L_p^2/\alpha_p$, where $\alpha_p = (\alpha_o + \alpha_m)/2$, α_m being the thermal diffusivity at temperature T_m.

These averages arise due to the fact that the properties of the unfrozen product correspond to $\xi > 0.25$, while the properties of a frozen product at a temperature varying between T_e and T_f approximately correspond to $\xi < 0.25$.

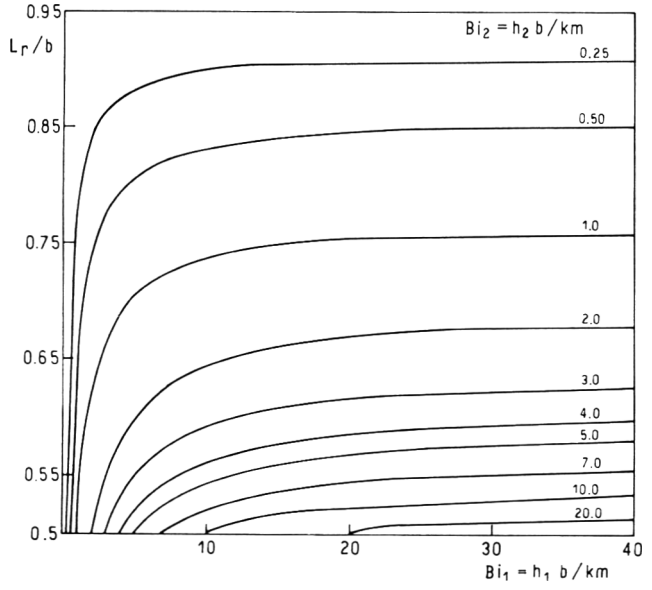

Fig. 3—Dimensionless position of the thermal center for asymmetry originated in different heat transfer coefficients.

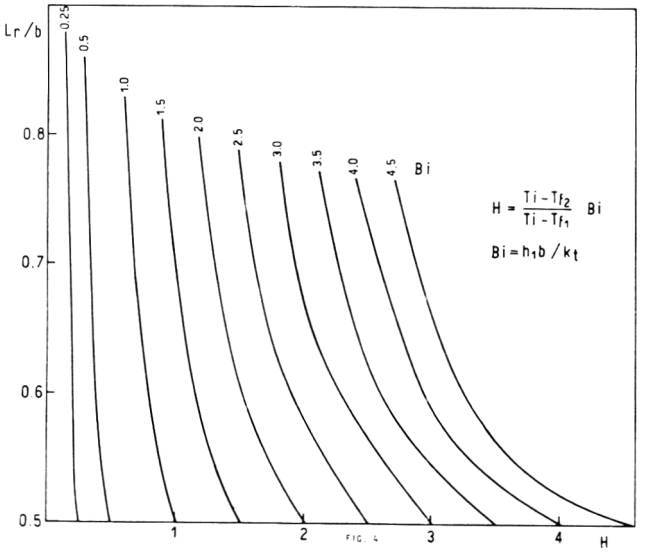

Fig. 4—Dimensionless position of the thermal center for asymmetry originated in different temperatures of the cooling media.

Calculating the change of phase time

The change of phase is considered to be produced entirely at the initial freezing temperature T_e and the change of phase time is calculated with the following equation:

$$t_{ce} = \frac{\rho_o\ Y_o\ \lambda_f^o\ \omega_m\ L_m^2}{(T_e - T_f)\ k_m} \left[\frac{1}{Bi_m} + \frac{1}{2}\right] \qquad (11)$$

where ρ_o and Y_o represent, respectively, the density and the water content of the fresh product, λ_f^o is the heat of solidification of pure water, $L_m = (L_p + L_r)/2$, $\omega_m = (\omega_f + \omega_r)/2$ is the average ice fraction between the values at temperatures T_f and T_r and $Bi_m = h\ L_m/k_m$; k_m being the thermal conductivity at the mean temperature T_m.

Calculating tempering time

During this period it is considered that the system starts at an initial uniform temperature T_e and cools to temperature T_r in the thermal center.

To calculate tempering time the graph of Fig. 7 which represents Eq (8) for $\delta = 0$ should be used. In that graph it is $\eta_r = (T_r - T_f)/(T_e - T_f)$.

To calculate $Bi_{r1} = h_1\ L_r/k_m$ or $Bi_{r2} = h_2(b - L_r)/k_m$ the final position of the thermal center L_r is used.

The basis of the calculation consists in evaluating the cooling time of a half-slab of L_r thickness with the conditions of side 1, or of $(b - L_r)$ thickness with the conditions of side 2, since the almost constant position of the thermal center detected in the tempering period can be assimilated to a zero flow condition at this point.

Consequently, with the value of τ_r obtained from Fig. 7 it is possible to calculate $\tau_r = \tau_r L_r^2/\alpha_m$.

EXPERIMENTAL

IN ORDER TO VERIFY the proposed model, laboratory experiments on the freezing of beef were performed under nonsymmetric conditions.

The runs were carried out on semimembranosus muscle stored at 4°C for 2 or 3 days after quartering. Cylindrical samples of beef with fibers perpendicular to the axial direction were cut and mounted in an acrylic sample holder. Through perforations made in the cylinder, copper-constantan thermocouples were mounted which permitted the temperature to be measured in prestablished positions.

On both ends of the cylindrical sample two heat exchanger plates were placed through which methanol coming from two thermostatized baths was circulated at controlled temperatures. In this way, nonsymmetric freezing could be attained by using different temperatures of the cooling media.

Acrylic slabs, which simulate interfacial thermal resistances, were placed between the exchanger plates and the meat. Nonsymmetric conditions were also possible by using slabs with different thickness in both sides.

The sample holder was isolated laterally with expanded polystyrene to ensure a unidirectional heat flow. In turn, the whole assembly was isolated from the environment.

The thermocouples were connected to a data logger which permitted scanning of temperatures at prestablished times.

The layout described makes it possible to simulate satisfactorily the freezing of meat in plate freezers where the thickness between plates is the same as the length of the cylinder.

Different initial temperatures were attained by storage of the mounted sample holder in controlled temperature rooms for one night.

From the measured temperature profiles the average heat transfer coefficient in each side was calculated from the equation:

$$\bar{h} = \int_0^{t_c} \frac{k\ (T)}{(T - T_f)} \left.\frac{\partial T}{\partial x}\right|_{x\ =\ o;\ b} dt \qquad (12)$$

The obtained values of h_1 and h_2 were used when comparing the numerical or simplified models with the experimental results.

The ranges of operating conditions used in experiments were from 9 to 18°C for the initial temperature T_i; from −30 to −43°C

for the cooling media temperature T_f; from 20 to 150 W/m^2 °K for the heat transfer coefficient h, values of b between 0.06 and 0.12 m, and Bi from 0.9 to 100.

RESULTS & DISCUSSION

AS MENTIONED, asymmetry in heat transfer was achieved either by placing different thicknesses of acrylic between the plate and the meat (keeping equal cooling medium temperatures), or by placing equal thicknesses of acrylic but using different plate temperatures.

Fig. 8b shows the temperature profiles obtained experimentally for a system where the asymmetry arises from different heat transfer coefficients (different acrylic thickness). Fig. 8a shows the profiles predicted by the numerical model.

It will be seen that the sequence followed by the profiles is completely analogous, even showing the displacement of the thermal center as described earlier. Nevertheless, a lower freezing rate is observed in the experimental system which involves freezing times (the time required for the thermal center to reach −18°C) about 5% higher than those predicted.

This difference can be ascribed to the existence of poor thermal contacts in the early stages of the experiments as well as to the influence of the heat capacity of the acrylic slabs used to simulate the thermal resistance. (A heat transfer coefficient involves a resistance without capacity.) Both effects lead to a heat transfer coefficient time dependent (increasing during freezing and tending toward an asymptotic value). The use of the average value of h calculated by Eq (12) account partially for these effects even though a faster cooling of the borders can be observed in the predicted profiles of Fig. 8a.

A similar effect (slightly shorter freezing times for the numerical model respect to the experimental data) was also detected when the asymmetry stemmed from different plate temperatures.

In those cases where no acrylic slabs were used (Bi_1 = Bi_2 = ∞) the coincidence between predicted and experimental profiles was improved.

Fig. 9 is a graph of freezing time values calculated by the simplified model as a function of the times predicted by the numerical method for a final temperature of the thermal center T_r = −18°C. The graph includes indiscriminately values calculated from the side of greater or less heat removal, as well as cases where the asymmetry was generated by different heat transfer coefficients or different temperatures of the cooling media.

It also includes experimental values obtained as described before. The parameter ranges used for the calculations are indicated in Fig. 9.

It will be observed that the agreement is satisfactory for the range of freezing times considered, the differences being below 9% in all cases. The arrangement of the points is independent of the origin of the asymmetry. Only a slight tendency is observed, depending on whether the calculation is made from the side of greater or lesser heat removal, the time calculated by the simplified model being higher than those obtained with the numerical model in the first case, and lower in the second. *—Continued on next page*

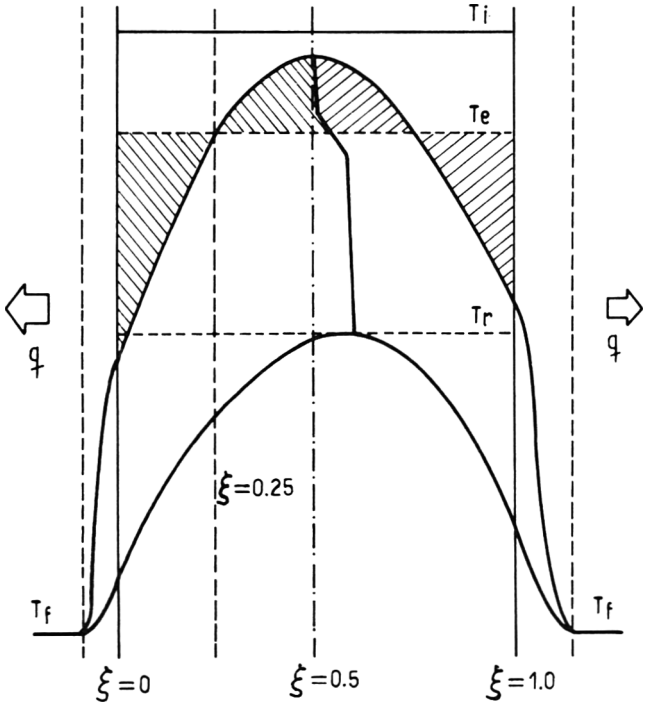

Fig. 5—Schema of temperature profiles at the end of the different periods.

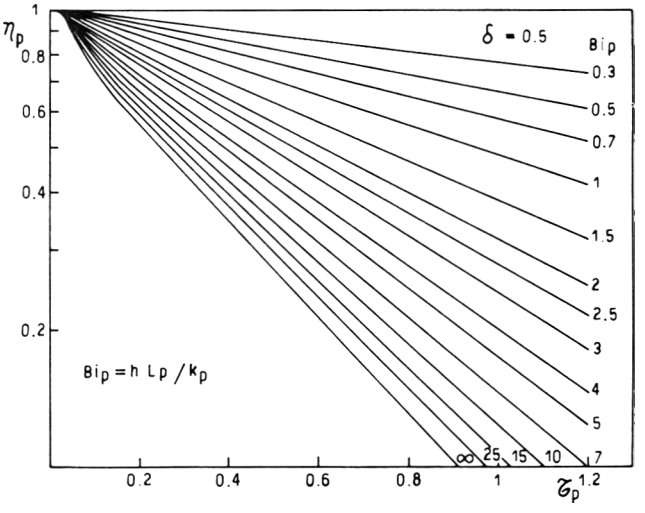

Fig. 6—Curves for pre-cooling time calculation.

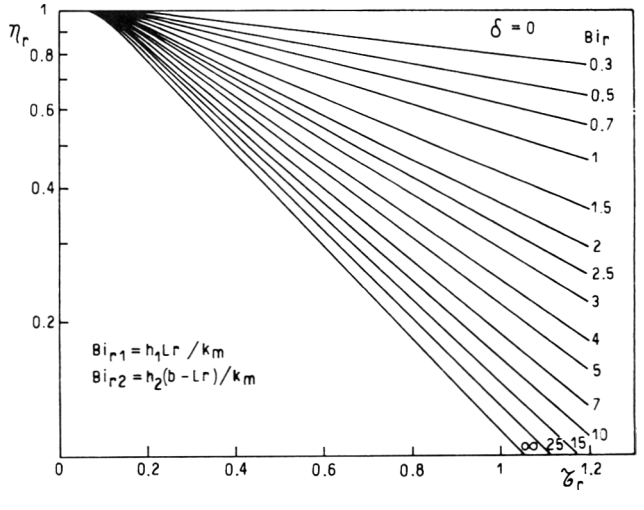

Fig. 7—Curves for tempering time calculation.

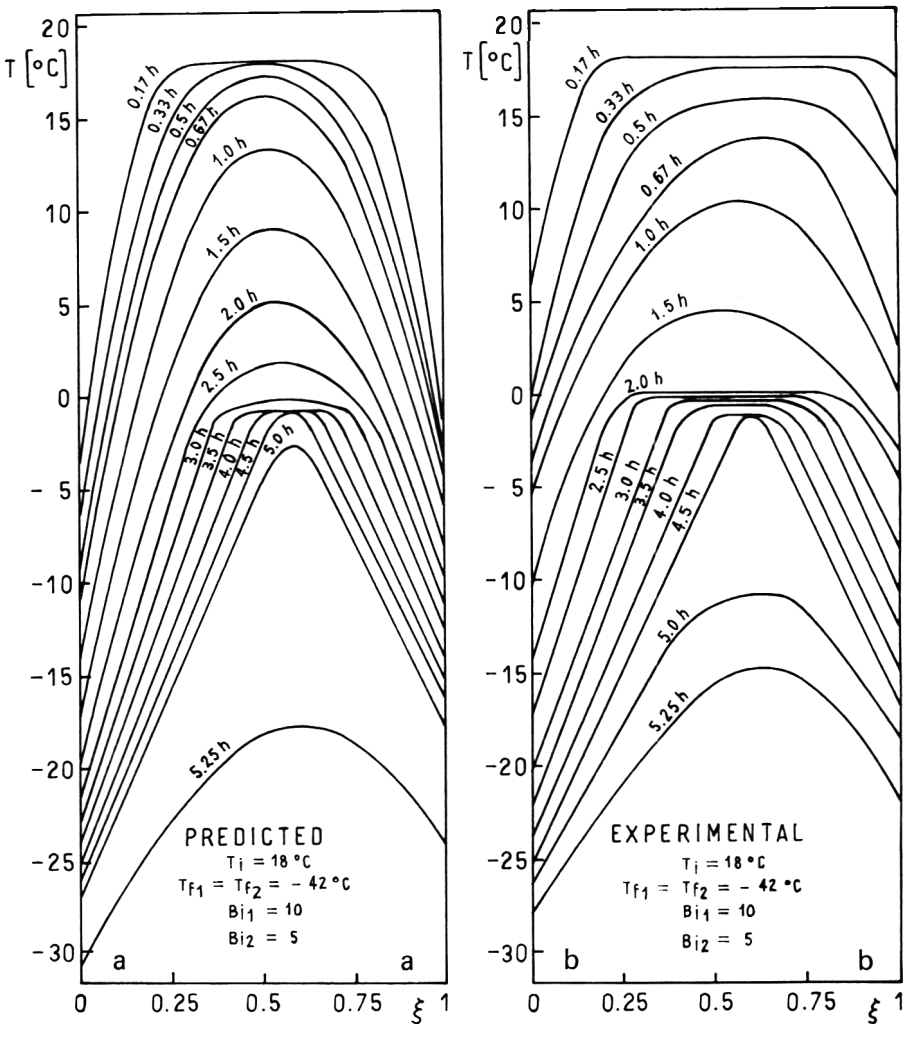

Fig. 8—Temperature profiles for nonsymmetric freezing: (a) Predicted by the numerical method; (b) Experimental.

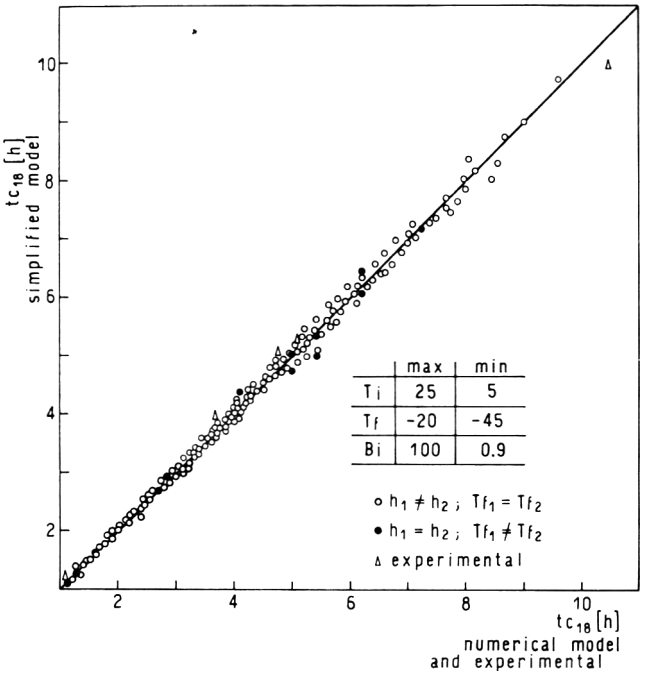

Fig. 9—Comparison of freezing times (t_{c18}) predicted by the simplified model with those experimentally obtained or numerically calculated.

CONCLUSIONS

(1) A numerical model is presented which simulates nonsymmetric freezing of meat in plate freezers.

(2) The model detects a variation of the thermal center throughout the freezing process which is ascribed to the nonlinearity of the system as a result of the change of phase in a wide range of temperatures and of variable thermal properties.

(3) Experimental results show, in the case of meat, a good agreement with the temperature profiles predicted by the model, as well as with the predicted location of the thermal center.

(4) The movement described by the thermal center makes it possible to establish assumptions regarding its position in the different periods. Thus, in the precooling period it remains in a symmetrical position (near the geometric center), while the final position (almost coinciding with that maintained during a large part of the tempering period) is predictable using heat transfer models without change of phase.

(5) On the basis of the assumptions mentioned in (4), a simplified model is proposed which is easy to use for calculating processing times in plate freezers.

(6) The times for heat transfer without change of phase (precooling and tempering periods) are found through the usual method for calculating cooling times.

(7) The model permits calculations for different initial temperatures and various final temperatures in the thermal center with errors below 9% within a wide range of operating conditions.

LIST OF SYMBOLS

- b = Thickness of the food slab (m)
- Bi = Biot number; $Bi = h\ b/k$
- Bi_1 = Biot number for left side of the slab; $Bi_1 = h_1\ b/k_o$
- Bi_2 = Biot number for right side of the slab $Bi_2 = h_2\ b/k_o$
- Bi_m = Biot number for change of phase period $Bi_m = h\ L_m/k_m$
- Bi_p = Biot number for pre-cooling period $Bi_p = h\ L_p/k_p$
- Bi_{r1} = Biot number for tempering period left side $Bi_{r1} = h_1\ L_r/k_m$
- Bi_{r2} = Biot number for tempering period right side $Bi_{r2} = h_2\ (b-L_r)/k_m$
- C_p = Apparent specific heat of partially frozen beef (kJ/kgK)
- $\underline{h}$ = Heat transfer coefficient (W/m^2K)
- h = Time averaged heat transfer coefficient
- h_1 = Heat transfer coefficient on the left side of the slab
- h_2 = Heat transfer coefficient on the right side of the slab
- H = Parameter defined in Eq. (9) $H = Bi\ (T_i - T_{f_2})/(T_i - T_{f_1})$
- k = Thermal conductivity ($W/m^\circ K$)
- k_o = Thermal conductivity of unfrozen product
- k_m = Thermal conductivity at temperature T_m
- k_p = Average thermal conductivity, $k_p = (k_o + k_m)/2$
- k_t = Thermal conductivity at the lowest cooling medium temperature
- L = Half thickness of slab
- L_m = Average position of the thermal center during the change of phase period $L_m = (L_p + L_r)/2$
- L_p = Position of the thermal center for pre-cooling period $L_p = b/2$
- L_r = Position of the thermal center for tempering period
- t = Time (s)
- t_{ce} = Change of phase time
- t_{cr} = Freezing time to attain T_r at the thermal center
- t_p = Pre-cooling time
- t_r = Tempering time
- T = Temperature ($^\circ K$)
- T_e = Temperature for freezing beginning
- T_f = Temperature of cooling medium
- T_{f_1} = Temperature of cooling medium at left side
- T_{f_2} = Temperature of cooling medium at right side
- T_i = Initial temperature
- T_m = Average temperature $T_m = (T_e + T_f)/2$
- T_r = Temperature attained by the thermal center at the end of freezing
- x = Spatial coordinate
- y = Spatial coordinate in the symmetric slab
- Y_o = Initial water content of food on wet basis

GREEK LETTERS

- α = Thermal diffusivity (m^2/s)
- α_o = Thermal diffusivity of unfrozen product
- α_m = Thermal diffusivity evaluated at T_m
- α_p = Average thermal diffusivity $\alpha_p = (\alpha_o + \alpha_m)/2$
- δ = Dimensionless spatial coordinate $\delta = y/L_p$ or $\delta = y/L_r$
- λ_n = Root in Eq. (8)
- λ_f^o = Heat of solidification of pure water (kJ/kg)
- η = Dimensionless temperature $\eta = (T - T_f)/(T_i - T_f)$
- η_p = Dimensionless temperature for pre-cooling period $\eta_p = (T_e - T_f)/(T_i - T_f)$
- η_r = Dimensionless temperature for tempering period $\eta_r = (T_r - T_f)/(T_e - T_f)$
- ω = Ice content of partially frozen food
- ω_m = Average ice content $\omega_m = (\omega_r + \omega_f)/2$
- ω_f = Ice content at temperature T_f
- ω_r = Ice content at temperature T_r
- ρ = Density (Kg/m^3)
- ρ_o = Density of unfrozen product
- τ = Dimensionless time
- τ_p = Dimensionless pre-cooling time; $\tau_p = \alpha_p\ t_p/L_p^2$
- τ_r = Dimensionless tempering time; $\tau_r = \alpha_m\ t_r/L_r^2$
- ξ = Dimensionless spatial coordinate $\xi = x/b$

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Surface Tension and Foaming of Protein Solutions

NAOFUMI KITABATAKE and ETSUSHIRO DOI

ABSTRACT

The relationship between surface tension and foamability of protein solutions was examined. Proteins including egg albumin, soybean protein, casein, milk whey protein and gelatin were used. Foamability was expressed as "foaming power" which corresponded to the ratio of gas volume to the liquid volume in the foam. The absolute surface tensions of protein solutions were not correlated to foamability, but the rate constant of surface tension decay of protein solutions was significantly correlated to foamability of protein solution.

INTRODUCTION

FOAMS are used to improve the texture, consistency, and appearance of food. Foam in food systems is commonly found in baked and confectionary goods. There is a need for a surface-active foaming agent to create foam. The most versatile foaming agents are those based on protein, but studies on protein foam have not progressed sufficiently to explain foaming behavior in terms of physical properties such as surface tension. Several independent studies have attempted to improve foaming properties through the empirical manipulation of compositional and procedural variables (Cherry et al., 1979; Richert, 1979; Yasumatsu et al., 1972a, b), but little systematic work has been done on basic studies of protein foams.

We investigated foamability, using various proteins. A vareity of empirical methods of foam preparation and measurement have been used by different authors (Nakamura and Sato, 1964; Waniska and Kinsella, 1979; Mita et al., 1977; Cherry and McWatters 1981), however, further work is necessary for better comparison.

Foam is a multiphase with gas (air or N_2) as the disperse phase and liquid (protein solution) as the disperse medium. Therefore, the performance of the foam can be predicted by a function composed of some physical properties of protein. We have also attempted to describe foamability more clearly; to show the general relationship between foam properties and physical properties of the protein solution. The equilibrium surface tension of the protein solutions was determined, and the surface behavior of various proteins have also been studied by measuring surface tension decay and by evaluating its kinetics.

MATERIALS & METHODS

Protein samples

Sodium caseinate (Emulac 50; Meggle Milchindustrie Gmbh & Co. Kg., Reitmehring, Fr-Germany) and milk whey (Type 50 and Type 50K; Meggle Milchindustrie Gmbh & Co. Kg., Reigmehring, Fr-Germany) were obtained by Calpis Food Industry Co. Ltd., Tokyo, Japan. Gelatin (alkaline gelatin) was from Yasu Chemical Industry Co. Ltd., Shiga, Japan. Immunoglobulin G (IgG), Lysozyme, hemoglobin and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO. Egg albumin (soluble type) was purchased from Nakarai Chemicals Co. Ltd., Kyoto, Japan. Soybean protein was prepared as follows. Undenatured hexane defatted soybean flour (obtained from Fuji Oil Co. Ltd., Osaka, Japan,) was suspended in 10-fold (w/v) distilled water, stirred for 30 min, then centrifuged at 12,000 x g for 10 min. The supernatant was acidified and adjusted to pH 4.5 with 6N HCl to precipitate the protein, and allowed to stand for 30 min, then centrifuged at 10,000 x g for 10 min. The precipitate was dissolved in distilled water by the gradual addition of 6N NaOH, and adjusted to pH 7.0. The volume of the solution was made the same as the original one with distilled water. Precipitation was conducted repeatedly and the protein solution of each precipitation was prepared by the same procedure. The soybean protein (Isolate protein) was freeze-dried. α_{s1}-Casein was prepared from fresh Holstein cow milk by the method of Zittle and Custer (1963) and the α_{s1}-casein fraction obtained was purified by DEAE-Sephadex A-50 chromatography. A 1-g sample was applied to a column (3.6 x 14.5 cm) previously equilibrated with 10 mM imidazole-HCl buffer, pH 7.1, containing 3.3M urea. The column was washed with 500 ml of the same buffer and with 1 liter of a linear gradient of NaCl (0–0.3M) in the same buffer. The obtained α_{s1}-casein fraction was dialized against distilled water exhausively at 4°C and adjusted to pH 7.0 by the addition of NaOH. The neutralized protein solution was freeze-dried to yield α_{s1}-casein powder. All purification operation except dialysis were carried out at room temperature.

Preparation of protein solution

A sample of the protein powder was dissolved in distilled water. After stirring for 1 hr at room temperature, the protein solution was adjusted to pH 7.0 by the addition of NaOH and centrifuged at 3000 x g for 10 min at 10°C to remove the insoluble fraction. Supernatant solutions were used through the following experiments. Protein concentration was determined by drying the sample to constant weight at 100°C and calculated as % weight/volume.

Stirring method

Fifteen ml of protein solution were placed in a 50 ml stainless steel container. A homogenizer (Ace type, Nihon Seiki Kaisha Ltd.) equipped with a constant-temperature cell compartment (25°C), consisting of a rotating six bladed knife suspended in the solution, was used. The rotor speed was adjusted to 10,000 rpm. After stirring for 1 min, enough of the foam to fill an Erlenmeyer flask of known weight and volume, was transferred immediately by pipette and weighed. "Foaming power" was defined by the expression:

$$100 \times \left(\frac{[L] + [G]}{[L]} - 1\right) \quad (1)$$

[L] + [G] and [L] were measured. [L] + [G] is the volume of foam. [L] is the volume of liquid phase of the foam, which is calculated when the weight of foam in the vessel and the density of the liquid are known. [G] means the volume of gas phase in the foam. (1) can be written as (100 x [G]/[L]) which exhibits the ratio of gas volume to liquid volume in the foam, therefore the "foaming power" is dimensionless.

Shaking method

Twenty-five ml of aqueous solution of each protein were shaken, in a 50 ml Teflon stoppered graduated cylinder (20 x 2 cm i.d.), for one min (30 strokes per min, 20 cm amplitude of shaking), using a "swing arm agitator" (Yayoi Medical Instrument Co., Ltd., Tokyo, Japan). After shaking, the cylinder was placed on a flat surface and the resulting foam volume and solution volume were recorded within 10 sec. The resulting foam volume was defined as "foam volume" which is another expression of foamability. At the

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same time "foaming power" was also estimated by this method. It was possible to compare the two different indications of foamability, i.e. "foaming power" and "foam volume" by this method.

Bubbling method

Ten ml of the protein solution were introduced into a cylindrical column (30 x 1.65 cm i.d.) maintained at 25 + 0.2° C, fitted with a sintered-glass at the bottom. Nitrogen gas was then passed through the filter and allowed to bubble up the solution at the rate of 15 ml/min for 3 min, after which the gaseous flow was stopped and the resulting foam and solution volume were measured. Only "foaming power" can be measured by this method as in the case of the stirring method.

Measurement of surface tension

The decrease of surface tension of protein solution with time was monitored with a Wilhelmy plate type surface tensiometer (Kyowa Model CBVP surface tensiometer type A3) equipped with a digital printer (Yokogawa portable printer Model 2543) and a constant temperature cell compartment. Changes in surface tension of 0.1 dyne/cm could be measured reproducibly. The vessel containing the solution was a dish 5.5 cm in diameter and 1.5 cm in depth. The solution was introduced into the vessel and after standing for about 20 min to equilibrate the temperature, a fresh surface was formed by removal of the surface of the solution by decantation. The change of surface tension was recorded every minute for 120 min after the new surface formation. The sample temperature was kept at 25 ± 0.2°C.

RESULTS & DISCUSSION

Measurements of foamability

The "foaming power" and the "foam volume" of various proteins were measured by the shaking method. Egg albumin, BSA, milk whey protein, gelatin, casein and soybean protein were used in concentration ranging from 0.3–3.0% (w/v). Although 30 protein solutions were used in the experiments, the values for egg albumin and BSA with concentrations above 1.0% (w/v), were excluded because of excessive foaming. There was no correlation between the values obtained by both the two expressions, that is, "foaming power" and "foam volume" ($r = 0.094$, $N = 20$). The cause of this poor correlation may be because the characteristics of the shaking method and the stability of the bubble wall in the column seems to influence the result largely.

In principle the stirring and bubbling method cannot be used for determining the "foam volume". Then in order to seek a better method for obtaining a reliable value of protein foamability, we used "foaming power" as indicator of foamability and examined (i) the comparison between the shaking and bubbling method, (ii) long periods shaking of protein solution, and (iii) the stirring method instead of the shaking method. The stirring used in the present experiments was much more vigorous than the shaking.

The "foaming powers" by the shaking and bubbling methods were correlated to each other ($r = 0.63$, $N = 20$). The values obtained by the bubbling method were about fivefold larger than those by the shaking method. The "foaming power" by the bubbling method may reflect the properties of protein foam stability. Therefore the use of the bubbling method is not suitable for study of foamability. By the shaking method the effect of foam stability on "foaming power" cannot be neglected, but it could be smaller than that by the bubbling method.

The effect of shaking time on the "foaming power" is shown in Fig. 1. A 1.0% (w/v) gelatin solution was used. The "foaming power" was reduced as the protein samples were shaken for long periods. It took more than 20 min shaking to reach the constant value. Similar results were obtained when other types of protein solution were used. Long time shaking would give "foaming power" which neglects the effect of foam stability as far as possible.

When stirring method was used, it took only 20 or 30 sec to attain the constant value as can be seen in Fig. 2. The results shown in Fig. 1 and 2, indicate that stirring for 1 min was sufficient to reach the constant value of "foaming power," but shaking for 1 min was not enough to make a foam which had the constant value of "foaming power." As shown in Fig. 1 and 2, the "foaming power" at short time by both the shaking and stirring method, gave higher values, since there were various sizes of bubbles including larger ones in the foam before attainment of the constant value. As the protein sample was shaken or stirred longer, the foam becamse homogenous small bubbles. The constant value obtained by the shaking method coincided well with that by the stirring method. The "foaming power" obtained by the shaking method and the stirring method

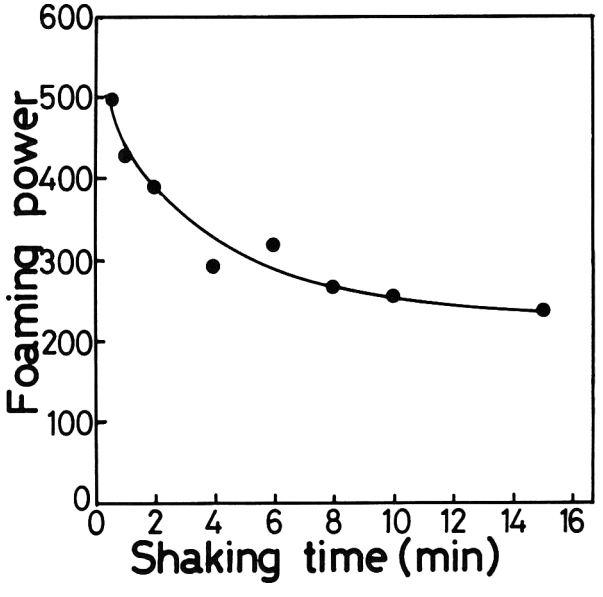

Fig. 1—Effects of shaking time on foamability; "foaming power." Gelatin solution (1% w/v) was used. The definition of "foaming power" and the method of measurement are described in text.

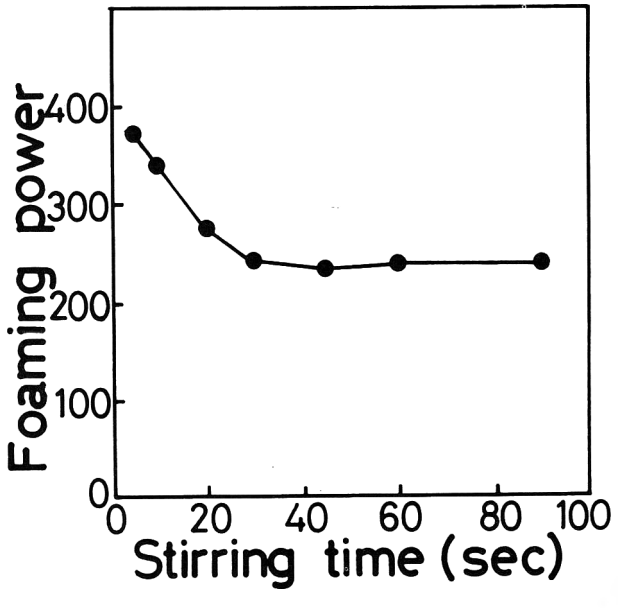

Fig. 2—Effects of stirring time on foamability; "foaming power." Gelatin solution (1% w/v) was used.

were 230.5 and 220.4, respectively. This suggests that the constant values obtained by both these methods are reliable and are suitable for the representation of the "foaming power".

The correlation coefficient between the "foaming power" by the shaking method and that by the stirring method was r = 0.52. The values obtained by the shaking method were twofold larger than those by the stirring method. This is because 1-min shaking was not enough and gave heterogeneous bubbles in the foam.

The experiments described above indicate that shaking the protein sample for more than 20 min and the use of the stirring method, are satisfactory for getting reliable values of protein foamability. However, the shaking method is time-consuming, and less convenient than the stirring method.

The stirring method to make foam is the best of the three methods described above, and the values obtained are the most reliable. Therefore, "foaming power" measured by the stirring method was used throughout the following experiments.

Table 1—"Foaming power" and surface tension of various protein solutions

Protein solution[a]	"Foaming power	σ_c[d] (dyne/cm)	k (min^{-1})
Gelatin	220.4	46.3	0.213
α_{s1}-casein	210.5	47.8	0.0445
Hemoglobin	204.6	41.8	0.0464
BSA	201.8	45.2	0.0475
Casein	198.2	46.4	0.0450
Milk whey (A)[c]	164.7	44.2	0.0345
Soybean protein	153.5	42.1	0.0327
Egg albumin	152.8	42.5	0.0288
Milk whey (B)[d]	144.8	44.6	0.0311
IgG	98.0	49.1	0.0293
Lysozyme	80.5	44.3	0.0183

[a] Protein concentration, 1% (w/v)
[b] Surface tension at 120 min after making the new surface
[c] Milk whey Type 50
[d] Milk whey Type 50K

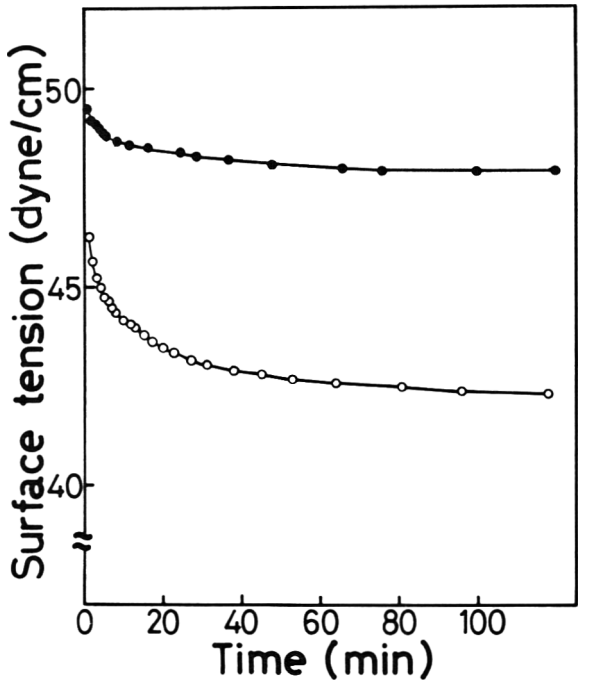

Fig. 3—Time dependence of surface tension for protein solution (1% w/v): ●, α_{s1}-casein; ○, egg albumin.

Surface behavior and foamability

The surface tension (σ) is an important physical property of foam. Generally it is known that the solution with lower σ has the larger foamability; however, it has been pointed out by Burcik (1950) that it was not so much the absolute value of σ which is crucial, but rather the time dependence of σ, that is, the dynamic surface tension during the foam formation.

In Table 1, "foaming power" and surface tension (absolute value) of various kinds of protein including food proteins are given. In the first column of Table 1, proteins were arranged successively from higher "foaming power" to lower ones, but σ was not parallel to "foaming power." It is apparent that the protein with lower σ does not always represent the high value of foamability. For example σs of soybean protein and egg albumin were very low, but "foaming power" of those proteins were not as large as that of hemoglobin. Though σ of lysozyme was lower than that of α_{s1}-casein, "foaming power" of lysozyme is much smaller than that of α_{s1}-casein. There was little correlation between the "foaming power" and the surface tension of solution.

In Fig. 3 the surface tension, which was measured with Whilhelmy plate method, has been plotted as a function of time up to 90 min for two proteins, α_{s1}-casein and egg albumin, from Table 1. The initial surface tension of the solvent (water) was 71.9 ± 0.2 dyne/cm at 25.0 ± 0.2°C. Surface tensions of the protein solutions in this experiment almost reached a constant value within about 120 min after making a new surface. It took different time period for each protein solution to reach the constant value of surface tension, which means that the rate of surface tension decay varied with the kind of protein.

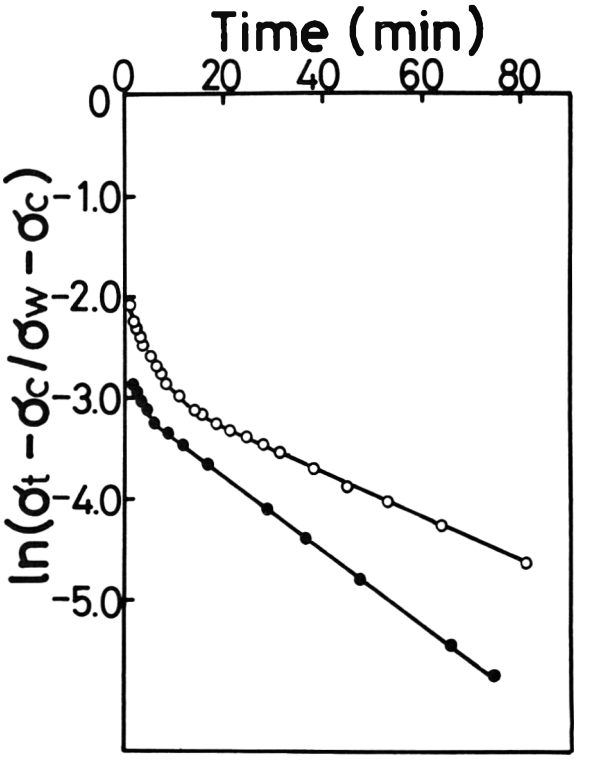

Fig. 4—Dimensionless surface tension $ln(\sigma_t-\sigma_c/\sigma_w-\sigma_c)$ as a function of time (t). 1% (w/v) protein solution was used: ●, α_{s1}-casein; ○, egg albumin.

In order to evaluate the kinetics of surface tension decay of the protein, k, the rate constant of surface tension decay was measured. There is no theoretical equation directly representing surface tension decay with surface aging, but some empirical equations have been reported (Nakamura and Sasaki, 1970; Frisch and Al-Madfai, 1958). Boutaric and Berthier (1939) reported that the rates of surface tension decay could be analyzed by a first order equation:

$$(\sigma_t - \sigma_c)/(\sigma_w - \sigma_c) = \exp(-kt), \quad (2)$$

Where σ_c is the surface tension value at 120 min after making new surface; σ_w is the surface tension value at time t = 0 that is equal to the surface tension of the solvent (water) so that σ_w is 71.9 dyne/cm, and σ_t is the surface tension at any time, t; k is the rate constant. Eq (2) can be written as

$$\ln(\sigma_t - \sigma_c/\sigma_w - \sigma_c) = -kt. \quad (3)$$

When Eq (3) is obeyed, $\ln(\sigma_t - \sigma_c/\sigma_w - \sigma_c)$ versus t gives a straight line. As is shown in Fig. 3, the plots give linear portion from 10 min to 60 min after creating the new surface. During first 10 min the line curved and the rate is greater than that of later portion. Calculation of k from the slopes of linear portions of Fig. 4 gives k = 0.0445 min^{-1} and k = 0.0288 min^{-1} for α_{s1}-casein and egg albumin, respectively.

k values of other proteins were obtained in this manner. These k values of various proteins are shown in Table 1 where the correlation between k and "foaming power" was good and the k vs. foamability plot is given in Fig. 5. The correlation coefficient for the linear part, except for gelatin, was 0.94. The proteins with high "foaming power" show high k values, i.e. the protein solutions with higher foamability need shorter time for the surface tension to reach a constant value. This indicates that it is necessary in the creation of foam containing a large volume of gas, to make the equilibrated surface condition with rapid adsorption, with little rearrangement, and with little orientation of protein molecules at the surface layer. Graham and Phillips (1979 a,b,c), MacRitchie (1978) and Phillips (1981) have reported that making a surface layer of protein solution was composed of several processes. During the initial stage the protein molecules arrived at the surface by diffusion and adsorption. Next, the protein molecules penetrated into the surface and were rearranged.

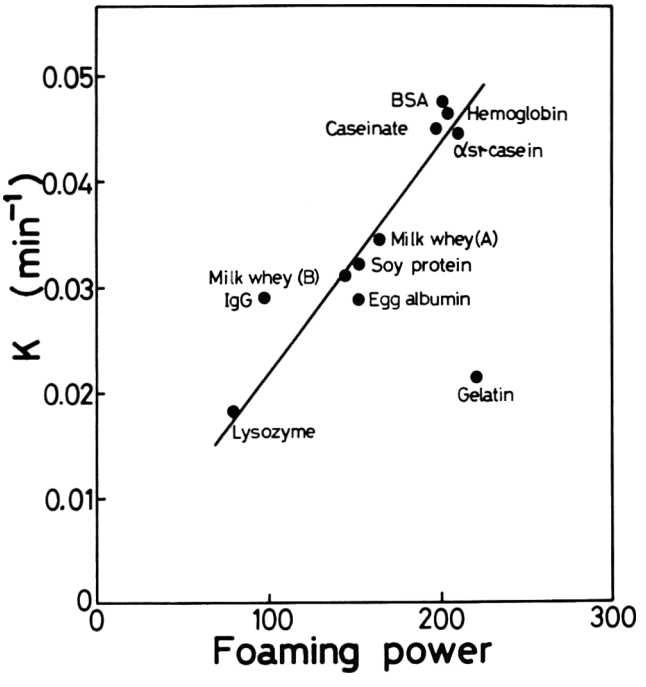

Fig. 5—Relationship between "foaming power" and the rate constant of surface tension decay of protein solution (1% w/v).

It is almost certain that protein molecules such as casein, having a flexible and random coil structure and amphipathic structure on the molecular surface, easily penetrate to the surface and needs no special unfolding at the air-water interface. Such protein gives large values of both k and "foaming power." Rigid globular moleculars such as lysozyme, which would be accompanied by surface denaturation upon penetrating the surface and would be arranged at the surface after penetration, represented low foamability and k values.

Egg albumin and soybean protein yielded low foamability and low rate constants of surface tension decay in spite of them being versatile foaming agents, and this is because they are able to form extremely stable foams rather than make a large volume of foam. The point of gelatin in Fig. 5 deviated from the fitted line. This would be because its molecular properties, including the distribution of molecular size and viscosity of gelatin solution, were quite different from those of other protein molecules (Courts, 1980).

Grahams and Phillips (1979) have measured the behavior of foamability and increase of surface pressure of three protein samples and suggested that foamability is related to the rate of decrease of the surface tension. Tornberg (1978a, b; 1979) discussed the relationship between the behavior of protein at the air-water interface, especially the surface tension decay and the emulsifying behavior of protein. Our results in this paper are basically in agreement with their qualitative findings; however we were able to get well defined correlation between foamability and surface behavior. Foamability of protein solution can be described quantitatively by measurement of the physical behavior of the solution. The rate constant of surface tension decay would be useful for the indication of foamability of protein food. Moreover the investigation about the surface tension decay made it possible to find a new method for the regulation of protein foamability. For example, if some treatment is devised to convert protein with low rate constant of surface tension decay, to protein with a high rate constant, it is expected that the foamability of the protein will be increased by this treatment.

In this report we discussed foamability. It is difficult to depict the foamability of protein solution, distinguished from foam stability, since the foam is a dynamic system and changes with time. Especially in the case of unstable form such as dilute protein solution, the foamability of the solution is equal to the stability of the foam formed. Therefore, the foam stability is also important to describe the foam characteristics and must be studied more precisely.

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Detection of Microbial Contamination Utilizing an Infrared CO_2 Analyzer

C. H. THRELKELD

ABSTRACT

Infrared (IR) analysis of headspace gas for the presence of CO_2 was evaluated as a method of nondestructively sampling and segregating microbiologically contaminated products. Approximately 1.8 million 7-oz bottles containing citrus juices and fruit drinks were inspected and segregated based on IR CO_2 analysis. Comparison of IR analysis with conventional plating methods indicated 89% of the samples determined to be positive by conventional methods were also detected by IR analysis. False negatives were found to be the result of nonfermentative microorganisms in over 80% of the contaminated samples not detected by IR analysis. Nonfermentative organisms were identified and effects on product were evaluated. The expected contamination rate was 5.3% compared to 6.3% contamination detected by IR analysis.

INTRODUCTION

MICROBIOLOGICAL CONTAMINATION continues to be a serious and costly problem to the citrus industry as well as the food industry in general. Rapid radiometric methods of detection have been utilized successfully for a variety of fresh and processed food products (Rowley et al., 1978; Stewart et al., 1980) including citrus (Hatcher et al., 1977). These methods make it possible for the processor to take corrective action sooner and thus greatly reduce the amount of contaminated product. Nevertheless, when microbial contamination does occur, detection and segregation of contaminated product by nondestructive methods offers significant advantages.

Microbial contamination of pasteurized, cold-filled (asceptic pack) citrus juices and fruit drinks provided an opportunity for evaluating a novel method for nondestructively analyzing each container for the presence or absence of spoilage organisms. Production of CO_2 is a characteristic of most microorganisms found to be associated with spoilage of citrus products (Faville and Hill, 1952; Buchanan and Gibbons, 1975; Hatcher et al., 1977). Infrared (IR) spectroscopy has been utilized in many scientific and industrial applications including medicine (Collier et al., 1955; Jones et al., 1979), biology (Hagar and Freeberg, 1979), microbiology (Parker, 1971), and furnace gas analysis (Hutchinson, 1977) to detect the presence of CO_2 both quantitatively and qualitatively. Since the contaminated product evaluated in this study was packaged in 7-oz bottles which contained a headspace in which CO_2 could accumulate if spoilage were occurring, IR analysis of container headspace was considered as a possible means of nondestructively examining each container within lots suspected of being contaminated.

Traditionally, sampling plans (USDA, 1973) are used which identify production lots containing contaminated product. These lots are then held for further sampling, reprocessing, or destruction depending upon the nature and/or severity of the contamination. Because microbial contamination is often intermittent and may not involve 100% of the containers within a contaminated lot, it would be beneficial to reprocess or destroy only that product which is actually contaminated. The purpose of this study was to determine the feasibility and effectiveness of identifying, by IR analysis, the presence of CO_2 in the headspace of individual containers suspected of being contaminated with microorganisms.

Research was conducted at Adams Packaging Assoc., 625 Bridgers Ave., Auburndale, FL 33623. Author Threlkeld's address is 324 Suwannee Road, Winter Haven, FL 33880.

MATERIALS & METHODS

Contaminated lots

Time periods in which contamination occurred were based on results of standard plate counts (Murdock et al., 1952) made from production line samples. Samples from each pallet (117 cases/pallet, 24 bottles/case) within defined time periods were subjected to further microbiological evaluation and the results served to identify contaminated lots. In order to insure that no contaminated product reached the consumer, pallets produced prior to and following contaminated lots were also subjected to microbiological evaluation. During this evaluation it was determined that contamination was intermittent and occurred at low frequency throughout certain production dates. All production from certain dates, therefore, was held for further evaluation by an IR CO_2 analyzer.

Infrared CO_2 analyzer

The infrared analyzer utilized in this study was the Anacon Model 206 Infrared Analyzer (Anacon, Inc.). The diagram in Fig. 1 illustrates the basic components of the system and the arrangement in which the headspace in each bottle was analyzed. Briefly, the analyzer is composed of a Measuring Unit consisting of the Infrared Source and Detector, and an Electronic Amplifier Control Unit (Fig. 1 Amplifier). A quartz halogen bulb produces energy which is passed through a filter wheel containing two filters. The output from the filter wheel consists of the sequence no energy, an IR reference wavelength of 2.7 micrometers, no energy, and an IR measuring wavelength of 2.6 micrometers in that order. This energy sequence passes through the sample to the detector where it is converted to an equivalent sequence of electrical pulses. The detector is synchronized with the IR source and is designed to separate the reference and measurement pulses which are compared and the difference is amplified and used to drive the output meter.

The Electronic Amplifier Control Unit front panel contains the power on-off switch, a Range Shift control which allows the meter to be set to zero, a 4-step sensitivity range control which includes x1, x2.5, x5, and x10, a sensitivity fine adjust allowing continuous sensitivity adjustment throughout the sensitivity ranges, a damping control which in effect averages short term fluctuations in sample measurement, a normal operation indicator light, and an output meter which reads 0%–100% in arbitrary units. Additionally, the unit is equipped with a calibration kit which allows simple self-testing and recalibration when necessary.

Initially, samples of both contaminated and uncontaminated product were sent to the manufacturer to determine the feasibility of the application. Analyzer settings were derived empirically and it was determined that a meter output reading of 45% of scale or above indicated a contaminated sample. This value was set slightly below the lowest level at which CO_2 was normally detected in contaminated samples. It was felt that although this precaution would result in some uncontaminated samples being rejected, it would virtually eliminate acceptance of contaminated samples.

IR analysis and sampling

Prior to analysis, cases of product suspected of being contaminated were removed from cold storage (2°C) and held at room

temperature (24–27°C) for a minimum of 10 days. This was necessary to allow fermentation with resultant CO_2 production to occur in contaminated samples. Containers were removed from the cartons and conveyed by a single conveyor to the IR analyzer. The full scale operation utilized two IR analyzers located on either side of the conveyor. Bottles were removed from the conveyor and placed on the rotating support as shown in Fig. 1. Care was taken in handling all containers since it was found that tilting the bottles resulted in a juice film on the inside of the bottles which gave high readings. For this reason, samples reading between 45% and 100% of scale were routinely set aside to allow drainage of juice from the sides of the bottle. Following this procedure, samples were rechecked and it was found that this technique eliminated many false positive results due to mishandling of samples. It was found that due to the optical properties of the glass it was necessary to rotate the bottles at 15–20 rpm for optimum results. The rotating support also positioned the bottle at the proper height and alignment for centering the IR beam. The IR beam diameter was modified by the manufacturer and measured 5/32 inch diameter and passed through the bottle neck 5/8 inch below the bottle top. The beam was actually adjusted to pass through the bottle 1/8 inch below the bottom of the lower bead in the bottle neck. Containers were left in position for approximately 3 sec (actual response time was approximately 2 sec). Samples containing CO_2 were identified by the illumination of a warning light (Fig. 1) placed in front of the rotating support of each analyzer. The light was set to illuminate whenever the meter output level reached 45% of scale which represented a positive detection of CO_2. This was advantageous because it eliminated the need to monitor the meter and in effect released the operator from having to make decisions on borderline readings. Samples that were positive (CO_2 detected) were destroyed while negative samples (no CO_2) detected. were conveyed to a casing area where they were cased and resealed utilizing the original cartons.

In order to ascertain the effectiveness of the IR analyzer in the full scale operation, two methods of sampling were utilized. First, every fifteen minutes a sample container representing a negative reading was removed from the conveyor after analysis and tested for the presence or absence of vacuum utilizing a Marshalltown Model 18 vacuum guage. Absence of vacuum or positive pressure would indicate an analyzer malfunction or insufficient time allowed for analysis. Second, sample containers representing both positive and negative readings were removed at random at a rate of approximately one every 30 min. These samples were examined for presence or absence of vacuum, and 1-ml portions of each sample were plated in orange serum agar (OSA, Difco) for microbiological analysis (Murdock et al., 1952).

Microbiological analysis

Orange serum agar (OSA) plates of samples selected as described above were incubated at 30°C for 48 hr after which they were read and either discarded or retained for further evaluation. Any plates showing growth that were from containers which IR analysis had determined to be negative (false negatives) were of particular interest. Selected colonies determined microscopically (Gram stain morphology) to be yeast were isolated on OSA slants and further identified to the species level utilizing the Uni-Yeast Tek system (Flow Laboratories) as described by Hill (1980). Colonies determined microscopically (Gram stain morphology) to be bacteria were isolated on OSA slants for further identification. Bacteria which were shown to be Gram positive nonspore forming rods were presumptively identified as being from the genus *Lactobacillus* based on colony morphology on OSA as described by Murdock et al. (1952). Gram negative nonfermentative bacteria were identified on the basis of biochemical characteristics as described by Tatum et al. (1974).

Additionally, samples showing growth in OSA plates after 48 hr incubation at 30°C were obtained from containers which IR analysis determined to be positive (true positives). These plates were also selectively evaluated as described previously to determine which microorganisms were responsible for spoilage.

RESULTS & DISCUSSION

Loss analysis

Expected versus actual percentage loss due to contamination is summarized in Table 1 by product type. The expected percent loss was derived from the results of plate counts performed on sample containers from lots known to be contaminated. The actual percent loss was derived from the number of cases inspected by the IR analyzer and destroyed compared to the total number of cases inspected (The total number of cases inspected for all product types was 73,609 or 1,766,616 containers).

It can be seen from Table 1 that in all products except Grape drink the actual loss was greater than the expected loss. Two factors probably contributed to the higher than expected loss. First, the selection of the meter output level of 45% of scale was intentionally set below the level of output normally produced by positive containers (most positives read between 60% and 100% of scale). Second, handling and conveying of product resulted in some containers being tilted with the juice producing a film on the interior of the bottle neck. These containers normally produced readings above 45% of scale and at least 10 min of drainage time was required prior to reinspection, or false positive results were obtained. This latter condition probably contributed the most to the increased actual loss rate. Actual loss of Grape drink due to contamination was considerably

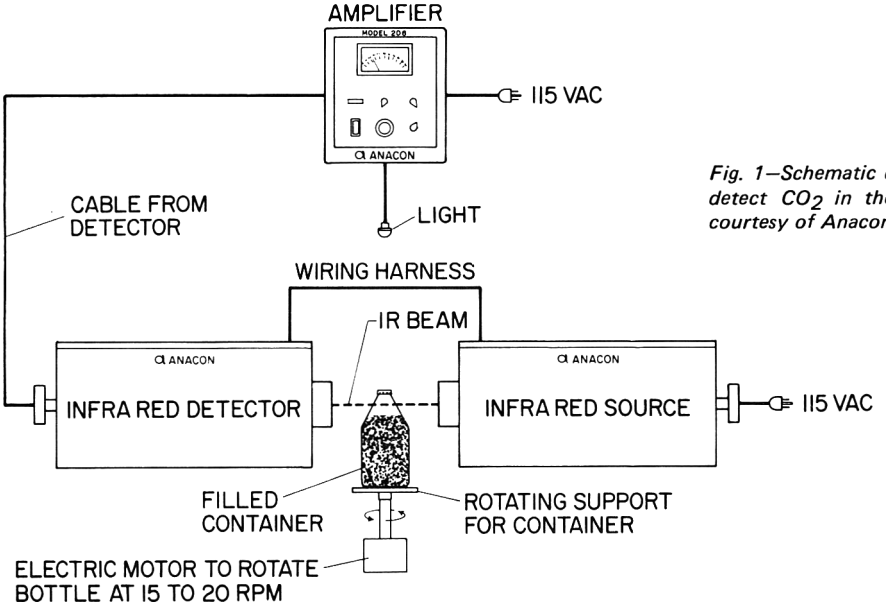

Fig. 1—Schematic diagram of Anacon Infrared Analyzer as set up to detect CO_2 in the headspace of fruit juice bottles. (Illustration courtesy of Anacon, Inc.)

lower than expected. This is probably due to a more restricted pattern of contamination. That is, contamination levels were high in suspect lots, but relatively low in the remainder of the product.

IR analysis vs conventional plating

In order to obtain an accurate comparison of IR analysis with conventional plating methods, 264 samples from contaminated orange juice and apple juice lots were carefully removed from cartons, analyzed, checked for vacuum, and plated in OSA. Care was taken to avoid tilting bottles, and analyses were timed to insure that the optimum time of 2–3 sec was allowed for each analysis. Table 2 contains the results of this comparison. It must be noted that great care was exercised to avoid false positives (samples showing $\geqslant$ 45% of scale but not showing counts when plated in OSA) due to tilting of bottles. During normal operations as many as 50% (data not shown) of the positives recorded by the IR analyzer rechecked within limits after allowing juice in the neck to drain for several minutes (10 min was recommended). False negatives were always a primary concern, and Table 2 indicates a rate of occurrence of false negatives very close to the rate found for all samples analyzed and plated. A total of 3,567 samples including those shown in Table 2 were analyzed by the IR analyzer, checked for vacuum, and plated in OSA. Conventional methods detected contamination in 742 of these samples of which 661 were also detected by IR analysis. There were 805 false positives, but allowance for drainage was not made for these samples and the overall comparison shown in Table 1 is probably more representative of the actual false positive rate (about 19%). There was a total of 81 false negatives in this group of which 66 were the result of nonfermentative organisms. Fifteen (15) of the false negatives showed absence of vacuum or positive pressure when tested prior to plating. Fortunately, the rate of occurrence of false negatives in which fermentation had occurred was vey low (0.4%). These false negatives were probably the result of insufficient time allowance for analysis. This is supported by the fact that no false negatives showing fermentation occurred during the controlled analysis shown in Table 2.

Identification of nonfermenters and spoilage organisms

The identification of those microorganisms responsible for false negative readings was considered important since any contamination not detected by IR analysis might be associated with off flavors and/or pressurized containers. From Table 3 and the previous discussion it can be seen that false negatives occurred in two groups. The largest group was made up of 66 samples which did not ferment the product. The largest proportion of this group included 51 samples and was represented by yeasts. Selected colonies were identified according to procedures explained previously and the following yeasts were identified; *Candida parapsilosis, Rhodotorula rubra,* and *Trichosporon pullulans.* From this group, *C. parapsilosis* and *R. rubra* were the most common isolates from nonfermented samples. Thirteen samples were found to contain Gram negative nonfermentative rods which were identified by methods discussed earlier as *Acinetobacter calcoaceticus* var. *anitratus.* Two samples in which fermentation did not occur were found to contain *Lactobacillus sp.* It is possible that very small amounts of CO_2 were produced since it has been shown (Hatcher et al., 1977) that some lactobacilli produce such small amounts of CO_2 that detection is difficult even with more sensitive radiometric techniques. It is interesting to note that yeasts were found in all types of product whereas *A. calcoaceticus* was not found in grape drink. *Lactobacillus sp.* were only present in orange and grapefruit juices.

The second group of false negatives evaluated in Table 3 was made up of 15 samples which were shown to have zero vacuum or positive pressure. Predominating this group were yeasts identified as *Saccharomyces cerevisiae.* These organisms were also the predominant organism found in true positive samples (samples registering greater than or equal to 45% of scale on the IR analyzer and showing microorganisms present when plated in OSA). Two samples from this group were found to contain *Lactobacillus sp.* The probable reasons for false negatives of this type have been discussed and involve insufficient time of analysis.

In order to determine if off odors or flavors existed due to the presence of nonfermentative organisms' samples containing these organisms were evaluated organoleptically with results indicating no significant flavor or odor problems. To reduce or eliminate the problem of fermented samples reaching the consumer, lots from which false negatives showing zero vacuum or positive pressure occurred were subjected to a second IR analysis. The results of this second IR analysis utilizing proper techniques revealed no false negatives with zero vacuum or positive pressure.

Sensitivity of IR CO_2 analysis

The application of IR analysis for CO_2 detection as

Table 1—Comparison of expected loss versus actual loss by product type

Type Product	% Loss expected[a]	% Loss actual[b]
Orange juice	2.0%	3.4%
Grapefruit juice	6.1%	6.7%
Grape drink	9.1%	4.4%
Apple juice	7.5%	9.6%
All products[c]	5.3%	6.3%

[a] Based on results of plating random samples from lots suspected of being contaminated. See text for further discussion.
[b] Product which when analyzed resulted in greater than or equal to 45% of scale meter output.
[c] Weighted average.

Table 2—Comparison of infrared CO_2 analyzer with conventional plating methods

No. samples analyzed and plated	Negative samples		Positive samples	
	IR analyzer	Plating	IR analyzer	Plating
264	256[a]	251	8	13

[a] Includes 5 false negatives of which all samples had a vacuum and were found to contain nonfermentative organisms. These as well as other false negative results are evaluated in detail in Table 3.

Table 3—Evaluation of false negatives[a]

Samples with vacuum[b]		Samples with no vacuum[c]	
No.	Type Organism	No.	Type Organism
51	Yeast sp.[d]	13	Yeast sp.[d]
13	*Acinetobacter calcoaceticus* var. *anitratus*	2	*Lactobacillus sp.*
2	*Lactobacillus sp.*		
66 Total		15 Total	

[a] False negatives defined in text.
[b] Samples which when tested prior to the plating showed a vacuum within the range recorded at the time of production.
[c] Samples which when tested prior to plating showed zero vacuum or positive pressure.
[d] See text for species of yeast identified.

described in this study was primarily qualitative in nature. However, an estimate of the minimum detectable CO_2 concentration can be made since there were many instances in which IR analysis detected the presence of CO_2 in samples which still retained a vacuum but showed microbial contamination when plated in OSA. By determining the reduction in vacuum (difference between minimum original vacuum recorded by the USDA and the vacuum recorded just prior to plating) due to the generation of CO_2, it was estimated that the minimum detectable CO_2 concentration in the headspace was 4.2% under the conditions of this study. This figure was based on a sample which showed a vacuum of 7 inches of Hg at the time of production and 6 inches of Hg at the time of IR analysis and plating. It was felt that this level of sensitivity was more than adequate for the application.

SUMMARY

IN SUMMARY, an IR CO_2 analyzer was utilized successfully to inspect 100% of product suspected of microbial contamination. When proper procedures were followed, contaminated product was detected and removed for destruction while good product was recovered for subsequent shipment to consumers. This system is advantageous because it allows for a 100% nondestructive inspection, is comparable to conventional plating methods in determining the presence of microbial contamination, and can be automated to a high degree. It is probable that automated container handling would have resulted in fewer false positives due to tilting of bottles as well as elimination of false negatives containing CO_2 which were the result of insufficient time of analysis. Disadvantages include the requirement of an IR detectable microbial product, a container or package adaptable to an IR transmission or reflection type analysis, and a considerable cost for equipment. Any product contaminated by fermentative microorganisms and packaged in glass containers having a headspace of approximately 0.5 inch could be inspected by the methods described here. Justification for the cost of equipment would depend on the amount of contaminated product and the expected recovery rate. It is quite possible that future application of IR analysis could include monitoring food process streams for indicators of microbial contamination.

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A Method for the Analysis of ATP Metabolites in Beef Skeletal Muscle by HPLC

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ABSTRACT

A method to determine inosine, hypoxanthine, NAD, AMP, IMP, IDP, ADP, adenylosuccinic acid, ITP and ATP contents in postmortem muscle by HPLC has been developed. The method consists of an homogenization of the muscle in 0.5M $HClO_4$ or 10% TCA, extraction of the compound with 0.5M tri-n-octylamine/Freon 113 and single injection chromatography on a Whatman Partisil-10/25 SAX column.

INTRODUCTION

DURING STUDIES (Currie and Wolfe, 1980; Nuss and Wolfe, 1981) of postmortem aging of muscle, a method to analyze the levels of ATP and its degradation products was required. The method of Khan and Frey (1971) using the UV absorbance ratios at 258 nm and 250 nm (recently reappraised by Attrey et al. (1981) measures the conversion of adenosine to inosine containing components. It does not reveal the changes that IMP is undergoing during postmortem aging nor the exact levels of ATP, AMP and ADP present in the muscle, hence was unsatisfactory. Recently several HPLC systems have been developed for the analysis of nucleotides. Riss et al. (1980) and vanHaverbeke and Brown (1978) have used strong anion exchange columns to identify the nucleotide contents of liver extracts and erythrocytes thus demonstrating the advantages of HPLC when applied to biological samples. With respect to the muscle system a procedure for the analysis of nucleotides from cardiac tissue (Jeungling and Kammermeier, 1980) and rat tissue (Shaw et al., 1979) using a combination of ion exchange and reverse-phase chromatography has been reported.

The present paper reports a rapid accurate single injection procedure for inosine, hypoxanthine, NAD (nicotinamide adenine dinucleotide), AMP (adenosine 5'-monophosphate), IMP (inosine 5'-monophosphate), IDP (inosine 5'-diphosphate), ADP (adenosine 5'-diphosphate) ITP (inosine 5'-triphosphate) and ATP (adenosine 5'-triphosphate). In developing this procedure we restricted ourselves to a single strong anion exchange column with the objective of separating those acid soluble ATP metabolites in meat reported by Bendall and Davey (1957) and Davey (1961). We have also examined several extraction procedures and include a discussion of disadvantages encountered in this report.

MATERIALS & METHODS

Chromatographic equipment

The high performance liquid chromatographic system (HPLC) used in this study was a Beckman Gradient Liquid Chromatograph Model 332 equipped with a Tracor Model 970 variable wavelength detector and a Hewlett Packard Model 3388A integrator. The HPLC was fitted with a Whatman Solvecon precolumn which is essential to prevent dissolution of the silica in the analytical column. The strong anion exchange column was a Whatman Partisil-10/25 SAX protected by a Whatman AS Pellionex SAX guard column.

The analytical column was not suitable for immediate use in that UV impurities were eluted during the upper part of the gradient. The regeneration procedure outlined by Whatman in their booklet on column care resulted in a stable baseline.

Solvent preparation

Aqueous solvents were prepared from KH_2PO_4 using water from a Millipore Milli Q system. Buffer A was 0.015M KH_2PO_4, 0.001M KCl at pH 4.1. The KCl concentration was obtained by adding 1 mL of 1N HCl to 1L of the KH_2PO_4 buffer and adjusting the pH to 4.1 with KOH. Buffer B was a 0.5M KH_2PO_4 solution at pH 4.5. All solutions were filtered through a 0.45 μm membrane filter (Millipore) before using. Problems with UV absorbing contaminants in the buffers which eluted during the upper part of the gradient were overcome by injecting 100 μL of 0.1M EDTA at the end of the day when baseline instabilities were apparent and then eluting with Buffer A at 0.7 mL/min overnight. The gradient was run the next morning after which additional samples could be run satisfactorily. We have found the small amount of KCl in Buffer A aids in the elution of the UV impurities.

Chromatographic procedure

The HPLC was programmed to elute the compounds of interest and be ready for reinjection within 60 min. The flow rate was 1.5 mL/min. The program consisted of Buffer A for 7 min, a gradient from 0–100% Buffer B in 10 min, 100% Buffer B for 18 min and a gradient from 100% to 0% Buffer B in 5 min. After 20 min of Buffer A the next sample (20μL) was injected.

Preparation of samples for injection

In preparing the standard solutions 10–15 mg of the appropriate standard was dissolved in water and made up to a 50 mL volume. Appropriate dilutions of these standards were made to provide solutions giving values of 1–15 nmoles/injection. Any effect that $HClO_4$ (perchloric acid) or TCA (trichloroacetic acid) may have on the standard components were accounted for by diluting the standard solutions with an appropriately concentrated acid to a final acid concentration of 0.5M $HClO_4$ or 10% TCA. The effect of extracting the nucleotide solutions with 0.5M tri-n-octylamine in Freon 113 (Riss et al., 1980) was examined using these standard solutions.

The meat samples (3–4g) were collected at various times postmortem, wrapped in tin foil, labelled with the Scotch C-31 labelling device, frozen and stored in liquid N_2 until the time of extraction of the nucleotides. ATP and its degradation products were extracted by either pulverizing the meat sample to a powder with a stainless steel mortar and pestle cooled in dry ice or by shaving small pieces off the sample and weighing 1.0–1.5g into the 50 mL container of the Sorvall omnimixer. The nucleotides were extracted by adding 10 mL of cold (2°C) 0.5M $HClO_4$ or 10% TCA to the sample and then homogenizing for 30 sec with the homogenizer container immersed in an ice bath. The contents of the homogenizer was filtered to remove the precipitated proteins.

Treatment of acid extracts

The first procedure followed was that of Davey and Gilbert (1976) using 1N NaOH to readjust the 0.5M $HClO_4$ extract to pH 7.0. The second procedure attempted was that of Chen et al. (1977) where the perchloric acid extract is neutralized with KOH and the precipitated perchlorate salt removed by filtration. The third procedure was the dilution of a more concentrated 10% TCA extract (1:1) and injection with the hope the TCA peak would not interfere. The final procedure adopted was the procedure of Khym

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(1975) as discussed by Chen et al. (1977) and Riss et al. (1980) in which a 0.5M tri-n-octylamine/Freon 113 solution (A) was used to extract the acids from the nucleotides. Equal volumes of solution A and the acid extracted nucleotides were mixed in a screw cap test tube and the contents centrifuged to promote layer separation. The top aqueous phase contained the nucleotides. No pH adjustments were required since the pH values of the extracts were consistently between 4 and 6 (Chen et al., 1977). With perchloric acid extracts, three layers were visible; the top layer was the aqueous layer, the middle layer consisted of a perchlorate-tri-n-octylamine complex and the bottom layer was Freon 113.

Calculations

The concentration of the ATP degradation products were calculated from the integrated peak areas of the 248 nm absorbing materials eluting from the column. The factors used to convert the areas into concentrations was the regression coefficient obtained from the calibration curves of the standard solutions. All calibration curves were linear in the 1–15 nmole/injection (20 μL) range. Although most of the work reported in this paper was performed with the detector at 248 nm the same linearity of response was observed at 258 nm with a corresponding difference in the factors used to convert the integrated peak areas into concentrations. The concentration of ATP and its degradation products in meat were expressed as mole/g frozen wet weight.

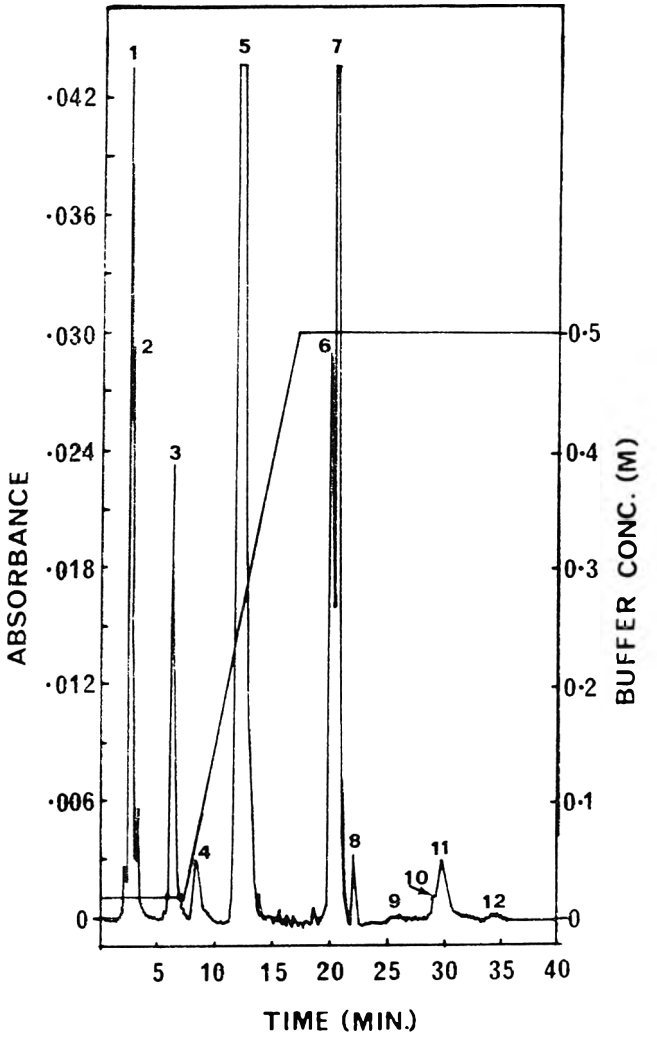

Fig. 1—Chromatogram obtained from a 3.5 hr postmortem muscle extract. The components identified are (1) inosine; (2) hypoxanthine; (3) NAD; (4) AMP; (5) IMP; (6) IDP; (7) ADP; (8) adenylosuccinic acid (9); ? (10); ITP (11); ATP (12) ?.

RESULTS & DISCUSSION

THE ELUTION PROFILE of a 3.5-hr postmortem meat sample is presented in Fig. 1. The muscle entered rigor rapidly with ATP levels at 0.1 μmole/g within 4 hr. This explains the relatively high levels of inosine, hypoxanthine and IMP at this time postmortem. The chromatographic procedure reported in this paper has allowed us to identify and quantitate inosine, hypoxanthine, NAD, AMP, IMP, IDP, ADP, adenylosuccinic acid, and ATP. Inosine and hypoxanthine are not completely resolved but reproducible results are obtained in repetitive runs (Table 1, 0.6 hr postmortem meat sample). At chart speeds of 0.5 cm/min the resolution of these very sharp peaks is more clearly observed. IDP and ADP do not show baseline separations; however, the ADP peak is very sharp and thus is separated sufficiently from IDP even at relatively high ADP concentrations so that quantitation of IDP is possible. The column must be clean of impurities from the phosphate buffers to prevent interference of these components eluted on the gradient with IDP. The chromatogram in Fig. 1 is unusual in that the IDP levels are greater than we usually have observed in postmortem muscle. Although the separation between ITP and ATP is greater (time scale) than IDP and ADP; the ATP peak is broad and thus ITP is not as well resolved. However, the small concentrations of ITP in meat are observed as a shoulder or a partially resolved peak. Two small peaks observed in early postmortem muscle which are lost during postmortem aging (approximately 26 and 34.5 min) have not as yet been identified. The levels of these two components have nearly been depleted in Fig. 1 but their locations are identified on the chromatogram.

In choosing the buffer systems used for the chromatoggraphy of ATP and its degradation products, several factors were considered. The first was to avoid a high concentration of halides which can corrode the stainless steel parts of the pumps. For this reason the procedure of Riss et al. (1980) was avoided. The small amount of KCl in buffer A was retained to aid in the elution of UV impurities in the phosphate buffers. The problem of UV impurities in phosphate buffers when using gradient elution with a pellicular anion exchange column was reported by Shmukler (1970). The method suggested for purifying the phosphate buffer involved the passage of the buffer over Dowex 1-X8 columns. We found purification of the Dowex 1-X8 column having an eluant $A_{254} < 0.005$ impossible and for this reason abandoned the procedure. An amberlite XAD-2 column was prepared in which the eluant A_{254} was less than 0.005. We passed a 0.5M KH_2PO_4 (HPLC grade) buffer through this column and achieved a 70% reduction in UV absorbance. However, the use of this buffer did not overcome the problem of irregularities on the baseline

Table 1—Reproducibility of extraction and chromatography of 1 0.6 hr postmortem meat sample

Compound	Concentration (μmole/g muscle wet wt) 1.07g Extract	1.61g Extract	Repeat 1.61g Extract
Inosine	0.08	0.08	0.07
Hypoxanthine	0.07	0.08	0.07
Nicotinamide adenine dinucleotide	0.59	0.60	0.61
Adenosine 5'-monophosphate	Trace	Trace	Trace
Inosine 5'-monophosphate	0.76	0.80	0.77
Adenosine 5'-diphosphate	0.71	0.76	0.75
Adenylosuccinic acid	Trace	Trace	Trace
Adenosine 5'-triphosphate	4.10	4.34	4.24

when the gradient reached about 0.4M KH_2PO_4 after the column had been used about 2 wk. We have found after the first regeneration of the new column (according to the Whatman procedure) the UV impurities are removed by simply injecting 100L of 0.1M EDTA and running buffer A through the column at 0.7 mL/min overnight. When we used a 0.015M phosphate buffer without the KCl little improvement in column performance occurred. With the KCl we have had excellent results in recovering column performance and have developed the procedure with KCl retained in buffer A.

Another problem faced was the separation of NAD, AMP and IMP. The major factor affecting their separation is the pH of buffer A. If the pH is 4.5 AMP and IMP overlap, a reduction in the pH to 3.7 results in the overlap of NAD and AMP. A pH of 4.1 allows all three components to be well resolved under the conditions chromatographed. It can be seen from the standard deviations of the retention times (Table 2) for NAD and AMP of samples chromatographed at approximately 1 hr intervals, the positions of these two peaks move about considerably. Clearly the column is not equilibrated. In fact the retention times for NAD and AMP are approximately 6.95 and 9.5 minutes for a well equilibrated column. Resolution of NAD, AMP and IMP can be obtained with repeat injections of less than 1 hr (although the retention times of NAD and AMP in particular are much shorter) but we have chosen the 1-hr interval as a convenient time interval for column equilibration, sample preparation, and reinjection.

When ATP measurements (enzymatic) were made previously in this laboratory the extraction of the nucleotides were according to the procedure of Lester and Gilbert (1976). The injection of a neutralized perchloric acid extract is not suitable for HPLC analysis on a SAX column since IMP is resolved into two peaks (a sharp initial peak and a broad second peak). By varying the pH of a standard IMP perchlorate solution different ratios of two and sometimes even three peaks of IMP were observed. No problems with neutralized solutions of the other extracted components were apparent.

The removal of the interfering perchlorate ion was attempted by the precipitation of the perchlorate ion using KOH instead of NaOH to adjust the pH to neutrality thus forming the insoluble potassium perchlorate salt (Chen et al., 1977). This procedure did not seem to remove all of the perchlorate ion since the problem of a dual peak for IMP was not overcome.

When a standard solution of IMP in 10% TCA was chromatographed the IMP was a sharp single peak. A diluted 10% TCA extract of meat was injected but the broad TCA peak interfered with the quantitation of AMP. However, the IMP peak was sharp and well resolved suggesting that the perchlorate ion was the reason for the double IMP peak.

We have found the 0.5M tri-n-octylamine/Freon 113 solution removes either TCA or perchloric acid from the nucleotides so that no problems with the symmetry of the peaks have been encountered. Extraction of the acids from standard solutions have shown excellent recovery with values in the order of that reported by Chen et al. (1977). In assessing the recovery of ATP and its degradation products from meat a comparison of four extract preparations were made. Injections of a 0.5M $HClO_4$ extract without acid extraction, a 0.5M $HClO_4$ extract with acid extraction (0.5M tri-n-octylamine/Freon 113), a 10% TCA extract without acid extraction and a 10% TCA extract with acid extraction (0.5M tri-n-octylamine/Freon 113). With the exception of AMP in the 10% TCA extract without acid extraction all the concentrations of ATP and its degradation products in the original meat sample (μgmole/g) could be compared. The areas of the two IMP peaks from the $HClO_4$ extract was totalled to obtain the concentration of IMP in this extract. All the values obtained were within the standard deviation found for repeated perchloric acid extractions of a meat sample followed by acid extraction and quantitation of ATP and its degradation products. In other words, errors produced from the lack of uniformity of the meat sample (e.g. fat, collagen levels), integration errors, treatment of the extracts (e.g. length of time in the liquid extract before injection) were all greater than differences contributed by the method of extraction.

Table 2—Retention times of ATP degradation products

Compound	Retention time[a] (Min)
Inosine	2.22 ± 0.05
Hypoxanthine	2.43 ± 0.09
Nicotinamide adenine dinucleotide	6.30 ± 0.33
Adenosine 5'-monophosphate	8.10 ± 0.46
Inosine 5'-monophosphate	12.17 ± 0.12
Inosine 5'-diphosphate	20.64 ± 0.23
Adenosine 5'-diphosphate	21.02 ± 0.20
Adenylosuccinic acid	22.60 ± 0.23
Adenosine 5'-triphosphate	30.38 ± 0.15
Inosine 5'-triphosphate	29.44[b]

[a] The mean retention time of 10 chromatograms of muscle extracted at different times postmortem. Values are the means of the retention times ± standard deviation.
[b] The retention time of a prepared standard.

The 248 nm wavelength chosen for this study was to increase the sensitivity to the inosine containing peaks. The adenine containing compounds have comparable extinctions at this wavelength. If increased sensitivity to the adenine containing nucleotides were desired, detection at 258 nm could be used since the response is linear at this wavelength.

Injections of standard NAD (oxidized) and NADH (reduced) showed that both forms have identical retention times. An NAD/NADH ratio would be valuable in determining the oxidative state of the cell. Unfortunately when a scan of NADH in water, 10% TCA and 0.5M $HClO_4$ were compared the acids were observed to oxidize the NADH to NAD. If a method to extract the nucleotides without oxidizing NADH were possible, a shift in the detector wave-length to 340 nm would allow an easy measure of the NADH concentration of this peak.

In conclusion we have reported a method for the analysis of ATP and its degradation products from beef skeletal muscle by HPLC. The advantage of this procedure is that it quantitates all of the adenine and inosine nucleotides with a single manipulation, something which cannot be done by absorbance ratios and enzymatic assays. It will provide a convenient method of examining each of the products of ATP degradation during postmortem aging with respect to its rate of degradation since the biochemical pathways are understood. In addition the affinity of the nucleotides for the acid precipitated components of muscle may be assessed with respect to postmortem aging. We hope that this rapid and accurate method will be of value to the meat research workers who are interested in nucleotides and metabolites in muscle.

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Determination of D-Amino Acids in Some Processed Foods and Effect of Racemization on *In Vitro* Digestibility of Casein

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ABSTRACT

Several processed foods and controls were analyzed by gas chromatography for the presence of D-amino acids. The concentration of each of the D-amino acids detected in the processed foods was approximately the same as that in the controls except that D-aspartic acid was significantly higher in dark toast surface and extruded soy flour. *In vitro* digestibility of casein which was alkali-treated to cause racemization, but without lysinoalanine (LAL) formation, was essentially the same as that of racemized casein containing LAL, but was lower than that of nonracemized control casein.

INTRODUCTION

FOOD PROTEINS are generally treated with heat and in some cases with alkali before consumption. Hayase et al. (1975) found that heating casein or lysozyme alone at high temperatures around 230–250°C for 20 min caused a considerable degree of racemization of amino acids. They also found a greater degree of racemization when glucose or methyl linoleate was present with casein during heat treatment (Hayase et al., 1979). Alkali treatments at moderate temperatures of less than 80°C are known to cause racemization of amino acids in food proteins (Hayashi and Kameda, 1980; Masters and Friedman, 1979; Provansal et al., 1975; Pollock and Frommhasen, 1968).

Along with racemization, alkali treatment also causes the formation of a number of cross-linked amino acids (Horn et al., 1941; Bohak, 1964; Ziegler et al., 1967; Asquith et al., 1969; Asquith and Otterburn, 1976; Friedman, 1978). Both phenomena have been suggested as the causes of decreases in *in vitro* and *in vivo* digestibility of alkali-treated proteins (de Groot and Slump, 1969; Friedman et al., 1981). However, the contribution of each phenomenon on digestibility has not been evaluated. This study was carried out, first, to determine the possible occurrence of D-amino acids in a number of processed foods and second, to investigate the effect of racemization alone on *in vitro* digestibility of a food protein.

EXPERIMENTAL

Food samples

A total of 12 foods were analyzed for D-amino acids. Six of these were processed or cooked foods and six were controls. The six processed foods were: peanut butter, taco shells, dark toast, extruded soy flour, fried hamburger and irradiated chicken. These foods were selected because they were exposed to either high temperatures or alkali during processing. The first two were commercial products purchased at a local supermarket; the next three were products prepared in our laboratory. The irradiated chicken was an experimental sample supplied by the U.S. Army Natick Research & Development Laboratory (Natick, MA).

Dark toast was prepared from white bread toasted for 1 min and 45 sec. Only the scraped surface which was in closest contact to the heat source was taken for analysis. Extruded soy flour was prepared according to Palkert and Fagerson (1980). Fried hamburger was prepared from raw ground beef fried for 4 min on each side in a pan having a surface temperature of about 250°C. Only the hamburger surface was taken for analysis. The processed chicken had been prepared by irradiation in the frozen state at a dosage of 4–5 megarads.

The six control foods were raw peanuts, corn meal, white bread, defatted soy flour, raw ground beef, and raw chicken. These were purchased locally. The defatted soy flour (200 mesh, Protein Dispersibility Index of 70) was obtained from Cargill Inc. (Minneapolis, MN).

Chemicals and reagents

Authentic D-, and L-amino acids were purchased from Sigma Chemical Co., (St Louis, MO), P.L. Biochemicals (Milwaukee, WI) and U.S. Biochemical Co. (Cleveland, OH). Isopropanol in 3N HCl was prepared according to Bengtsson and Odham (1979). Pentafluoropropionic anhydride (PFPA) was purchased from Pierce Co., (Rockford, IL) and from Regis Chemical Co. (Morton Grove, IL). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) was purchased from Eastman Kodak Co. (Rochester, NY). Citraconic anhydride, chymotrypsin type II, trypsin type IX, peptidase type III, and protease type VI were all purchased from Sigma Chemical Co. Lysinoalanine (LAL) was purchased from Miles Lab. (Elkhart, IN). Other reagents were all of analytical grade.

D-amino acid analysis

Duplicate samples of each food containing approximately 75 mg of protein were hydrolyzed in 300 ml of 6N HCl with a stream of bubbling nitrogen at 110°C for 24 hr. Each hydrolyzate was filtered through a sintered glass funnel, rotary evaporated under partial vacuum at 80°C until approximately 50 ml remained and then at 40°C until dryness. The dried hydrolyzate was dissolved in 50 ml of sodium citrate buffer pH 2.20. Ten ml were taken and concentrated to about 1 ml in a rotary evaporator at 45°C. Five ml of 0.1N HCl and 0.50 ml of the internal standard, gamma-amino-n-butyric acid (GABA; 0.1975 mg/ml) were then added. The sample was passed through a cation exchange column as described by Zumwalt et al. (1970). The amino acids retained on the column were eluted with 7N ammonium hydroxide. The eluted sample was rotary evaporated at 45°C until dryness and was derivatized with 3.0 ml of the acidic isopropanol at 100°C, while stirring, for 1 hr. The solvent was evaporated with a gentle stream of nitrogen at room temperature and 1g of liquid PFPA and 3.0 ml of methylene chloride were added. After 1 hr at room temperature, excess solvents were evaporated with nittogen and 500 μliters of chloroform were used to dissolve the derivatized sample. Samples of 0.1–1.0 μliter were injected into a gas chromatograph (Varian 3700) with a split ratio of 20:1. The amino acids were separated in a glass capillary column, Chirasil-Val (Applied Science, State College, PA), 25m by 0.25 mm. The flow rate of the helium carrier gas was 1 ml/min. The GC oven was generally held at 90°C for 4 min then programmed at 4°C/min to 200°C. The injector and the flame ionization detector temperatures were both at 250°C. The identification of a D- or L-amino acid was done by matching its retention time to that of the derivatized authentic D- or L-amino acid. Peak areas were integrated by a Spectra Physics data processor (SP 4000). The percentage of a D-amino acid were calculated from its peak area divided by the sum of peak areas of the D- and L- amino acid, and the result multiplied by 100.

Preparation of racemized protein

The protein used in this part of the study was vitamin-free casein (Fisher Co., Medford, MA). A 2.0% casein suspension in distilled water containing 0.02% sodium azide was prepared and adjusted to pH 8.0 ± 0.2 with NaOH. The casein was solubilized by heating in a water bath at 80°C, while stirring, for 30 min. This stock casein

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solution was used for preparing two different samples of racemized casein and two different controls. Each was prepared in duplicate.

Sample I was casein citraconylated to protect the epsilon-amino groups of lysine residues, alkali-treated and then decitraconylated. It was prepared by adding five, 60-μliter portions of citraconic anhydride to 20 ml of the stock casein solution at room temperature while stirring. The ratio of the amount of anhydride to the number of lysine residues was about 10:1. The pH of the solution was maintained at 8.0 ± 0.5 with 1.0N NaOH after each addition and the reaction was judged complete when there was no further change in pH. The sample was then dialyzed (Spectrapor membrane tubing having a molecular weight cutoff of approximately 3500, Sprectrum Medical Industries, Inc., Los Angeles, CA) against distilled water pH 8 for 12 hr. After dialysis, sufficient NaOH was added to bring the solution to 0.1N with a resulting pH of 12.5. The concentration of casein at this point was 4.5 mg/ml. The sample was held in a water bath at 65°C for 3 hr. After the alkali treatment, the sample was adjusted to pH 3.8 with HCl and held at 37°C for 3 hr for decitraconylation (Brinegar and Kinsella, 1980). The sample was resolubilized by adjusting the pH to 8.0 with NaOH and then dialyzed against distilled water for 11 hr. The extent of citraconylation and decitraconylation was measured by the determination of amino groups using procedure 2 of Fields' TNBS methods (Fields, 1971). The assay components were 1.1 mg of 0.1M sodium borate in 0.1N NaOH, 0.30 ml of sample (diluted to 1.0 mg/ml), 20 μliters of 0.1M sodium sulfite and 20 μliters of 1.1M TNBS. Protein was determined by the Biuret method (Gornall et al., 1949).

The second sample, sample II, was prepared from the stock casein solution adjusted to the same concentration as sample I, and then alkali-treated as described above, but with prior citraconylation. After the alkali treatment, it was adjusted to pH 8.0 with HCl and dialyzed against distilled water for 11 hr.

Samples I and II were analyzed for D-amino acids and lysinoalanine (LAL) gas chromatographically. The identification of LAL peaks was done by matching their retention times with those of the derivatized authentic LAL. Both samples were also subjected to an *in vitro* enzyme digestion method as described below. A summary of steps used for preparing samples I and II is shown in Fig. 1.

Two controls were also prepared. Control A was prepared in the same way as sample I was but without the alkali treatment step. Control B was the stock casein solution itself. Both controls were analyzed for D-amino acids and were also subject to the *in vitro* digestion.

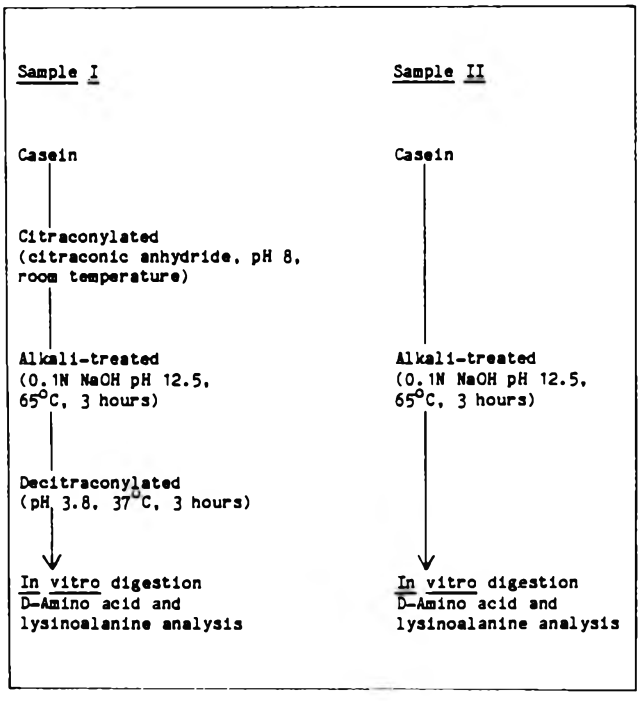

Fig. 1—Summary of steps used in preparation of samples I and II.

In vitro digestion

The *in vitro* digestion was performed according to the multienzyme method of Satterlee (1979) in which sodium caseinate was used as a reference for obtaining proper enzyme activities and the pH after 20 min of reaction was recorded. Results from this method have been shown to correlate well with the apparent digestibility of many foods as determined by rat studies (U. Nebraska Food Protein Research Group, 1979).

RESULTS & DISCUSSION

D-amino acid analysis of processed and control foods

A representative gas chromatogram of 15 derivatized standard D- and L- acids and the internal standard (GABA) is shown in Fig. 2. The D-peak of an amino acid always

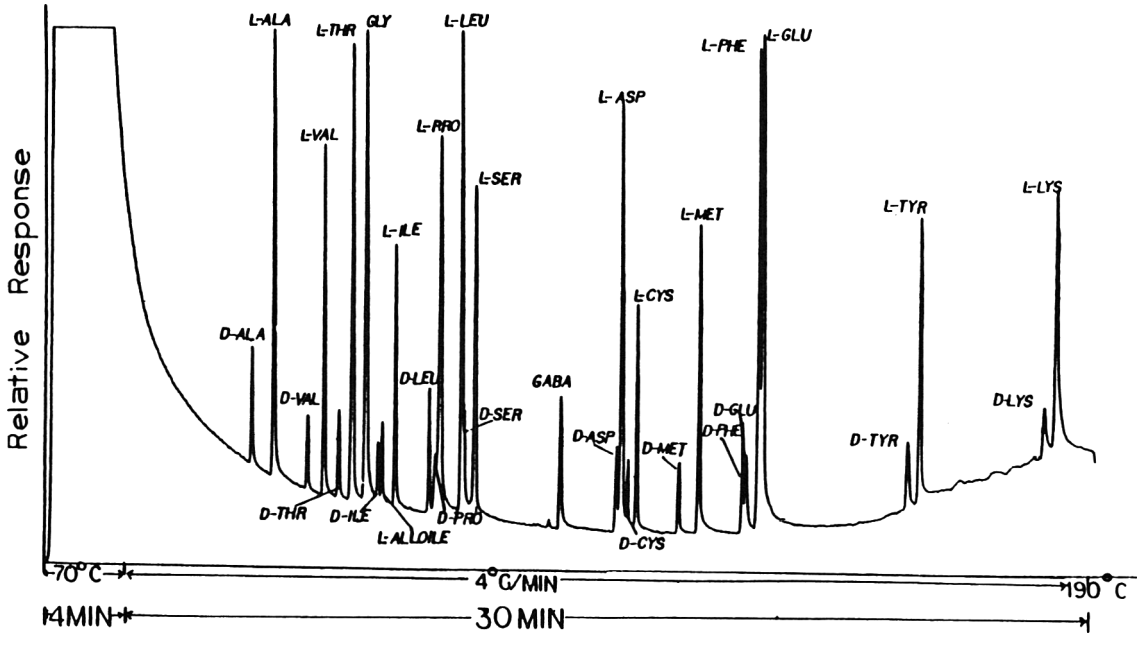

Fig. 2—Typical gas chromatogram of 15 derivatized standard D- and L-amino acids and the internal standard (GABA).

emerges before the corresponding L-peak. L-amino acids may preferentially interact with the L-valine moiety of the stationary phase and so emerge later. Three other amino acids, tryptophan, arginine, and histidine could not be derivatized by the procedure described and so were not analyzed. Fig. 3 is a representative gas chromatogram of a processed food. Typically, eight D-amino acids were detected in either a control or processed food. These include alanine, valine, leucine, proline, aspartic acid, methionine, phenylalanine, and glutamic acid. The areas of the D-amino acid peaks are very small compared with those of the L-amino acids. Fig. 4 shows a representative gas chromatogram of a control food. The amounts of D-amino acids found in all processed and control foods are summarized in Table 1. These data show that both groups have approximately the same concentration of each of the D-amino acids, with the exceptions of aspartic acid. The amounts of D-aspartic acid in dark toast surface (10.5%) and in extruded soy flour (7.6%) were higher than those of their controls (5.6% and 4.4%, respectively).

In general, since the concentrations of D-amino acids in both groups were approximately the same, it cannot be concluded that the racemization of L-amino acids to D-amino acids in the processed foods was caused by the processing or cooking methods. Rather, sample preparation procedures (hydrolysis, evaporation etc.) to which all foods were subjected, were likely responsible for the small degree of racemization of amino acids in both groups. This was also confirmed when 14 free L-amino acids were subjected to the hydrolysis procedure (6N HCl, 110°C for 24 hr) as shown in Table 2. Other authors have also reported racemization of free L-amino acids or bovine albumin subjected to similar conditions (Manning, 1970; Bayer et al., 1980). The presence of D-aspartic acid in the dark toast surface in higher concentration than that in white bread indicated that relatively long toasting (e.g. 1 min 45 sec) could cause a small degree of racemization of aspartic acid. It should be pointed out that the portion of toast analyzed was the scraped surface which constituted approximately 10% of the total weight. Therefore, when taking the whole toast into account, the degree of racemization would be correspondingly smaller than that in the toast surface. The results also indicated that the extruding conditions used for making the extruded soy flour could casue a small degree of racemization of aspartic acid. Commercial processing methods of textured soy products probably vary from one

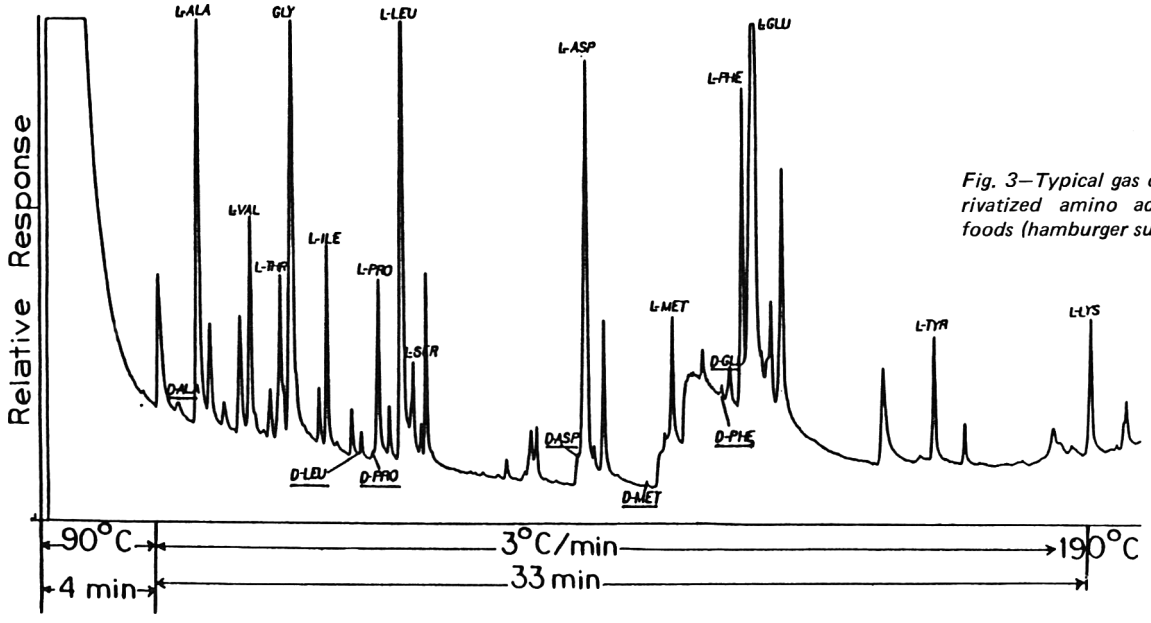

Fig. 3—Typical gas chromatogram of derivatized amino acids in a processed foods (hamburger surface).

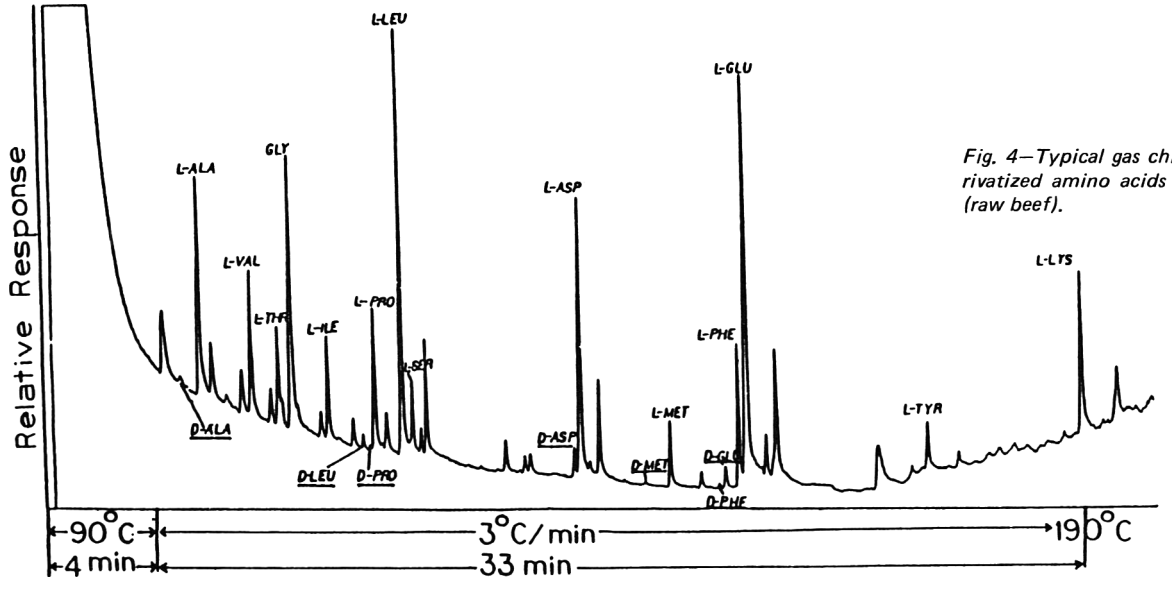

Fig. 4—Typical gas chromatogram of derivatized amino acids in a control food (raw beef).

Table 1—Relative amounts of D-amino acids in processed and control foods

Amino acid	Toast surface	White bread	Extruded soy flour	Soy flour	Peanut butter	Raw peanuts	Taco shells	Corn meal	Hamburger surface	Raw beef	Irradiated chicken	Raw chicken
	Percent D-amino acids[a]											
D-ala	2.8	2.4	2.7	2.5	3.1	2.8	3.2	2.3	2.8	3.2	1.6	1.9
D-val	1.1	0.9	0.8	1.0	1.2	1.2	1.5	1.3	1.5	1.6	1.2	0.8
D-leu	2.7	3.2	2.7	1.4	2.2	2.4	3.8	2.5	3.2	3.1	2.1	2.5
D-pro	2.1	0.9	1.6	2.3	1.5	1.7	1.0	1.5	1.8	2.0	1.7	1.5
D-asp	10.5	5.6	7.6	4.4	4.9	4.8	5.8	5.2	5.5	6.2	6.1	4.4
D-met	1.7	2.3	—[b]	—[c]	2.0	2.8	1.1	2.4	2.9	2.4	1.8	1.2
D-phe	2.4	2.3	2.4	2.8	2.7	2.9	—[d]	1.5	2.7	2.8	1.5	2.5
D-glu	3.2	2.8	3.9	3.1	3.7	4.3	3.8	3.4	2.4	4.9	1.9	2.7

[a] Each value is an average of duplicate samples:

$$\% \text{ D-amino acid} = \frac{\text{peak area of D-amino acid}}{\Sigma \text{ peak areas of D- and L-amino acid}} \times 100$$

[b] Peak not integrated by the data processor
[c] Peak could not be found, e.g. due to noise interference
[d] Peak appeared to consist of more than one compound

type of product to another. The lower the temperature (and pH) used for processing the textured soy products, the less likely that racemization will occur (Masters and Friedman, 1979).

Effect of racemization on in vitro digestibility of casein

Representative gas chromatograms of alkali-treated casein (samples I and II) are shown in Fig. 5 and 6. In addition to all the D-amino acids shown on the chromatograms, D-allo-threonine and D-serine were also detected when a smaller amount of sample was injected into the GC and the GC oven was temperature programmed at 3°C per minute as shown in Fig. 7.

The main difference between samples I and II was that LAL was not detected in sample I, but was detected in sample II at a concentration of 5.1 mg per 100 mg casein. This indicated that citraconylation of casein prior to the alkali treatment could prevent LAL formation. As would be expected no LAL was detected in either control A or control B since they were not alkali-treated. Table 3 shows the amounts of D-amino acids in Samples I, II and the controls. The concentrations of D-amino acids in both Samples I and II were approximately the same. They were markedly higher than those of the controls, indicating that the casein in Samples I and II was partially racemized.

Table 4 shows the results of the *in vitro* digestion. The digestibility of control A (casein citraconylated and then decitraconylated) was lower than that of control B probably because some of the lysine results were still citraconylated. This was confirmed by measurements of free amino groups with TNBS. (Table 5). Sample I had higher 20-min pH than those of controls A and B, and therefore lower digestibility. Even though the conditions used for decitraconylation of sample I and control A were the same, TNBS measurements showed complete decitraconylation of sample I (compared with sample II which was not citraconylated). The apparently complete decitraconylation of sample I might be for the reason that it was exposed to the alkali treatment which could affect the subsequent decitraconylation. Sample II had essentially the same digestibility as that of sample I. Both samples I and II showed a marked decrease in the *in vitro* digestibility when compared with the controls.

In addition to LAL and/or D-amino acids, one additional peak was detected in the alkali-treated samples (samples I and II) but not in either control A or control B. This peak appeared between L-tyrosine and D-lysine (Fig. 7). It was most likely L-ornithine since its retention time corresponded very closely to that of standard L-ornithine. Ziegler et al. (1967) reported that alkali treatment of proteins could cause partial degradation of arginine resulting in the formation of ornithine.

Table 2—Relative amounts of five D-amino acids formed after subjecting 14 free L-amino acids to 6N HCl at 110°C for 24 hr followed by cation exchange treatment[a]

Amino acid	% D-amino acids[b]
D-ala	1.4
D-leu	1.8
D-asp	4.8
D-phe	2.7
D-glu	3.4

[a] Nine other D-amino acids were not detected
[b]

$$\% \text{ D-amino acids} = \frac{\text{peak area of D-amino acid}}{\Sigma \text{ peak areas of D- and L-amino acid}} \times 100$$

Table 3—Relative amount of D-amino acids in Samples I, II and Controls[a]

Amino acid	Sample I	Sample II	Controls[c]
	Percent D-amino acids[b]		
D-allo-thr	42	44	0.0
D-ser	29	33	0.0
D-asp	24	24	5.6
D-met	21	23	2.3
D-phe	16	26	2.2
D-glu	14	14	2.9
D-ala	11	10	1.9
D-tyr	7.9	8.2	1.9
D-leu	5.3	5.1	2.7
D-lys	4.6	5.1	0.7
D-val	2.0	2.2	1.0

[a] Each value is an average of duplicate samples;
[b]

$$\% \text{ D-amino acid} = \frac{\text{peak area of D-amino acid}}{\Sigma \text{ peak areas of D- and L- amino acid}} \times 100$$

[c] Values are averages of Control A and Control B

There was also another change observed in the alkali-treated casein samples, i.e. the TNBS measurements increased from 0.898 to 1.117 (Table 5). This suggesed that some new amino groups were formed upon alkali treatment of casein, possibly from the hydrolysis of some peptide bonds. Results from SDS gel electrophoresis of casein samples supported this hypothesis since native casein bands were not detected in the alkali-treated samples. However, the Biuret test results (Table 6) show that the majority of protein (93%) in samples I and II was not lost through dialysis. Moreover, it is likely that only a few bonds were hydrolyzed by the alkali treatment or else the products

would have passed through the dialysis bag which had a cut-off of 3500 daltons. Hence the digestibility results of alkali-treated samples are still valid for comparison with those of the controls.

Since LAL was not formed in sample I, the results suggested that the D-amino acids were the major reason for the decrease in *in vitro* digestibility. In addition, LAL apparently did not contribute to the decrease in *in vitro* digestibility of the alkali-treated casein since sample I, which did not contain LAL, showed essentially the same digestibility result as that of sample II, which did.

CONCLUSIONS

IT APPEARS from this study that it is unlikely that conventional processing or cooking methods will cause extensive racemization of protein amino acids in foods. However, the results also suggested that extensive racemization due to a more severe treatment could cause a significant decrease in the *in vitro* digestibility of a food protein.

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Table 4—In vitro digestiblity of casein samples

Sample	20 min pH[a]	% Digestibility[b]
Sodium caseinate	6.36	91.4
Control B	6.34	91.8
Control A	6.54	87.3
Sample I	6.96	77.8
Sample II	6.89	79.4

[a] Each value in an average of at least two determinations
[b] Percent digestibility = 234.84 — 22.56(X), where X=20 min pH

Table 5—TNBS measurements of casein samples[a]

Sample	Abs. at 420 nm
Control B	0.898
Control A	0.672
Sample I	1.109
Sample II	1.117

[a] Each sample was diluted to 1.0 mg/ml; each value is an average of duplicate samples

Table 6—Biuret test of casein samples[a]

Sample	mg Protein/ml
Control B	2.96
Control A	3.04
Sample I	2.82
Sample II	2.76

[a] Each value is an average of duplicate samples

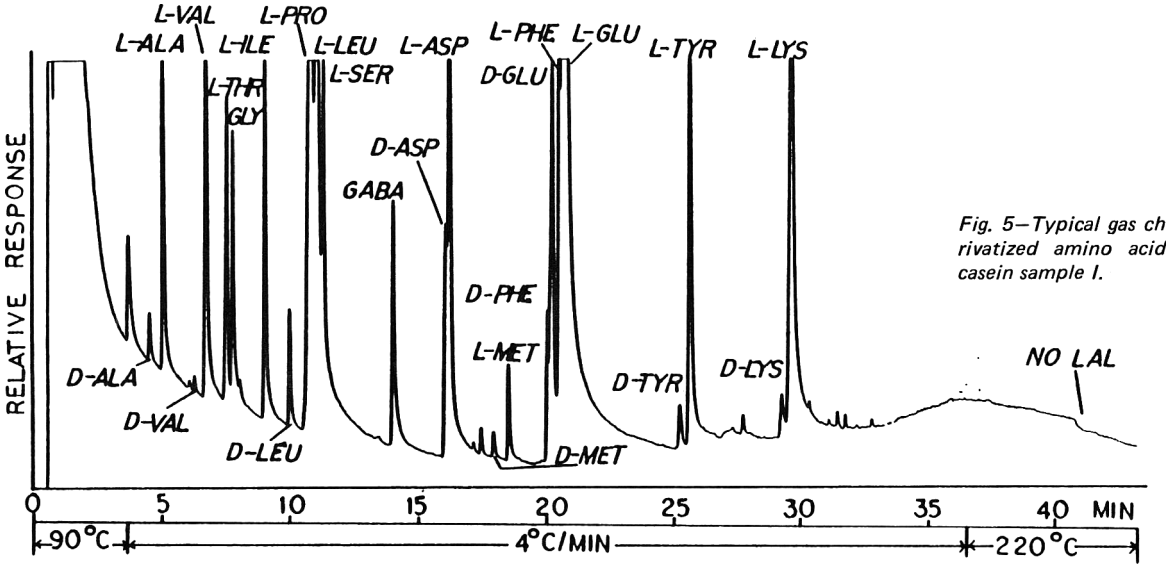

Fig. 5—Typical gas chromatogram of derivatized amino acids in alkali-treated casein sample I.

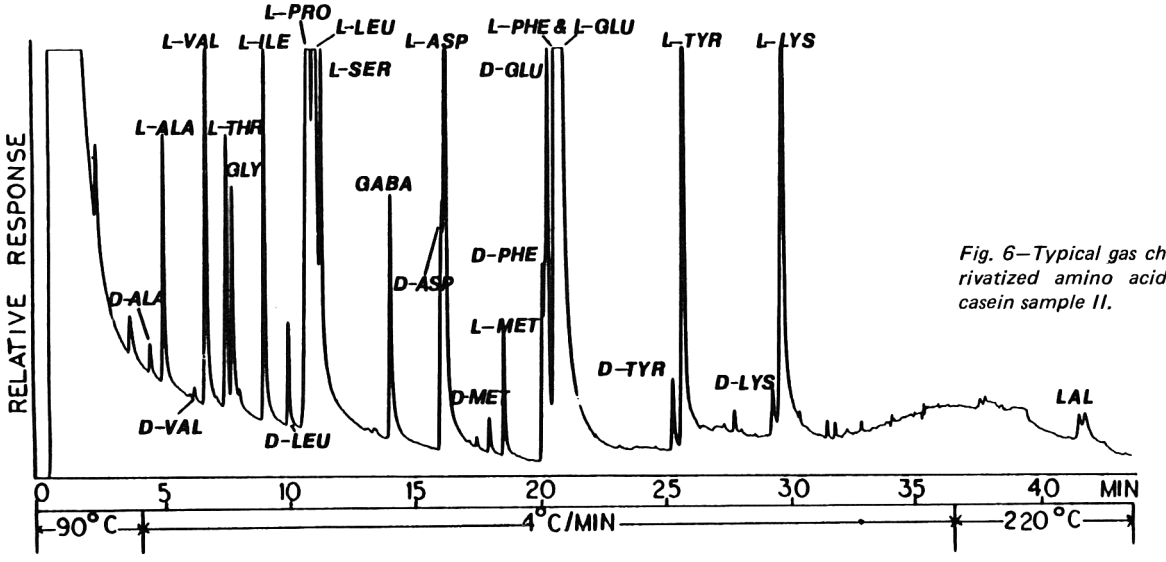

Fig. 6—Typical gas chromatogram of derivatized amino acids in alkali-treated casein sample II.

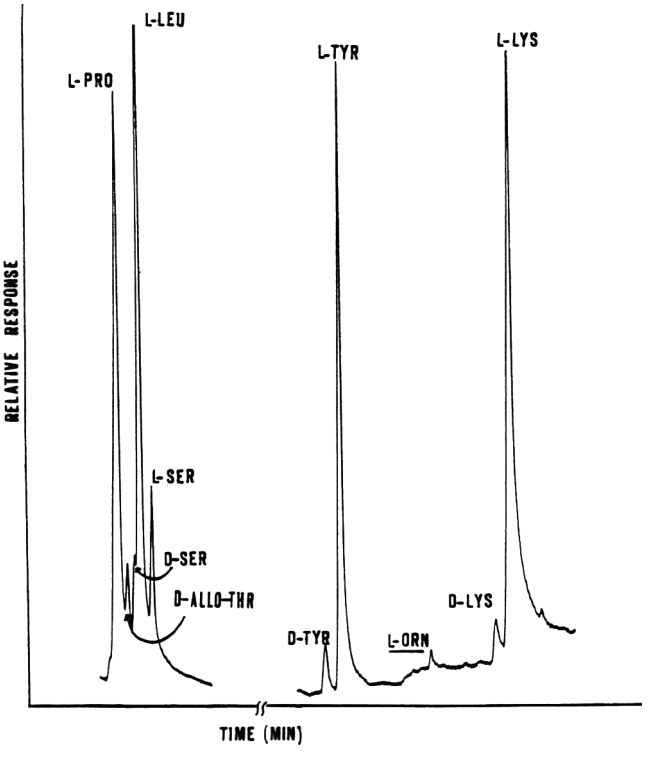

Fig. 7—Composite gas chromatogramof derivatized amino acids from two different runs of alkali-treated casein, showing the presence of D-serine, D-allo-threonine, and L-ornithine.

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A Model System for Curd Formation and Melting Properties of Calcium Caseinates

JOANNE C. HOKES, M. E. MANGINO, and P. M. T. HANSEN

ABSTRACT

A lipid-free model system was developed to aid in the study of melting and other functional properties of calcium caseinates in their application to imitation processed cheese. The model system was formed by the controlled addition of water to an alcohol suspension of the protein. At a critical water concentration, a curd was formed spontaneously. The recovered curd was melted and the area of the melt determined (melt area index). The amount of water added, rate of water addition and the source of caseinate affected the melt area index. A protein fraction, recovered from the supernatant, was rich in γ-casein and also contained minor amounts of β-, α_S- and κ-caseins.

INTRODUCTION

Substantial amounts of calcium and sodium caseinates are currently being used for imitation processed cheese (IPC). However, it seems likely that the demands for casein for this purpose may eventually exceed the available supply (Graf, 1979). The production of IPC depends upon the formation of a protein matrix from calcium caseinate which will undergo thermomelting similar to its processed cheese counterpart.

Replacement of caseinates by other proteins to alleviate this projected shortage has been unsatisfactory so far because the other proteins have not been able to meet the desired textural features and functional properties of IPC (Chang, 1976; Chen et al., 1979). The reasons for this are not clearly understood but relate in part to the poorer emulsification and curd forming characteristics of plant proteins.

The calcium salt of casein differs in physical properties from the sodium salt. Calcium caseinate is poorly soluble in water where it forms a cloudy dispersion. Sodium caseinate, on the other hand, is water soluble forming viscous solutions at low concentrations. A critical ratio of the monovalent and divalent ions is important to the functionality of natural cheese (Keller et al., 1974; Sirry and Shipe, 1958), and processed cheese (Vakaleris et al., 1962). If only calcium caseinate is used in an IPC formulation, a poorly emulsified and low melt product results due to its low solubility. Sodium caseinate is a better emulsifier but tends to produce irregular melting patterns, undesirable in cheese products. A melting index for processed cheese spread was noted to be a result of the ratio of the insoluble nitrogen to total casein nitrogen (Vakaleris et al., 1962). The greater the soluble nitrogen content, the higher the melt area index was.

In an attempt to develop a model that would predict the meltability of proteins in IPC we observed that when water was added to an alcoholic suspension of calcium caseinate, a curd was formed spontaneously. The curd exhibited thermoplastic deformation which was markedly altered by different solvent systems and the incorporation of electrolytes. The purpose of this research was to identify the variables affecting curd formation and resulting meltability.

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MATERIALS & METHODS

Samples and reagents

Spray-dried calcium caseinate manufactured in New Zealand was used. All solutions were prepared with reagent grade chemicals in double distilled, demineralized water.

Formation of a calcium caseinate curd

Ten ml of absolute ethanol were measured into a 100 ml beaker containing 1.5g calcium caseinate. The slurry was agitated with a 1-inch magnetic stirring bar while 15.5 ml of water or selected electrolyte solution was added at a constant rate over one minute at ambient temperature. A single agglomerated curd was formed.

Melt area

The curd was removed from the alcohol/water solution, shaped into a ball and placed in a petri dish for 30 minutes to permit syneresis. The weight of the curd after syneresis was recorded as the wet weight. The drained curd was then melted in a microwave oven (Sanyo EM8600) for 15 sec at full power (650 Watts). The area of the melted curd was measured with a planimeter.

Microscopy

Samples for light microscopy were taken during the early stage of curd formation, when less than 4 ml of water had been added. The curd was placed on a glass slide and viewed through a Leitz Dialux light microscope equipped with polarizing filters.

For scanning electron microscopy, cubes of curd were fixed in 2% gluteraldehyde (adjusted to pH 6.7 with 2, 4, 6-trimethylpyridine) for 1 hr at room temperature. Samples were washed, dehydrated in a graded ethanol series and air dried in a dessicator. The preparations were mounted with liquid graphite, and coated with carbon and gold prior to examination in a Cambridge S4-10 scanning electron microscope.

Electrophoresis

Polyacrylamide gel electrophoresis was performed according to Kiddy (1975) with modifications employed for its application to disc rather than slab gels.

RESULTS

Variables affecting curd formation

Calcium caseinate is insoluble in ethanol and only partially soluble in water. When water was added to an ethanol/caseinate dispersion, a curd was formed spontaneously (Fig. 1). The curd formation proceeded along two separate stages: (1) At early stages of hydration, flocculation similar to that in the renneting of milk occurred; (2) As hydration proceeded, the system separated into a single protein agglomerate and a supernatant.

The calcium caseinate curd was cohesive, similar to fresh cheese curd, but exhibited melting properties more similar to processed cheese (Fig. 1). Variations in melt area were observed in different lots of calcium caseinate suggesting that this parameter might prove useful as an index of functional performance. *—Continued on next page*

To assess the effect of variables on the characteristics of curd formation and on subsequent melt area, a factorial experiment was designed. Two types of caseinate with known functionality in IPC were chosen. These were labeled 'good' and 'bad', indicative of their overall performance in IPC but with emphasis on emulsion quality. The three components in the spontaneous curd formation, calcium caseinate, ethanol and water were varied over three, two and three levels respectively (Table 1). Melt areas were expressed as cm^2 per gram of caseinate. Analysis of variance of melt area (Table 1) showed the quality and amount of caseinate and the amount of water to be significant with 'good' caseinate producing the greater melt area. The significant interaction term between water and type of caseinate indicated that the relationship between these variables was the controlling factor in the spread of the melted curd. Within the levels chosen, the amount of ethanol was not significant. A standardized formula for curd formation was chosen as 15.5 ml water, 1.5g caseinate and 10 ml ethanol. Under these conditions and within a single lot of caseinate, the melt areas were reproducible within 0.655 cm^2 per gram of caseinate (1.4% of area).

The rate of water addition was found to be a factor in the resulting melt area. When 15.5 ml of water were added over time periods up to 2.5 min, both the wet weight of the curd and melt area increased with time of addition. The melt areas were spherical except when the rate of addition was less than 25 sec or when the caseinate was added directly to a mixture of ethanol and water (considered as 0 time). In these two cases the melt was directional, producing oval profiles.

Although a cohesive protein aggregate was formed, some protein remained suspended in the supernatant in an insoluble form. Electrophoretic patterns (Fig. 2) showed that the supernatant was rich in γ-casein and a number of minor milk proteins. No differences were noted in the amount or composition of the supernatant fraction between 'good' and 'bad' caseinates.

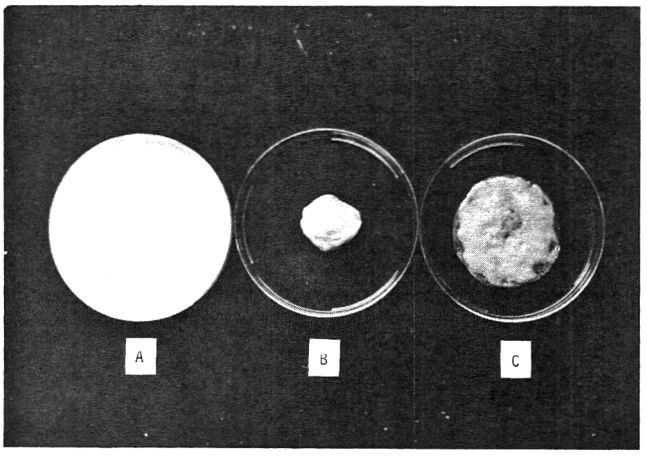

Fig. 1—Calcium caseinate in three different physical forms: (A) ethanol dispersion; (B) agglomerated curd; and (C) melted curd.

Table 1 — Analysis of variance for the melt area index[a] class level information

Class	Levels	Values
Ethanol (E)	3	9, 10, 11 (ml)
Water (W)	2	13.5, 15.5 (ml)
Casein (c)	3	1, .5, 1.5 (g)
Quality of Casein (Q)	2	'Bad', 'Good'

Source	df	ANOVA SS	F Value	Significance
E	2	0.216	1.63	NS
W	1	2.190	33.03	1%
C	2	1.554	11.71	1%
Q	1	13.868	209.02	1%
EXW	2	0.031	0.24	NS
EXC	4	0.188	0.71	NS
WXC	2	0.004	0.03	NS
EXQ	2	0.112	0.85	NS
CXQ	2	0.171	1.29	NS
WXQ	1	0.694	10.47	2%
EXWXC	4	0.429	1.62	NS
EXWXQ	2	0.245	1.85	NS
WXCXQ	2	0.278	2.10	NS

[a] The melt area index was normalized by dividing the area by grams of caseinate (cm^2/g).

Variables affecting thermoplastic properties

Substitution of other water miscible solvents for ethanol also resulted in curd formation, however, the thermoplastic nature of the curds was quite different (Table 2). In ethanol, the melted curd was opaque and nonadhesive upon cooling. Dioxane produced a more hydrated, adhesive curd which became clear and even more adhesive upon melting. Methanol produced a melt similar in appearance to ethanol, though the melt area was relatively smaller. This may partially be explained by the lesser degree of solvation as measured by the wet weight. Acetone produced a curd that was intermediate in melt area and the curd was somewhat

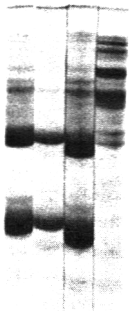

Fig. 2—Polyacrylamide gel electrophoresis: (a) calcium caseinate; (b) rennet treated calcium caseinate; (c) ethanol/ water curd proteins; and (d) ethanol/water supernatant proteins.

Table 2 — Comparison of curd formation in four solvent systems

Solvent	Dielectric constant 25°C	Curd wet wt (g)	Melt area (cm^2)	Supernatant protein (mg)
Methanol	32.6	3.51	7.19	7.4
Acetone	20.7	3.82	9.91	13.1
Ethanol	24.3	4.16	11.94	14.3
Dioxane	2.2	5.00	14.22	23.8

adhesive after melting and was blotched with opaque and clear areas.

As a solvent is diluted through the addition of water, the mixture increases in polarity, but the availability of the water will vary with the particular solvent characteristics. Therefore, polarity of the pure solvent may not properly reflect the polarity of the aqueous mixture. Curd wet weight, melt area and amount of supernatant protein were positively correlated. Water immiscible solvents caused a flocculation of the caseinate but final curd formation was prevented by phase separation. This same reaction was seen with vegetable oil, but by increasing the temperature, a curd very similar in appearance to that produced in ethanol was formed.

The effect of electrolytes on curd properties was studied using a cube-plus-octohedron rotatable design (Table 3). This design was chosen because of its suitability for sequential experimentation. The independent variables selected were phosphate, sodium and pH since these have been cited as influencing the functionality of processed cheese products. Monosodium and disodium phosphate solutions were prepared and combined to obtain the desired pH and phosphate levels. Sodium concentration was then adjusted through the addition of sodium chloride.

A matrix of correlation coefficients for both the independent and dependent variables is shown in Table 4. Melt areas were positively correlated to pH and phosphate levels whereas sodium effects were insignificant at the levels studied. Solvent uptake was also positively correlated to these two variables and in addition showed a strong positive correlation to melt area. There was no correlation between the amount of supernatant protein and the three independent variables. Loss of protein to the supernatant and the combined effects of phosphate and pH on solvation were the major contributors to melt area differences in this statistical design.

The melt area, supernatant protein and wet weight were all positively correlated with the level of sodium chloride addition up to 3.1 mmoles, when other electrolytes were not present (Fig. 3). At 7.75 mmoles a flocculation occurred but the material did not spontaneously coalesce as in the samples with less NaCl. Electrophoretic patterns again showed primarily γ-casein in the supernatant with some minor milk proteins. The level of β-casein in the patterns increased progressively with increasing levels of sodium chloride. The α_s-casein band was not visible until the salt concentration reached 3.1 mMole sodium chloride. The electrophoretic patterns suggest that agglomeration was inhibited at higher sodium chloride levels by the dissolution of α_s and/or β-casein from the curd resulting in poor cohesive properties. This sodium chloride effect was not apparent in the design which included phosphate and pH adjustment.

Table 3 — Ranges of variables in the cube-plus-octohedron rotatable design

Level of variables	Variable		
	pH	Phosphate (meq)	Sodium (meq)
+1.682	6.68	0.93	1.13
1	6	0.8	0.988
0	5	0.6	0.788
−1	4	0.4	0.588
−1.318	3.32	0.27	0.45

Microstructure

The structural transition from discrete particles to agglomerated curd is illustrated by the photomicrographs in Fig. 4 (polarized light microscopy) and Fig. 5 (scanning electron microscopy). Spray-dried calcium caseinate particles retained their integrity in alcohol showing slightly collapsed spheres with a hollow core that has been observed in fractured particles. Particle size analysis of dry powder and powder dispersed in alcohol yielded similar values with mean diameters of approximately 60μ. Upon the addition of water to the alcoholic slurry, agglomeration became evident while discrete particles were still present, although distorted (Fig. 4B and 5B). Under polarized light the curd

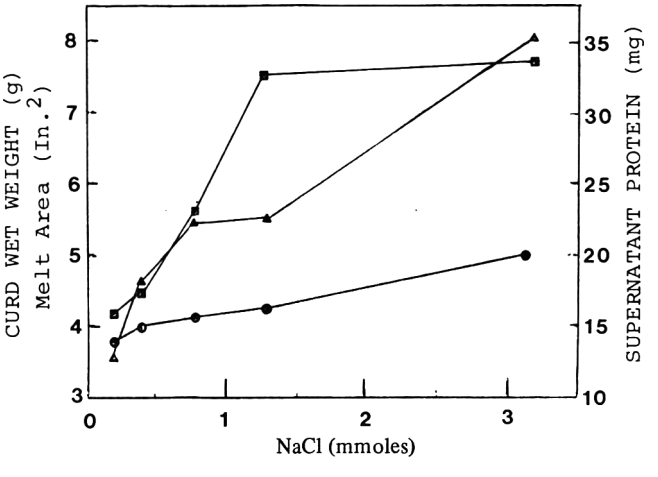

Fig. 3—Effect of sodium chloride concentration on curd wet weight (●); melt area (▲); and supernatant protein (■).

Table 4 — Correlation coefficients of variables in the cube-plus-octahedron rotatable design

	Melt	pH	Phosphate	Sodium	Solvent uptake	Supernatant protein	Curd dry wt	Phosphate X pH
Melt	1							
pH	+ 0.40	1						
Phosphate	+ 0.57	– [a]	1					
Sodium	–	–	–	1				
Solvent uptake	+ 0.94	+ 0.38	+ 0.49	–	1			
Supernatant protein	+ 0.58	–	–	–	+ 0.55	1		
Curd dry wt	– 0.44	– 0.69	–	–	– 0.46	– 0.39	1	
Phosphate X pH[b]	+ 0.74	+ 0.50	+ 0.86	–	+ 0.67	–	–	1

[a] Correlations not significant < 0.10% are marked with a dash
[b] Represents an interaction term

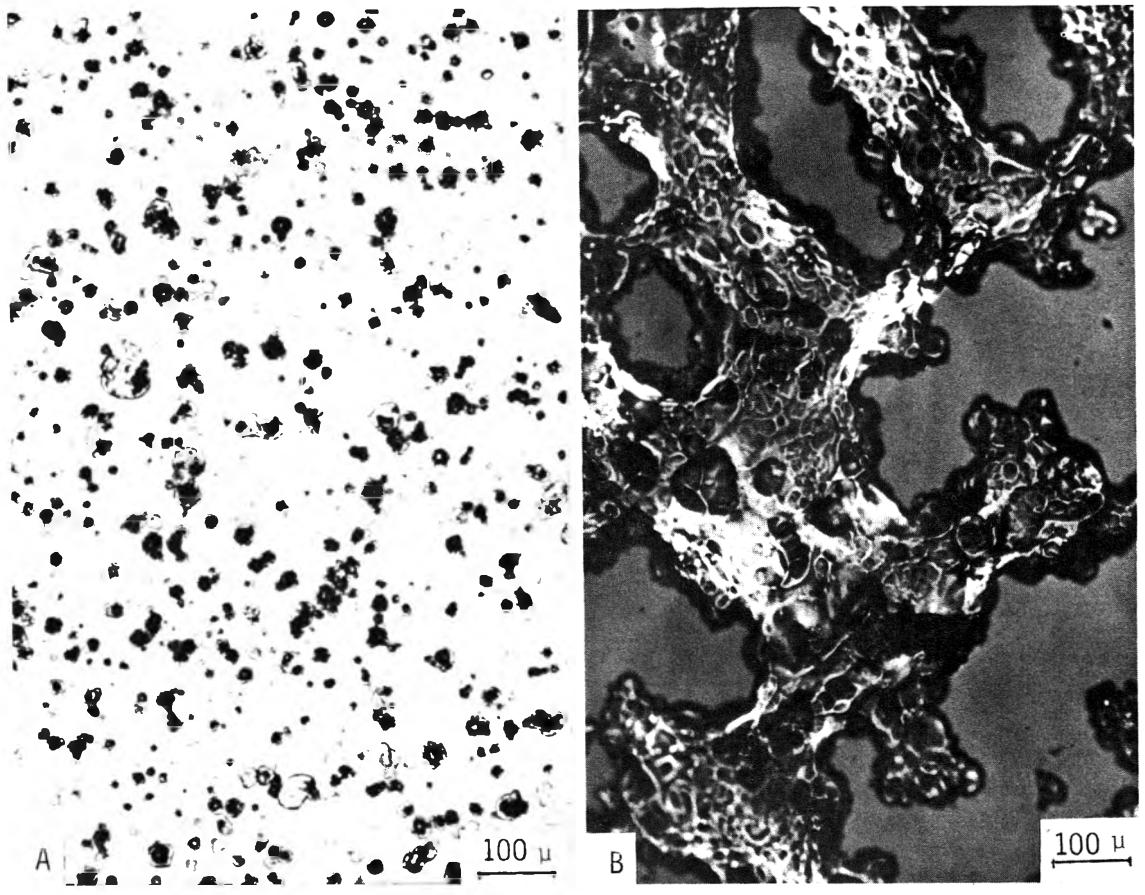

Fig. 4—Polarized light micrographs: (A) spray-dried calcium caseinate in ethanol; (B) calcium caseinate curd.

appeared birefringent indicating an ordered fiber structure. Such an ordered arrangement may have been caused by the stirring action used during curd formation which tends to stretch and align the fibers. However, regions of birefringence could still be observed when the curd was formed without shear.

SEM of the curd (Fig. 5) shows a sequence in which caseinate particles initially stick together but subsequently become grossly distorted and elongated into strands or bundles of fibers. Very small spherical particles are evident on the surface of the curd fibers (Fig. 5D). These particles increase in number when NaCl is introduced (Fig. 6), reflecting a disaggregating effect of the monovalent ions.

Curd formation is temperature dependent. At 4°C flocculation occurred, but agglomeration to a single curd was not seen until the temperature was increased. Fig. 7 shows the amorphous nature of the curd which was formed and chemically fixed in the cold in comparison with the more ordered structure of curd formed at ambient temperature (Fig. 5B). As the temperature of formation was increased above ambient, less water was needed for the reaction. This phenomenon indicates that hydrophobic interaction may be a driving force for the formation of a cohesive protein mass.

DISCUSSION

A SPONTANEOUS CURD was formed by the addition of water to an ethanol/calcium caseinate slurry. This phenomenon appears to be a result of a change in the surface properties of the caseinate particles and was illustrated by the sticky, yet intact particles, seen during agglomeration.

The mechanism by which the curd was formed in this system is of interest because it appears to provide a model for understanding how a cheese matrix is generated from caseinate blends in IPC. The fact that a coherent curd is formed in an alcohol/water mixture of approximately 40% reflects a structural alteration of the spray dried calcium caseinate particles when the polarity of the solvent is changed. There are two conditions that must be met for the formation of this protein curd:

1. The presence of a divalent cation in the protein/solvent mixture.
2. Changes in solvent polarity from relatively nonpolar to a more polar mixture.

It is hypothesized that when casein particles are suspended in nonpolar solvents an entropy driven rearrangement of their structure occurs in which some previously buried hydrophobic groups are exposed to the nonaqueous solvent. A concomitant strengthening of ionic bonding would also occur due to the decreased dielectric of the medium and thus any calcium bridging between protein particles would be made even more tenacious.

As water is added to the suspension, the exposure of hydrophobic groups becomes increasingly energetically unfavorable. At some critical water content these groups must either return to the interior of the protein molecule or associate with similar groups in an attempt to counteract the ordering of water molecules that occurs in their presence.

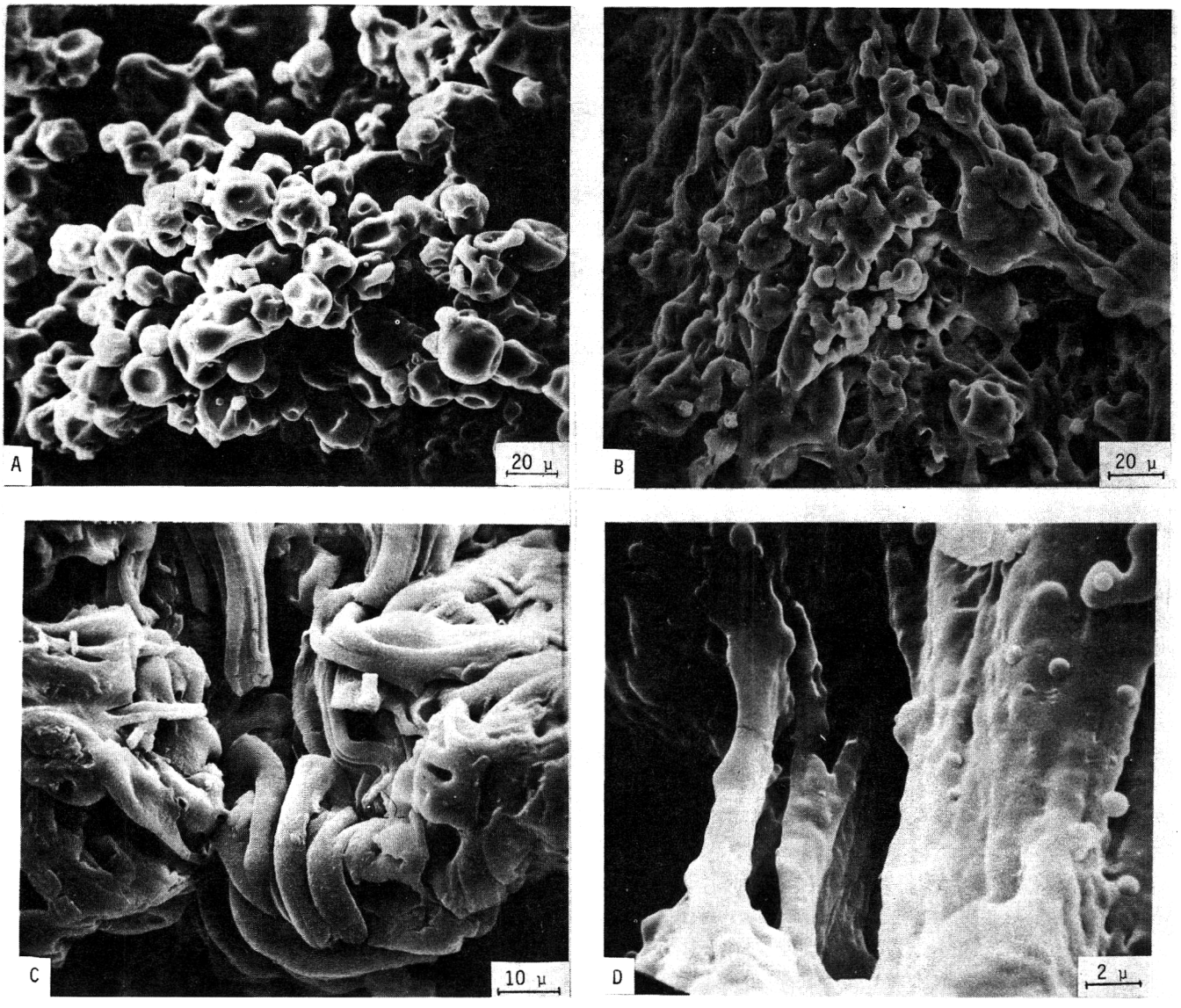

Fig. 5—Scanning electron micrographs of the progression in calcium caseinate curd formation: (A) spray-dried calcium caseinate particles; (B) early stage of curd formation; (C) caseinate curd following syneresis, magnification of 855X; (D) caseinate curd following syneresis, magnification of 4,250X.

When no crosslinked protein is present (sodium caseinate) a curd is not formed and it appears that the hydrophobic groups simply return to the interior of the protein structure. In the presence of calcium, the proteins are probably held in closer proximity and the hydrophobic groups find it easier to self associate rather than to rearrange the shape of the protein molecules. This process leads to the formation of fibers consisting of hydrophobically linked protein molecules with their polar groups on the surface.

The inability of caseinates to form a curd at 4°C is consistent with an entropy driven mechanism of formation as is the temperature dependence of the amount of water required for the agglomeration to occur. At low temperatures the free energy gained by hydrophobic interactions is too low to favor curd formation. At higher temperatures, the strength of hydrophobic interactions would be increased and thus the presence of less water would be required to cause their self association. The observation that curd formation could be induced by a number of different solvents, including vegetable oil, is consistent with the concept that intimate mixing of calcium caseinate with liquids of low polarity alters the surface properties of the particles and predisposes them towards agglomeration when water is added.

This study demonstrated that monovalent ions exerted a disaggregating effect on the curd. This was revealed by the structural changes in the curd when sodium chloride was incorporated and by the failure of sodium caseinate to produce a cohesive curd in this system. Electrophoresis showed that disaggregation by sodium chloride was accompanied by the loss of α_s and β-casein from the curd. These effects reiterate that calcium-bridging is an important structural element for cohesiveness in the curd. Calcium bridging between negatively charged groups is important in drawing together the different protein moieties which are then more likely to self associate rather than to solubilize and thus yield a cohesive structure. The effect of monovalent ions may well be one of breaking calcium cross-bridges and allowing the proteins to solubilize before a cohesive mass can be obtained.

The melting properties of the curd, reported as the melt area, proved to be an indicator of the functionality of different sources of calcium caseinate. Though the explana-

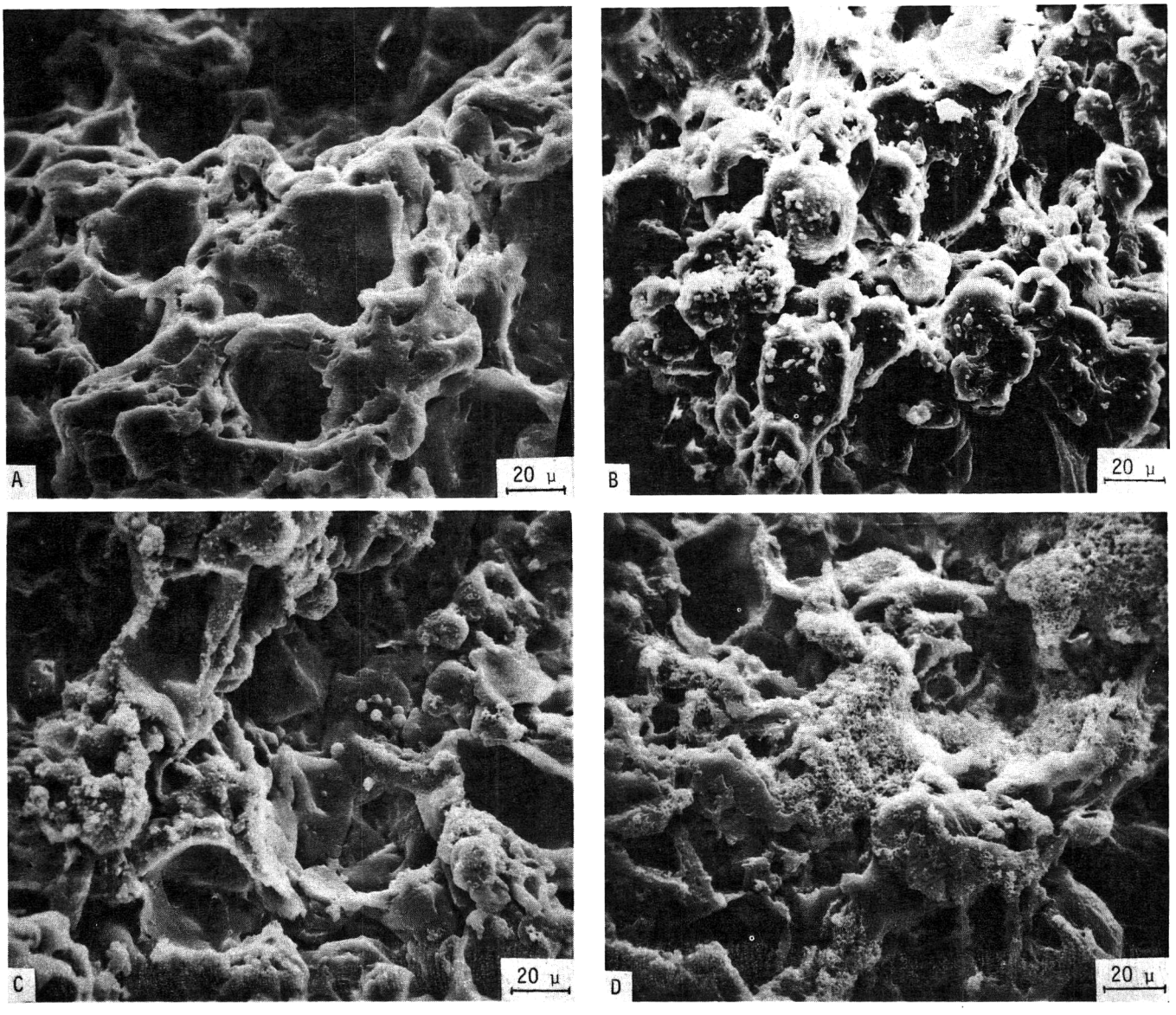

Fig. 6—Scanning electron micrographs of calcium caseinate curd with increasing concentrations of sodium chloride: (A) curd produced without additional sodium chloride; (B) curd produced with 0.775 mMoles sodium chloride added; (C) curd produced with 1.55 mMoles sodium chloride added; (D) curd produced with 3.10 mMoles sodium chloride added.

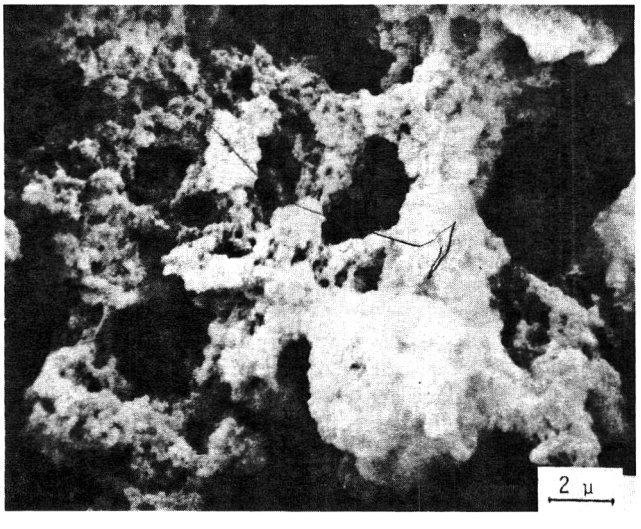

Fig. 7—Scanning electron micrograph of the caseinate curd formed at 4° C.

tion for this is as yet unclear, it is possible that it reflects subtle differences in molecular attractions within the caseinate particles. In IPC, excessive calcium bridging may impose a limit on the degree of swelling and hydration of the calcium caseinate particles and indeed on the spreading of the melting curd. However, the controlled addition of sodium caseinate and sodium chloride undoubtedly provides the necessary elements for attaining the proper adjustment of an IPC system.

Further research is necessary to establish if differences in melt and differences in the functional performance of different calcium caseinates are related to a variable amount of the hydrophobic proteins which were found in the supernatant after curd formation. The amount of excluded protein may be expected to vary with the particle size and specific surface. Besides, it was observed that γ-casein was a major constitutent of this fraction. γ-caseins are known to be proteolytic products of β-casein, corresponding to the more hydrophobic portion (Swaisgood, 1973). Greater quantities of these more hydrophobic proteins may impart different functional properties to an IPC. Proteolysis has been shown to affect the properties of conventional cheese

—Continued on page 1249

Conditions for the Formation of Heat-Induced Gels of Some Globular Food Proteins

PER-OLOF HEGG

ABSTRACT

The quality of thermally induced aggregates of the globular proteins conalbumin, serum albumin, β-lactoglobulin and lysozyme has been examined at various salt concentrations and pH values. The properties of the aggregates were characterized by their dry matter content. The results are given as simple phase diagrams. The following areas of dry matter content were found: solubility; transparent and opaque gels (dry matter content of 5–9%); precipitates (dry matter content above 9). Gels were formed only close to conditions of solubility. Only serum albumin was found to be a protein with good gelling properties. A small gelling area was registered for β-lactoglobulin, while no gelling was observed for conalbumin or lysozyme under the conditions examined. No common simple physical characteristic of the proteins used could be correlated to good gelling behavior.

INTRODUCTION

THE IMPORTANCE of gel formation in food is well known in, for instance, many meat, egg and milk products. In these products proteins are considered to be the main texture building component. The mechanism of protein gelling is, however, poorly understood and theories on gel formation are instead to be found in carbohydrate chemistry (c.f. Rees 1972). It is conceivable, however, that some of these theories could be applied also to protein chemistry.

It has been reported earlier that the egg white protein ovalbumin forms excellent heat induced gels under certain conditions. These conditions, however, must be exactly defined in terms of, for instance, salt concentration and pH. In the case of ovalbumin transparent gels are only found between pH 10 and 11 at physiological salt concentration. At another salt concentration transparent gels are formed within a different pH-interval (Hegg et al., 1979). Other proteins seem either not to form gels by heating (Hegg, 1978) or the conditions required for gel formation are quite different when compared to ovalbumin.

This investigation was initiated to map the conditions required for globular proteins to form thermally induced gels. It would be of considerable value in food technology if a common environmental condition or certain protein characteristics could be correlated with the ability to form heat induced gels. To this end the following common food proteins, which have different qualities in many respects, were selected: conalbumin and lysozyme from egg white, serum albumin from bovine blood, and β-lactoglobulin from milk. It should be stressed that the protein preparations used were extremely pure and well characterized.

EXPERIMENTAL

Protein preparations

Bovine serum albumin (lot no. 16C-7201), conalbumin (lot no. 34C-8200) and lysozyme (lot no. 74C-8041) were all obtained from Sigma Chemical Co. Serum albumin was free of bound fatty acids and conalbumin from bound iron, as determined by differential scanning calorimetry (Gumpen et al., 1979, Donovan and Ross, 1975). Both preparations were saltfree. Lysozyme was desalted prior to use according to Hegg et al. (1979). β-Lactoglobulin was obtained saltfree by preparation from fresh whole raw milk (Hegg, 1980). None of the used preparations were contaminated by other proteins as determined by SDS-polyacrylamide gel electrophoresis.

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Determination of water-holding capacity

The experiments were performed at the fixed protein concentration of 44 mg/ml. Protein concentrations were determined according to the following: β-lactoglobulin $A^{1\%}_{1\ cm} = 9.6$ (Townend et al., 1960), serum albumin $A^{1\%}_{1\ cm} = 6.67$ (Reynolds et al., 1967) conalbumin $A^{1\%}_{1\ cm} = 11.3$ (Glazer and McKenzie, 1963) and lysozyme $A^{1\%}_{1\ cm} = 26.9$ (Ogasahara and Hamaguchi, 1967). All values are given at the wavelength of 280 nm.

Protein solutions were prepared as earlier described (Hegg et al., 1979). They were heated from 25–95°C with a rate of 10°C/min. Heat treatments were carried out in glass tubes (7 x 75 mm) containing 1 ml of protein solution (Hegg et al., 1979). After heating to 95°C the tubes were immediately cooled in ice water and centrifuged at 30,000 x *g* for 45 min. The percentage of thermally aggregated protein was calculated from the decrease in absorbance of the supernatants at 280 nm. In most cases aggregation was complete (> 90% insoluble protein) at 95°C.

The water-holding capacity of the aggregates formed was characterized by their dry matter (d.m.) content. The dry matter content (%) was calculated from $(C_o \cdot V_o \cdot a)/(W_a)$ where C_o is the initial protein concentration (mg/ml); V_o, the initial solution volume (ml); a, percentage aggregation; and W_a, the weight of the aggregate (mg). A discussion of the d.m. content is given elsewhere (Hegg et al., 1979). The reproducibility was ±3.5% calculated on the measured d.m. contents. In the few cases when complete aggregation was not reached at 95°C the figure for d.m. content is marked with an asterisk. Complete solubility was defined as no observed phase separation after centrifugation.

RESULTS

Conalbumin

When no sodium chloride was added to the conalbumin solution, thermally induced aggregates were only formed within the pH-interval from around 6 to pH 8.5 (Fig. 1). Consequently, aggregation in a saltfree protein solution only occurred around the isoelectric point of the protein (approximatively pH 6 for conalbumin). The aggregates obtained were characterized by their dry matter content and these values were all high in the pH-interval described above. High dry matter values indicate that the aggregates visibly appear as "white loose precipitates" which are effectively packed together by centrifugation. No transparent gels, which are characterized by dry matter values of around 5% or opaque gels with dry matter contents between 5 and 9% were observed.

The limits between solubility and aggregation gradually moved towards lower and higher pH-values, respectively, when the salt concentration was increased. As can be seen from the incomplete aggregation near the boundary (dry matter figures marked with an asterisk) there was a gradual transition from solubility to complete aggregation. With the exception of a few points, the dry matter values in Fig. 1 are all above 13, i.e. the aggregates visibly appeared as precipitates. No systematic tendency to a decrease or in-

crease in dry matter value was found with changing conditions. The quality of the heat induced aggregates of conalbumin thus seems to be largely independent of salt concentration and pH.

The very steep course of the solubility/aggregation line observed for conalbumin at acidic pH-values was not observed earlier for ovalbumin. The boundary between solubility and aggregation seems to be closely related to the titration curve of the protein (Hegg, 1979). Conalbumin has a pH-induced transition between pH 3.5 and 5, and thus a profound alteration in the amount of titratable groups in this pH-interval (Wisnia et al., 1961).

Serumalbumin

Heat induced aggregation in saltfree solution of serumalbumin occurred within a more narrow pH-interval than for conalbumin. The isoelectric point is located around the middle of this interval (Fig. 2).

Serum albumin has a similar pH-induced transition at acidic pH-values as conalbumin. Thus, the course of the boundary between solubility and aggregation at acidic pH-values is the same for these two proteins. At alkaline pH-values they differ, however, and serum albumin is soluble under conditions ranging from pH 6.5 in saltfree solution to pH 11 in 2% NaCl. This difference compared to conalbumin is mainly due to the fact that serum albumin undergoes structural transitions also at alkaline pH-values (Steinhardt and Reynolds, 1969).

The most important difference between the two proteins is, however, the extremely large gelling area for serum albumin at neutral and weak alkaline pH-values (Fig. 2). At physiological salt concentration, for instance, gels are formed in the wide pH-interval 6.5–9.5. Both transparent and opaque gels are found within this interval. Transparent gels are characterized by a dry matter content around 5% and these gels are nearly always found close to the boundary between solubility and aggregation. This is probably due to the higher net charge repulsion between the protein molecules required for the formation of a gel with transparency (Hegg, 1978).

Precipitates of serum albumin were always observed near the isoelectric point regardless of the salt concentration used. This is in agreement with the results on conalbumin. Progressively lower dry matter contents were obtained towards the solubility/aggregation limit on the acidic side and even at these acidic pH-values a narrow gelling area could be distinguished. A gradual decrease in dry matter content was also registered from the isoelectric point towards alkaline pH-values. This tendency was, however, casually broken at pH 8, where lower values were found at all salt concentrations. The reason for this is probably due to the structural transition mentioned above.

β-Lactoglobulin

β-Lactoglobulin is the most abundant whey protein and has an isoelectric point of around 5.2 (McKenzie, 1971). The protein has a pH-induced transition above pH 7 (McKenzie et al., 1967), which accounts for the solubility characteristics (Fig. 3) similar to those registered for serum albumin. The solubility/aggregation boundary at acidic pH-values more reflects the shape of a normal titration behavior (Hegg, 1978).

Gel formation occurred close to the aggregation/solubility boundary both on the acidic and alkaline side of the isoelectric point. Both these areas of gel formation were, however, very narrow. Furthermore, only opaque gels were obtained. The behavior earlier found for ovalbumin (Hegg et

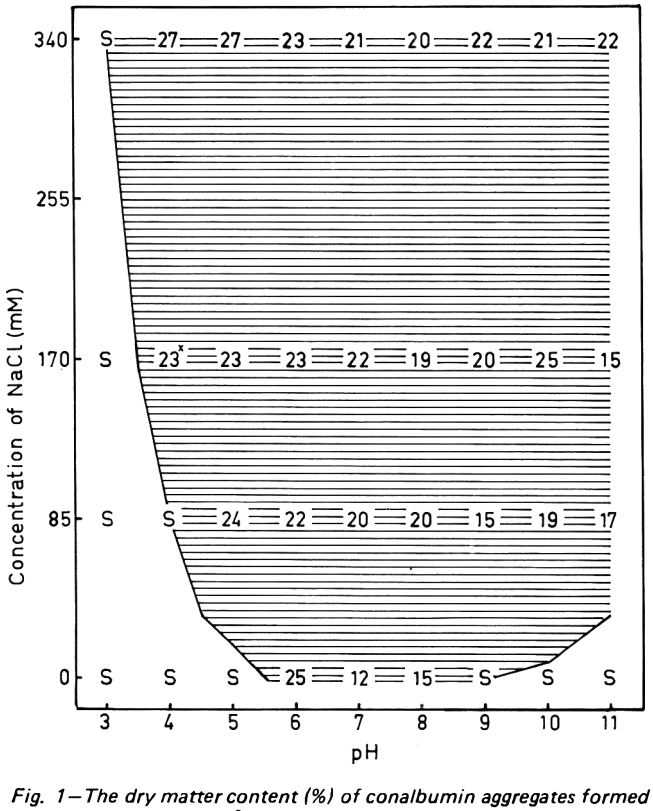

Fig. 1—The dry matter content (%) of conalbumin aggregates formed through heating to 95°C in different concentrations of NaCl between pH 3 and 11. Heating rate was 10°C/min. Dry matter contents are given with figures at each pH for 0, 85 mM, 170 mM and 340 mM added NaCl. S indicates solubility. The lines differentiate areas of different dry matter content; /// 5–9% and ≡ above 9%. The unhatched area indicates that thermal aggregation does not occur. Dry matter contents when aggregation was incomplete have been marked with an asterisk.

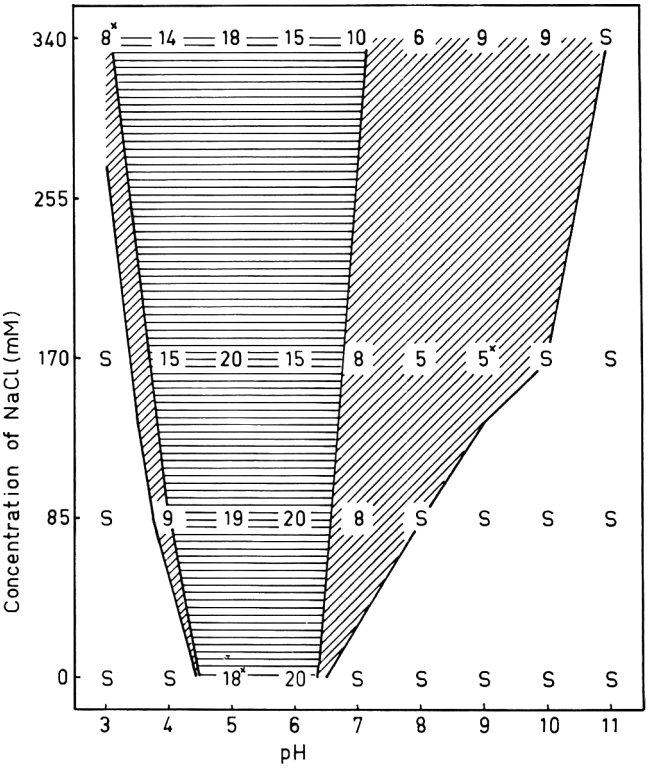

Fig. 2—The dry matter content (%) of serum albumin aggregates formed through heating to 95°C in different concentrations of NaCl between pH 3 and 11. For details, see legend to Fig. 1.

al., 1979) that at any salt concentration the dry matter contents were at highest around the isoelectric point and then gradually decreased towards lower and higher pH-values was observed also for β-lactoglobulin. Compared to the other proteins in this investigation, the dry matter contents were generally lower for β-lactoglobulin, and dry matter values above 20% were found only in saltfree solution.

Lysozyme

Lysozyme is the smallest globular protein used in this investigation with a molecular weight of 14 500 (Dayhoff, 1972). The protein has a small solubility area, and is soluble only on the acidic side of the isoelectric point (Fig. 4). This is due to the high isoelectric point (pH 10.5–11.0) of the protein (Gilbert, 1971).

The dry matter contents found in the aggregates were all very high, i.e. only precipitates were formed. No gel formation, either of transparent or opaque gels, could be detected. Furthermore, there was an extremely slow transformation from solubility to complete precipitation for lysozyme. Several of the dry matter values in Fig. 4 are marked with an asterisk which indicates incomplete aggregation.

DISCUSSION

THE SOLUBILITY AREAS for the proteins given in Fig. 1–4 show the same general appearance. From the isoelectric point, these areas expand with a decreasing or increasing pH and shrink with an increasing salt concentration. Thus, thermal non-aggregating conditions are largely determined by the state of ionization of the titrable groups of the proteins, and the differences found in extension of the boundaries between solubility and aggregation mainly reflect different net charges of the proteins under various conditions (Hegg et al., 1979). The effect of salt and pH on the attractive forces is small in comparison with the effect on the repulsive net charge. Consequently, it would be possible to predict the boundaries between solubility and aggregation for any globular protein by knowing titration curves, amount of salt present in the sample and simple physical data, as the isoelectric point, pH-induced transitions etc.

Ovalbumin has earlier been reported to be a protein with good gelling properties, i.e. gels were formed at a wide pH- and salt concentration range (Hegg et al., 1979). Serum albumin (Fig. 2) has gelling properties comparable to those of ovalbumin. The gelling area for serum albumin is, however, displaced towards lower pH values and higher salt concentrations, and the area where transparent gels were formed was also more narrow.

β-Lactoglobulin has an isoelectric point close to that of serum albumin and a similar pH-induced transition at alkaline pH values. For β-lactoglobulin, however, only a very small gelling area was detected (Fig. 3). Obviously, these simple physical characteristics could not be used as a correlation to good gelling property of a protein.

Areas of gel formation could not be distinguished for conalbumin (Fig. 1) or lysozyme (Fig. 4). Our data do not indicate a simple physical characteristic in common to account for their lack of ability to form gels thermally. It must be pointed out, however, that only a fixed protein concentration of 4.4% has been used in this investigation. A higher concentration would possibly facilitate the formation of a three-dimensional protein network and thus the formation of a gel structure. It cannot be excluded that a critical concentration for gelling exists for every protein and that this concentration in the case of conalbumin and lysozyme is very high. The kinetics of heating is another factor that affects the gelling behaviour. A low rate of heating generally has a positive effect on gel formation (Hegg, 1978). From these points of view the experimental conditions selected in this investigation are quite unfavor-

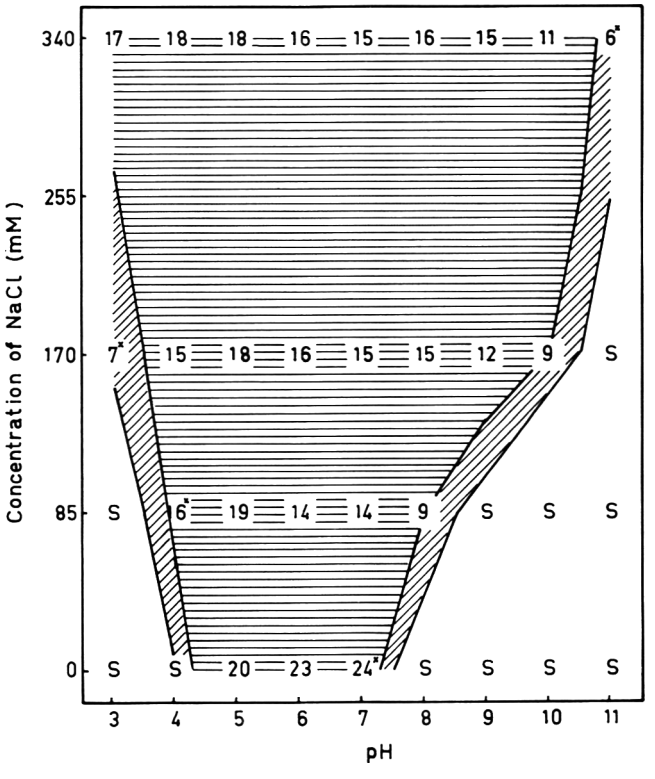

Fig. 3—The dry matter content (%) of β-lactoglobulin aggregates formed through heating to 95°C in different concentrations of NaCl between pH 3 and 11. For details, see legend to Fig. 1.

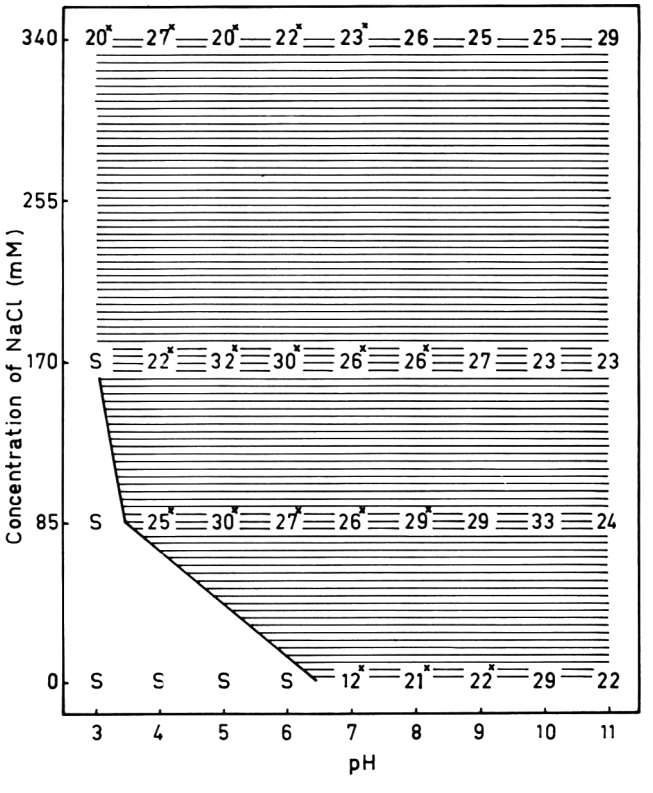

Fig. 4—The dry matter content (%) of lysozyme aggregates formed through heating to 95°C in different concentrations of NaCl between pH 3 and 11. For details, see legend to Fig. 1.

able for gel formation since they were designed to sort out a protein with good gelling properties from a poor one.

Disulphide bridges and sulphydryl groups have been suggested to be important for crosslinking of proteins. The proteins used in this investigation differ widely in their content of these groups and thus no correlation between disulphide or sulphydryl content and the ability to form thermally induced gels was found. Some other proteins apart from those reported on here have also been investigated. Notably, myoglobin containing no disulphide bridge or free sulphydryl group (Mahler and Cordes, 1966), was found to be an extremely potent gelling protein, further indicating that these parameters are unimportant in gel formation of these proteins. The forces which keep the framework of the gel together must instead be found in the hydrophobic and hydrogen bonds, which become available during thermal denaturation of the globular proteins. These forces counteract the repulsive net charge of the proteins, and a delicate balance between attractive and repulsive forces seems to be a prerequisite for the formation of a gel framework (Hegg et al., 1979). The differences in the ability to form gels might reflect different types of intermolecular interactions in the aggregates of the proteins examined. It has been proposed that β-sheet hydrogen bonding might be important in aggregate formation (Clark et al., 1981). The β-sheet content in the native state of serum albumin, for instance, is reported as low and that of β-lactoglobulin as high (Wasylewski, 1979). There is therefore no indicaton that a high content of intramolecular β-sheet structure in the native state would facilitate the formation of intermolecular β-sheet formation in the denatured state. Instead, the potent gelling proteins serum albumin, ovalbumin and myoglobin seem to have a high helical content in the native state (Joly, 1965; Chen et al., 1974). Since the poor gelling protein lysozyme also has a high helix content (Chen et al., 1974) this seems not to be decisive factor in gel formation either.

The contribution from the hydrophobic forces to the formation of the gel framework is difficult to assess since hydrophobicity is not easy either to calculate or to measure. None of the proteins used are known to have an extreme content of hydrophobic amino acids. Furthermore, the location of the hydrophobic amino acids within the molecule is probably more important for the development of intermolecular interactions than the total content. Further speculation in this matter is not meaningful, since, except for lysozyme, the protein structures for the model proteins examined are unknown.

In conclusion, it seems possible to predict the conditions required for aggregation and solubility of a globular protein but hard to identify a special protein characteristic which is crucial for gel formation. If gel formation occurs, however, this is found close to the boundary between aggregation and solubility.

Studies at various protein concentrations might possibly add further clues to the mechanism of protein gelling. As no characteristic property of the proteins used in this investigation could be correlated with a good gelling behaviour it could not be excluded that most proteins might form thermally induced gels. If so, the question of gelling might be reduced to find the right condition for the actual protein.

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Diffusional Behavior of Tripalmitin in a Freeze-Dried Model System at Different Water Activities

W. NAESENS, G. BRESSELEERS, and P. TOBBACK

ABSTRACT

The diffusional behavior of tripalmitin (TP) in a low moisture model system composed of microcrystalline cellulose and gum arabic was found to be very dependent on the water activity (a_w), the temperature and the presence of paraffin oil (PO). The complex mechanism of mobilization of food components in dry systems, particularly that of TP in our system, has been discussed in detail. Such knowledge is especially important in relation to the reactions that occur in foodstuffs during dehydration and subsequent storage. As a typical example, the results on TP diffusion indicate that the restriction of enzymatic activity in dry systems may not solely be due to the diffusional limitations of the reactants.

INTRODUCTION

IT IS WELL-KNOWN that in dry systems most physical properties, including the diffusivities of food components, are strongly dependent on the water content. However, only limited information is available with respect to diffusional problems in partially dried systems (Karel, 1975; Leniger and Bruin, 1977; Simatos et al., 1981). Bomben et al. (1973) mentioned a possible decrease by a factor 10^7 of the water diffusion coefficient from very high moisture to nearly dry material. Although the reported decreases of the water diffusion coefficient are not always so dramatic as the above figure, a decrease by several orders of magnitude is not exceptional in the low moisture range (Fish, 1958; Menting et al., 1970). Diffusion coefficients of volatile compounds generally drop to a much greater extent. This fact was the basis of the so-called selective diffusion theory as proposed by Thijssen and Rulkens (1968) in order to interpret the volatiles retention during drying of foodstuffs. Quantitative data on diffusion are lacking especially for non-volatile compounds in dehydrated systems (Naesens et al., 1981).

The progress of reactions in dehydrated systems in relation to the availability of the water is not yet fully understood. As a typical example, the restriction of enzymatic conversions in dried foodstuffs still remains to be elucidated. This has been attributed by many authors to the restricted ability of the substrate to diffuse to the active site of the enzyme (Drapron, 1972; Brockmann and Acker, 1978; Potthast, 1978). Even for hydrophobic substrates, enzymatic activity increases with water content. Therefore, Brockmann and Acker (1978) considered water as an universal vehicle. Usually no activity is observed in the region of the so-called BET-monolayer adsorption of water. According to Acker and co-workers, this is due to the inability of the water in the monolayer to act as a mobilizer. The authors also indicated the impact of the physical state of fat substrates on their susceptibility to enzymatic degradation (Acker and Wiese, 1972a, b; Brockmann and Acker, 1977, 1978). The reaction rate of lipase increases rapidly above the melting point of the fat substrates. According to the authors, liquid fats are able to move to the enzyme on their own account thus being susceptible to enzymatic attack even at extremely low moisture content. Solid substrates, when dissolved in such liquid phases as e.g. glycerin or paraffin oil, are transformed to a similar extent. Unfortunately, Acker never measured the mobility of fat substrates.

Dried foods are mostly multicomponent heterogeneous systems, built up by particles which are more or less bound together and giving the porous product a certain coherence. As a model for such a product, we studied a freeze-dried system composed of microcrystalline cellulose and gum arabic. The diffusion of tripalmitin (TP) in this sytem was measured as a function of water activity (a_w) and temperature. The influence of paraffin oil (PO) in the system on the TP mobility was also investigated. The aim of the present study was to provide information on the question to which extent water is able to mobilize food ingredients in partially dried systems. The second objective of our study was to compare the observed diffusional behavior of TP with the available data in literature on the extent of its enzymatic breakdown in low moisture systems.

EXPERIMENTAL

A 10% (w/v) AQUEOUS SOLUTION of gum arabic (Merck) was centrifuged at 900g for 20 min. The clarified solution was used to emulsify the fat. ^{14}C-TP (Radiochemical Centre, Amersham) was diluted with carrier TP (Merck) to an approximate specific activity of 18 μCi/mmol. 45 mg of this ^{14}C-labeled TP, dissolved in 1 ml chloroform, was emulsified for 2 min with 10 ml of the 10% aqueous solution of gum arabic and 10 ml of bidistilled water using an Ultra-Turrax Mixer Emulsifier (Janke and Kunkel KG, Type TP 18/10, Staufen, W. Germany) at maximum speed. Subsequently, the emulsion was well mixed for 3 min with 15g microcrystalline cellulose (Avicel, Merck) and 60 ml bidistilled water by means of the Ultra-Turrax at maximum speed. Similar suspensions were prepared without TP. When PO was included in the model system, the emulsion was prepared as above but it was started from 45 mg ^{14}C-labeled TP and 900 mg PO (commercial grade) dissolved in 2 ml chloroform.

The suspensions were poured into petri-dishes to a depth of about 3 mm, quickly frozen with liquid air and freeze-dried (Secfroid, Lyolab C 3023, Lausanne, Suisse) at a pressure of less than 0.1 Torr in such a way that the maximum temperature never exceeded 25°C. The freeze-dried systems were stored at the desired temperature and equilibrated above saturated salt solutions or above anhydrous P_2O_5.

The diffusion experiments were carried out as described previously (Naesens et al., 1981). According to this method, an amount of model system without diffusant and equilibrated at a given a_w was pressed into a glass cylinder (length 7 cm; inner diameter 1.2 cm) which was stoppered at one end. An equal amount of diffusant-containing model system equilibrated at the same a_w was deposited on the first layer in the same manner and the cylinder was stoppered at the other end. The cylinder was stored at the desired condition of relative humidity and temperature. For the sake of clearness, the geometry of the diffusion set-up is shown in Fig. 1.

After a given period of time, the diffusion system was analyzed as described previously and the concentration distance curve was plotted as the relative concentration (C/C_0) versus distance (x), relative to the plane x = 0. This plane was located by computation so that the amount of diffusant passing across the plane to one side is equal to the amount of diffusant disappearing from the other side. The apparent diffusion coefficients were calculated from the

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symmetric profiles using the curve-fitting procedure based on the sum of least squares.

All a_w measurements were carried out at the equilibration temperature on a part of the diffusion system which was discarded afterwards. The electrical hygrometer (Nova Sina AG, SMT-B with sensor eZFBA$_4$, Zürich, Suisse) was first calibrated with saturated salt solutions of known a_w (Greenspan, 1977). Water sorption isotherms of the model system with and without PO were determined at 50°C. For this purpose, nonlabeled systems were prepared and equilibrated as described above. Part of the sample was used to determine the a_w. The water content was determined by weight. To this end, the rest of the sample was weighed before and after drying at 105°C for 3 hr and subsequent storage over anhydrous P_2O_5 for 5 days. The water content was expressed on a fat free basis.

RESULTS & DISCUSSION

IN ALL THE DIFFUSION RUNS as they are shown in Fig. 2 to 6, measurements were carried out at seven different a_w values, ranging from a_w 0.0 up to about a_w 0.95. For the sake of clearness, the diffusion curves intermediate to the ones drawn in the graphs were left out. The calculated apparent diffusion coefficients of the symmetric profiles at 50°C of TP diffusion in both the PO-containing and the PO-free model system are given in Fig. 7. To render our results comparable to those reported in literature, the numerical values of the diffusion coefficient are given in Table 1 together with the a_w and the moisture content of the system. It must be noted that the water content was calculated on a fat free basis since it is commonly accepted that fats have no or only a minor influence on the water sorption capacity of the system. It was found that the isotherms of the model systems with and without PO coincide. The values of water content given in Table 1 have been interpolated from this isotherm.

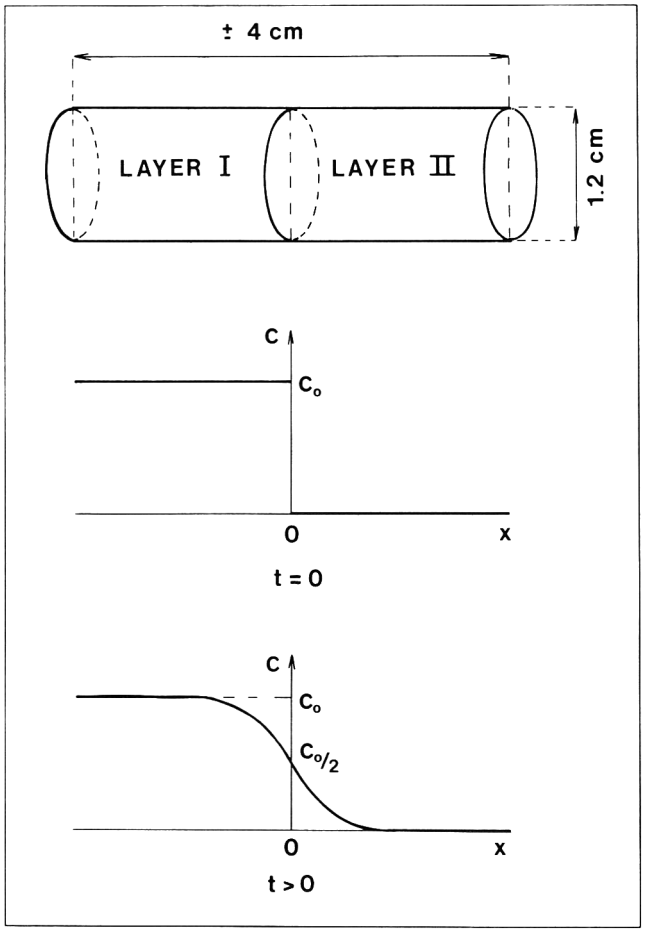

Fig. 1—Schematic representation of the diffusion set-up performed in cylinders in which a diffusant-containing layer I is in close contact with a layer II containing no diffusant (top). The geometry of the set-up corresponds to an extended source of infinite extent. The concentration of the diffusant (C) versus distance (x) is shown at both time t = 0 (center) and t > 0 (bottom). C_0 stands for the initial concentration of the diffusant in the diffusant-containing layer and x = 0 is the position of the initial interface between the two layers at time = 0.

Influence of temperature and paraffin oil

The diffusing substance, TP, develops a symmetric concentration-versus-distance curve in the PO containing system at 50°C (Fig. 2). This is indicative for a mobility of the lipid based on pure diffusion despite the complexity of the system. As shown in Fig. 3 and 4, the diffusion of TP in the same system is not only retarded with decreasing temperature, but also results in asymmetric diffusion profiles. The increased complexity of the system with the decrease in temperature can reasonably be explained by a progressive solidification of TP, which, due to its chemical nature, is probably in close contact with the PO. As was easily checked, the TP/PO-ratio used in the diffusion runs allows TP to be completely soluble in PO at 50°C. At lower temperatures, TP is above its saturation point in PO which gives rise to a heterogeneous population of TP crystals in the PO liquid phase. Due to the high mass of these TP aggregates as well as to the presumably high viscosity of the

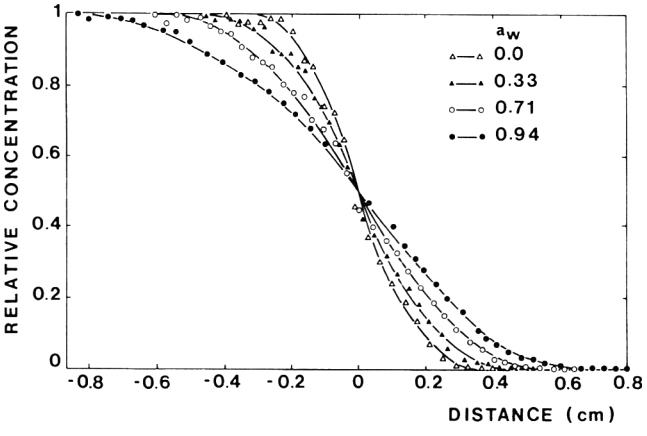

Fig. 2—Diffusion curves at 50°C of TP after 45 days in the PO-containing model system at different water activities.

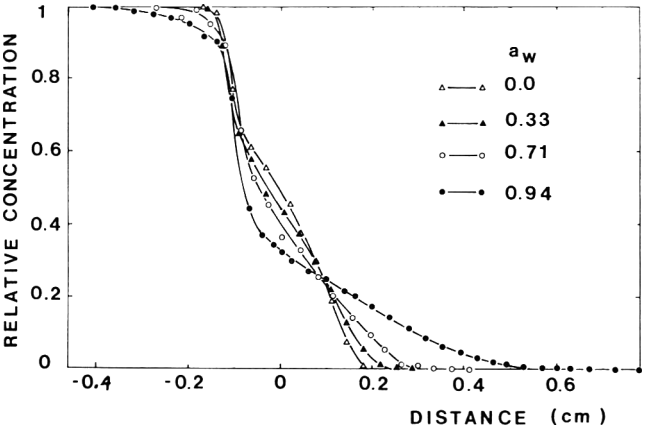

Fig. 3—Diffusion curves at 40°C of TP after 45 days in the PO-containing model system at different water activities.

PO-TP mixture, the particles have a very restricted mobility. In this context, it may be instructive to remind that the Brownian motion of large molecules is caused by the thermal energy of the solvent molecules, driving them forward against the viscosity of the medium as resistance-factor. The idea that TP gives rise to asymmetric profiles when it is above its saturation point in PO was confirmed by the observed development of a symmetric diffusion profile at 25°C when the ratio of TP/PO was sufficiently lowered as to dissolve all the TP added in the phase at the temperature under consideration.

Diffusion of TP in a model system free of PO also gives rise to well-developed symmetric profiles at 50°C (Fig. 5). This is rather surprising because the normal melting point of TP is about 66°C. As was the case for the PO-containing system, the diffusion curves at 25°C are again asymmetric especially at high a_w (Fig. 6). The diffusional behavior of TP in this PO-free system cannot be explained in definite terms. The TP may be present as small solid particles floating on the water film sorbed by the solid matrix, and being

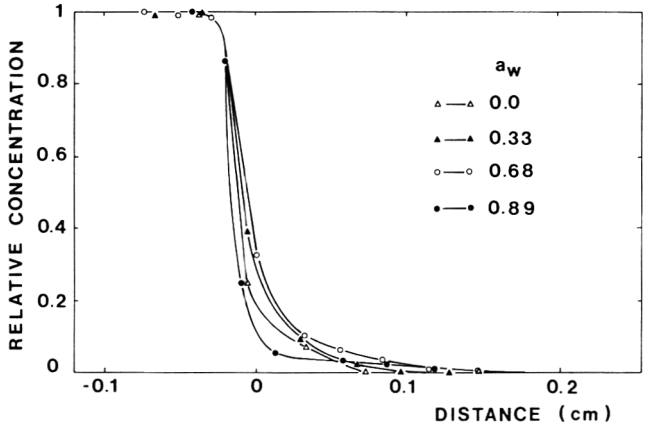

Fig. 4—Diffusion curves at 25°C of TP after 45 days in the PO-containing model system at different water activities.

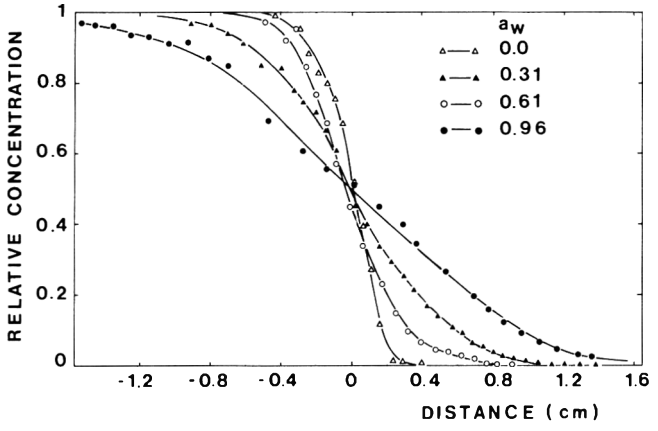

Fig. 5—Diffusion curves at 50°C of TP after 45 days in the PO-free model system at different water activities.

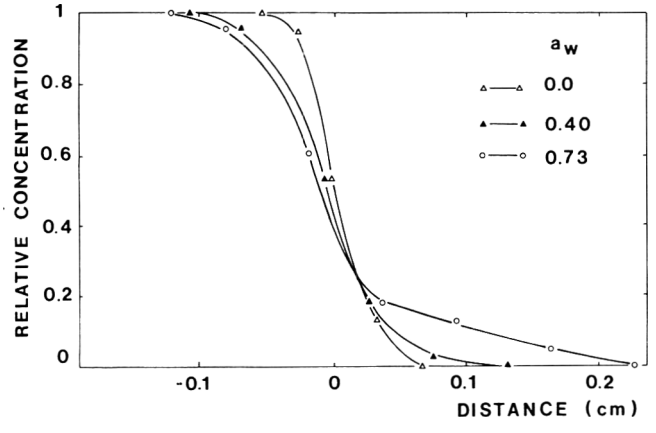

Fig. 6—Diffusion curves at 25°C of TP after 45 days in the PO-free model system at different water activities.

Table 1—Numerical values of the TP diffusion data given in Fig. 7 as related to the corresponding water content of the diffusion systems with and without PO at 50°C

Model system	a_w[a]	Water content[b]	D x 10^{10} (cm^2 sec^{-1})
Without PO	0.0	0.0	36
	0.14	3.6	45
	0.31	5.9	88
	0.48	7.9	120
	0.65	9.9	260
	0.82	12.4	350
	0.96	21.3	830
With PO	0.0	0.0	25
	0.16	3.9	41
	0.33	6.1	40
	0.52	8.4	62
	0.71	10.8	77
	0.86	13.7	87
	0.94	18.8	137

[a] As measured by means of the calibrated Sina hygrometer
[b] Expressed as g H_2O/100g fat free dry solids

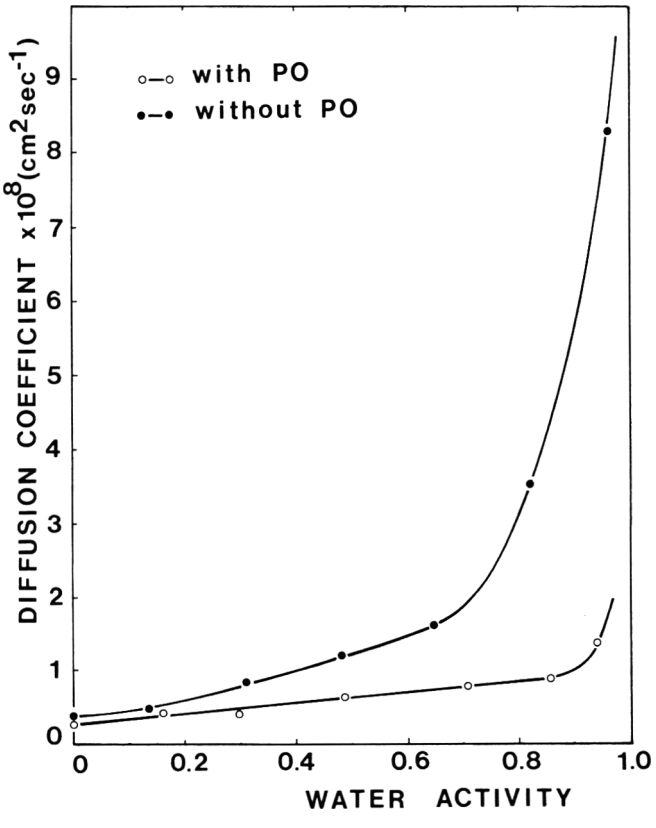

Fig. 7—Diffusion coefficients of TP at 50°C in the model system with and without PO as a function of water activity.

agitated by Brownian motion as a result of the thermal agitation of the water molecules.

Before analyzing the influence of a_w on diffusion, it is of interest to consider the markedly higher diffusion of TP in the PO-free model system (Fig. 2 and 4 as compared to Fig. 5 and 6 respectively; also Fig. 7 and Table 1). We have previously reported that the diffusion of a large molecule such as TP in the PO-containing system is remarkably high (40×10^{-10} cm^2 sec^{-1} at a_w 0.33 and 77×10^{-10} cm^2 sec^{-1} at a_w 0.71), probably due to a relative small interaction between TP and the matrix (Naesens et al., 1981). The still higher mobility of TP in the absence of PO (Table 1) is surprising because one could expect that TP is not transported in such a system as single molecules but rather as multimolecular aggregates. In view of the above proposed diffusion mechanism, the lower diffusion rate of TP in the PO-containing system may be due to the affinity of TP for the PO phase and thus to the presumed high viscosity of the PO-TP system, even at 50°C.

It should also be mentioned that it is surprising that TP in a PO-free medium and equilibrated above P_2O_5 (a_w 0.0) is still mobile. Although all manipulations of the diffusion set-up were carried out as fast as possible, it was unavoidable to prevent water-sorption, albeit in trace amounts, by such extremely hygroscopic system. It should however be stressed, as we checked, that the amount of sorbed water remained definitely below the BET-monolayer value of the system, which is about 4.9g water/100g fat free dry solids. Diffusion below the BET-monolayer has never been measured by the NMR-based technique described by Duckworth et al. (1976). This may be indicative of the lack of sensitivity of the latter method at very low moisture content.

Influence of water activity

The extent to which moisture can support the mobility of food components is undoubtedly strongly influenced by the extent to which water is bound to the matrix. The most widely used measure to characterize the degree of water binding within foods is the relative water vapor pressure which, as has been indicated by Gal (1967), approximates quite well the thermodynamic concept of a_w. Therefore, it is logical to consider diffusion in terms of a_w: the higher a_w, the higher the random motion of the water molecules which results in a higher Brownian motion of the diffusants. Alternatively, the enhanced diffusion at higher a_w may also be explained by a decrease in viscosity of the medium as was done by Lee and Labuza (1975) and by Duckworth et al. (1976) for water soluble constituents. In this context, it is remarkable that many of the diffusion data in literature are related to moisture content instead of water activity. This is especially true since it is generally agreed that the progress of reactions during drying and subsequent storage, which are related to the diffusional behavior of the reactants, is best represented in terms of a_w. Moreover, it has been observed that solute mobility as measured by ESR (Simatos et al., 1981) and NMR (Duckworth, 1981) is related to a_w rather than to moisture content. For this reason, the data in this study are mainly related to a_w.

From our results, it is apparent that the diffusion of the hydrophobic molecule TP increases with increasing a_w. As presented in Fig. 7 and Table 1, this is especially pronounced in a system free of PO. This observation is in agreement with our view on the diffusion behavior of TP and the influence of PO on it. When TP is withdrawn by PO from the water present in the system, its mobility becomes to a minimal extent dependent on a_w. On the other hand, TP in a system free of PO is highly influenced by the degree of freedom of the water molecules as characterized by a_w. As shown by Fig. 7, the diffusion coefficient of TP in the PO-free system increases almost exponentially as related to a_w. Such an exponential relationship can also be deduced from the data on water and aceton diffusion at 21.5°C in a similar system given by Menting et al. (1970), but the exponential increase of these components are even more pronounced. This may be the case with all water soluble components as compared to the hydrophobic TP.

The mechanism by which diffusion is controlled by water may also be approached from another angle. It is well-known that water plays a key role in the retention of organic volatiles. The rate of volatiles loss from freeze-dried carbohydrate solutions containing entrapped volatiles is greater at higher humidities and reaches a level of retention which is inversely proportional with the equilibration a_w. This phenomenon has been interpreted in terms of the ability of water to disrupt the hydrogen-bonded network formed by the hydroxyl groups of the carbohydrate matrix, allowing in this way the volatiles to escape from the so-called microregions (Flink and Karel, 1972; Flink, 1975). Further, the observed retentions are in agreement with a mathematical analysis based on an increase of the diffusion coefficients with an increase in the moisture content (Omatete and King, 1978). Similarly, the diffusion of TP in the dried model system may be considered to be strongly dependent on the capability of water to loosen the hydrogen-bonded network by a dynamic process of formation disruption of hydrogen bonds.

The influence of water on the diffusion of food components is presumably much more complex than the conclusions that can be deduced from the ideas set forward above. The diffusion may also be influenced by changes in the characteristics of both the diffusant and the supporting matrix as related to hydration. The actual concentration, the charge or the physical state of the diffusant may change with a_w. The structural framework of the matrix may be altered by swelling, plasticizing or collapse phenomena. Also, the interaction capacity of the matrix for the diffusant may be different at different hydration levels.

It can be concluded from this that water has a multifarious impact on the diffusional behavior of food components. Since all diffusants and all foodstuffs as diffusion mediums have their own special characteristics, it is likely that there is no simple relationship between mobility and a_w. It is worthwhile mentioning the suggestion put forward by Simatos et al. (1981) that solute mobility may be a more direct criterion for food stability than a_w. From this point of view, the growing interest for data on the mobility of food components in relation to stability of foods is quite understandable (Duckworth et al., 1976; Duckworth, 1981, Naesens et al., 1981; Simatos et al., 1981).

Relation to enzymatic activity

Brockmann and Acker (1977, 1978) claimed the mobility of the substrate to be the determining factor in the enzymatic reactions in dry systems. The results presented here will be further discussed in the perspective of this hypothesis.

The increase in diffusion mobility of TP with rising a_w is in accordance with the work of Acker and co-workers carried out on lipase and lipoxygenase (Acker and Wiese, 1972a, b; Brockmann and Acker, 1977). Although they did not provide proof, these authors nevertheless put forward quite rightly the universal role of water as a vehicle, even for hydrophobic substrates. On the other hand, it is not straight-forward from these observations that there is a relation between diffusion and enzyme activity. Some serious drawbacks as to such relation can be stated. Acker and Wiese (1972a, b) claim a low activity of lipase on TP to be due to the low mobility of the solid fat. Our results however show a reasonable diffusion of TP at all water

activities, even below the BET-monolayer for water, a region where Acker did not observe enzymatic activity. It must be kept in mind that the enzymatic activity on a solid interface, as is the case with TP, is very low as compared to a liquid one. It has been shown by Desnuelle and Savary (1963) that a liquid substrate is a prerequisite for a good activity of lipase in a liquid medium. This is consistent with strong evidence that there is a need for a disordered hydrocarbon region in order to achieve hydrophobic bonding between proteins and lipids (Rand, 1976). TP is thus a poor substrate for lipase, unless it is incorporated in mixed glycerides of lower melting point. This finding has also been observed by Acker and co-workers in dehydrated systems, but it was interpreted in a different way (Acker and Wiese, 1972a, b). The rise in activity observed by Brockmann and Acker (1978) after incorporation of PO or glycerol in the system may be explained on the same basis of liquidity at the interface. This is further supported by the observation of a decreased mobility of TP after addition of a so-called mobiliser as PO.

Finally as can be concluded from this study it is not likely that the minor enzymatic activity at low moisture content is only due to an immobility of the substrate. In this context, it should be mentioned that several authors have suggested that enzymes at low a_w have no optimal hydration and conformation in order to be catalytic efficient (Purr, 1966; Drapron, 1972; Silver, 1976).

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(Vakaleris et al., 1962) and cheese made from retentates (Sood and Kosikowski, 1979).

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Effect of Bile Salts and Bile Salt Mixtures on the Recovery of *Yersinia enterocolitica*

C. L. YU, M. C. HARMON, and B. SWAMINATHAN

ABSTRACT

The effect of sodium salts of cholic, deoxycholic, chenodeoxycholic, glycocholic and taurocholic acids and of Bacto-bile salts, Bacto-bile salts #3 and Bacto-oxgall on the recovery of nine strains representing seven serotypes (0:3, 0:8, 0:9, 0:11, 24, 0:12, 25, 0:16, 0:17) of *Yersinia enterocolitica* was determined. Serotypes 0:3, 0:8, 0:9 and 0:17 were resistant to 3% sodium taurocholate, 3% Bacto-bile salts or 3% Bacto-oxgall. Serotypes 0:11, 23, 0:12, 25, 0:16 and 0:17 were significantly inhibited by 3% sodium deoxycholate, 2.5% chenodeoxycholate and 3% Bacto-bile salts #3. Differences in the inhibitory effects of bile salt mixtures could not be explained only on the basis of their bile acid composition as determined by gas-liquid chromatography.

INTRODUCTION

YERSINIA ENTEROCOLITICA is emerging as an organism of considerable public health significance in various parts of the world, most notably Europe, Canada, South Africa and Japan. *Y. enterocolitica* has been isolated from the environment (Schiemann, 1978) and a variety of foods such as milk and milk products, meats and poultry, and vegetables. (Aldova et al. 1975, Black et al. 1978, Hanna et al. 1976, Schiemann and Toma, 1978). Therefore, food may play a role in the transmission of *Y. enterocolitica* to humans.

Methodology for the isolation of *Y. enterocolitica* from foods is still in a stage of active development. There are no good selective enrichment media for the isolation of *Y. enterocolitica* at this time. However, several selective differential plating media have been developed for *Y. enterocolitica*. Many of these selective media contain bile salt mixtures or a component of bile, such as deoxycholate, to inhibit interfering bacteria. Wauters (1973) reported that *Y. enterocolitica* serotype 0:3 and 0:9 were resistant to as high as 3% deoxycholate and recommended that Salmonella-Shigella agar supplemented with 2% deoxycholate be used for the isolation of *Y. enterocolitica* from clinical specimens. Mehlman et al. (1978) recommended that phosphate buffered saline containing 0.15% Bacto-bile salts #3 and 1% sorbitol be used for the selective enrichment of *Y. enterocolitica* from foods. However, a recent report by Restaino et al. (1980) indicated that some serotypes of *Y. enterocolitica* may be sensitive to even low concentrations of Bacto-bile salts #3.

The objective of this investigation was to determine the effect of salts of bile acids and of commercially available bile salt mixtures on the recovery of typical and atypical strains of *Y. enterocolitica*.

EXPERIMENTAL

Bacterial strains

Nine strains of *Y. enterocolitica*, representing seven serotypes were used in tihis study. Sources of these strains are shown in Table 1. Serotypes 0:3 and 0:9 are the predominant pathogenic serotypes in Europe, Canada, Japan, and South Africa. Serotype 0:8 is primarily confined to the U.S. Serotypes 0:11, 24, 0:12, 25, 0:16 and 0:17 are representative of atypical strains isolated from water and many foods. Stock cultures were grown and maintained on tryptic soy agar slants at room temperature (25°C). Inocula were prepared by transfering a loopful of stock into 5 ml tryptic soy broth and incubating for 24 hr. at 25°C.

Chemicals

The effect of five sodium salts of bile acids and three commercial bile salt mixtures on the recovery of *Y. enterocolitica* were compared. These included sodium cholate (Sigma Chemical Company, St. Louis, MO.), sodium deoxycholate (Difco Laboratories, Detroit, MI.), sodium chenodeoxycholate (Calbiochem-Behring Corp., La Jolla, CA.), sodium taurocholate (Difco), sodium glycocholate (Calbiochem), Bacto-bile salts (Difco, Lot No. 620952), Bacto-bile salts #3 (Difco, Lot No. 657562) and Bacto-oxgall (Difco, Lot No. 674301).

Methodology

Sodium taurocholcate, sodium glycocholate and Bacto-oxgall were added to tryptic soy broth (Difco) at 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3% levels before sterilization of the medium by autoclaving. The media were inoculated with one loopful of the appropriate strains of *Y. enterocolitica* and the inoculated media were incubated at 25°C in static condition. The recovery of *Y. enterocolitica* in the media was evaluated by measuring the optical density in a Spectronic 20 spectrophotometer at 520 nm after 12, 24, 36, 48 and 72 hours of incubation. Duplicate experimental trials were performed to obtain the data. Since, a precipitate was formed in media containing chenodeoxycholate after bacterial growth, the effect of chenodeoxycholate on the recovery *Y. enterocolitica* was determined by plating appropriate dilutions of the broth containing different concentrations of chenodeoxycholate on tryptic soy agar. Colonies were counted after incubation for 48 hr at 25°C. Sodium cholate, Bacto-bile salts and Bacto-bile salts #3 were incorporated at 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% levels into tryptic soy agar. One tenth milliliter of a 10^{-6} dilution of a 24-hr culture of *Y. enterocolitica* was spread plated on these media. Sodium deoxycholate was incorporated into brain heart infusion agar at 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% levels and the pH of the medium was adjusted to 7.5 after

Table 1—Strains of Yersinia enterocolitica used in the study

Strain #	Serotype	Source	Remarks*
IP134	0:3	G. Wauters, Belgium	*Y. enterocolitica sensu stricto**
731707	0:8	T. J. Quan, Colorado	*Y. enterocolitica sensu stricto**
721621	0:8	T. J. Quan, Colorado	*Y. entercolitica sensu stricto**
288-77	0:8	M. Shayegani, N.Y. State Dept. of Health	*Y. entercolitica sensu stricto**
IP383	0:9	G. Wauters, Belgium	*Y. entercolitica sensu stricto**
IP891	0:11, 24	G. Wauters, Belgium	*Y. kristensenii*
IP490	0:12, 25	G. Wauters, Belgium	*Y. kristensenii*
IP1475	0:16	G. Wauters, Belgium	*Y. frederiksenii*
IP855	0:17	G. Wauters, Belgium	*Y. intermedia*

* These organisms have recently been classified into four species (Brenner et al., 1980).

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sterilization to keep deoxycholate in solution. Triplicate plates were used in each trial (Table 2).

Evaluation of modified CIN media

Pure cultures. Cefsulodin-irgasan-novobiocin (CIN) agar (Schiemann, 1979) was modified respectively by incorporating 2% sodium cholate, 3% sodium taurocholate, 3% Bacto-bile salts or 3% Bacto-bile salts #3 instead of 0.2% Bacto-bile salts. For each bile salt or bil salt mixture, the selected level represented the highest concentration tolerated by a majority of strains of *Y. entercolitica* in pure culture studies. One tenth milliliter of a 10^{-6} dilution of a 24 hr. culture of *Y. enterocolitica* was spread plated on these media and colonies were counted after incubation for 48 hr at 25°C. Brain heart infusion agar was used as the control medium for comparisons.

Mixed cultures in pork. CIN agar containing 3% Bacto-bile salts was further evaluated for its efficiency in the isolation of selected *Y. enterocolitica* serotypes (three strains of 0:8 and one of 0:12, 25) from experimentally inoculated ground pork. Ground pork homogenate was prepared by homogenizing 50g of fresh ground pork (obtained from a retail store in Lafayette, IN) with 450 ml of Butterfield's phosphate buffer (PBS) in a Waring blender at low speed for 2 min. The homogenate was filtered through two layers of cheesecloth and the filtrate was dispensed aseptically in 1 ml amounts into sterile 18 x 150 mm test tubes. Two milliliters of a 10^{-4} dilution of a 24-hr culture of *Y. enterocolitica* (approx. 10^4 cells) and 2 ml of sterile PBS were added and the contents of the tube were mixed. The mixture culture (0.02 ml) was streaked on three plates of each of CIN agar and CIN agar prepared with 3% bile salts. The plates were incubated at 25°C for 48 hr and were examined for the presence of *Y. enterocolitica* like colonies and for the extent of the presence of interfering organisms.

Gas chromatographic analysis of bile salt mixtures

The components of the commercial bile salt mixtures were separated and identified by a modification of the method of Brydon et al. (1979). The bile salt mixtures were deconjugated by cholylglycine hydrolase and extracted with diethyl ether and ethyl acetate. Extracted bile acids were methylated with diazomethane generated from a mixture of MNNG (n-methyl-N-nitroso-N'-nitroguinidine)-ether-NaOH. The methyl esters were acetylated for 2 hr at 0°C with 0.5 ml of a freshly prepared mixture of acetic acid-acetic anhydride-70% $HClO_4$ (Story, 1981).

Separation of the methyl acetate derivatives was carried out on a Hewlett Packard 5710A Gas Chromatograph equipped with 6 ft glass column packed with 3% SP-2100 on 100/120 mesh Supelcoport. Nitrogen was used as carrier gas at a flow rate of 30 ml/min. The temperature program was initiated at 230°C and increased at a rate of 1°C/min to a final temperature of 260°C. The bile acids were identified by comparing the retention times of components of bile salts with those of standards using 23-nordeoxycholic acid as the internal standard.

RESULTS

THE EFFECT of sodium taurocholate, sodium glycocholate and Bacto-oxgall on the recovery of *Y. entercolitica* was evaluated by comparing the optical density of inoculated media in different concentrations with that of controls. (Table 3) Sodium taurocholate had no significant inhibitory effect except that serotypes 0:8, 0:11, 24 and 0:16 were inhibited to 18%, 23%, and 24% of the numbers obtained in the control medium, respectively, by 3% taurocholate. Serotype 0:3 and 0:17 were resistant to 2.5% glycocholate, while serotypes 0:8 and 0:16 were inhibited to the extent of 29% and 32%, respectively, by 2.5% glycocholate. The recovery of serotypes 0:9, 0:11, 24, and 0:12, 25 were inhibited to the extent of 20% by 2.5% glycocholate. Bacto-oxgall had an effect similar to that of sodium taurocholate. Only serotypes 0:8, 0:11, 24, and 0:16 were slightly (less than 20%) inhibited by 3% Bacto-oxgall in tryptic soy broth.

The recovery of *Y. enterocolitica* in media containing sodium cholate, sodium deoxycholate, sodium chenodeoxycholate, Bacto-bile salts and Bacto-bile salts #3 were determined (Tables 4–8). Percent recovery was defined as the ratio of the number of colonies observed on the test medium to the number of colonies observed on the control medium. Sodium cholate had a moderately inhibitory effect on the typical strains at the 2% level and was highly inhibitory to serotype 0:11, 24 even at the 1% level (Table 4). Sodium deoxycholate showed slight to moderate inhibition on the three typical strains, but was highly inhibitory to all the atypical strains at the 0.5% level (Table 5). Sodium chenodeoxycholate was the most inhibitory of the salts of bile acids examined in this study (Table 6). Only serotype 0:8 was resistant to 2.5% chenodeoxycholate. Serotypes 0:11, 24, 0:12, 25 and 0:16 were completely inhibited by 0.5% chenodeoxycholate in tryptic soy broth. Bacto-bile salts had slight inhibitory effects on the recovery of serotype 0:3, 0:8, 0:9 and 0:17, and moderate inhibitory effects on the recovery of 0:11, 24, 0:12, 25 and 0:16 (Table 7). Bacto-bile salts #3 at 3% concentration was highly inhibitory to serotype 0:8 and the atypical strains of *Y. enterocolitica* (Table 8). Serotype 0:9 showed the greatest resistance to Bacto-bile salts #3.

—Continued on next page

Table 3—Effect of sodium taurocholate, sodium glycocholate, and Bacto-oxgall on the recovery of Y. enterocolitica

Strain	Percent recovery[a] 48 hr @ 25°C		
	3% Sodium taurocholate	3% Sodium glycocholate	3% Bacto-oxgall
0:3	108	102	113
0:8 (731707)	82	71	83
0:9	98	78	118
0:11, 24	77	80	81
0:12, 25	93	80	91
0:16	76	68	82
0:17	111	97	110

[a] Percent recovery = [(O.D. in test medium)/(O.D. in control medium)] x 100

Table 2—Parameters used to determine inhibition of Y. enterocolitica

Media	Bile acid	Concentration (%)	Criteria for measuring inhibition
Tryptic soy broth	Sodium taurocholate	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0	Spectronic 20 (A_{520})
	Sodium glycocholate	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0	
	Bacto-oxgall	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0	
Tryptic soy agar	Chenodeoxycholate	0, 0.5, 1.0, 1.5, 2.0, 2.5	Plate counts
	Sodium cholate	0.25, 0.5, 0.75, 1.0, 1.5, 2.0	
	Bacto-bile salts	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0	
	Bacto-bile salts #3	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0	
Brain heart infusion	Sodium deoxycholate	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0	Plate counts

Table 9 shows the counts of *Y. enterocolitica* on various formulations of CIN agar for different strains. Serotype 0:12, 25 was completely inhibited on CIN agar containing 3% bile salts #3. Statistical analysis showed that there were significant differences between the strains and also between the formulations of media tested. Newman-Keul's range test indicated that there was no significant difference ($p < 0.05$) between the number of colonies formed by strains of *Y. enterocoliticia* on CIN agar containing 3% bile salts and the regular formulation of CIN medium. However, there were significant differences between CIN + sodium cholate, CIN + sodium taurocholate, CIN + bile salts #3 and CIN media.

Outgrowth of background organisms from pork was also observed on CIN agar containing 3% bile salts. Increasing the bile salt concentration in CIN agar did not result in increased inhibition of competing pork organisms (data not presented).

The relative concentrations of different bile acids in the three bile salt mixtures are shown in Table 10. Bacto-bile salts consisted primarily of chenodeoxycholate, hyodeoycholate and α-muricholate, and was very low in cholic and deoxycholic acids. In contrast, Bacto-oxgall and Bacto-bile salts #3 were high in deoxycholic and cholic acids.

Table 4—Effect of sodium cholate on the recovery of Y. enterocolitica

	Percent recovery[a] 48 hr @ 25°C					
	Conc of Sodium cholate (g/100 ml)					
Strain	0.25	0.5	0.75	1.0	1.5	2.0
0:3	–[b]	89	–	76	77	73
0:8 (731707)	–	91	–	79	76	62
0:9	–	89	–	81	69	54
0:17	–	86	–	80	65	43
0:11, 24	92	56	22	17	–	–
0:12, 25	79	66	44	48	–	–
0:16	95	80	73	56	–	–

[a] Percent recovery (PR) = CFU/plate on test medium/CFU/plate on control medium x 100
PR = 91—100, nonsignificantly inhibited; 75—90, slightly inhibited; 50—74, moderately inhibited; <50, highly inhibited.
[b] No value determined.

Table 5—Effect of sodium deoxycholate on the recovery of Y. enterocolitica

	Percent recovery[a] 48 hr @ 25°C					
	Conc of deoxycholate (g/100 ml)					
Strain	0.5	1.0	1.5	2.0	2.5	3.0
0:3	90	94	96	91	88	83
0:8 (731707)	72	79	79	72	78	72
0:9	57	59	82	73	67	71
0:11, 24	6	7	8	11	4	5
0:12, 25	0	0	0	0	0	0
0:16	5	2	5	5	6	6
0:17	5	3	0	0	0	0

[a] Percent recovery = $\frac{\text{CFU/plate on test medium}}{\text{CFU/plate on control medium}} \times 100$

Table 6—Effect of sodium chenodeoxycholate on the recovery of Y. enterocolitica

	Percent recovery[a] 48 hr @ 25°C				
	Conc of Sodium chenodeoxycholate (g/100 ml)				
Strain	0.5	1.0	1.5	2.0	2.5
0:3	110	110	101	12	13
0:8 (731707)	51	121	86	69	93
0:9	3	6	9	6	7
0:11, 24	0	0	0	0	0
0:12, 25	0	0	0	0	0
0:16	0	0	0	0	0
0:17	25	19	6	0	0

[a] Percent recovery = $\frac{\text{CFU/plate on test medium}}{\text{CFU/plate on control medium}} \times 100$

DISCUSSION

BILE SALT INHIBITION of *Y. enterocolitica* is dependent on the serotype and the chemical nature of bile salt. Typical strains of *Y. enterocolitica,* serotypes 0:3, 0:8 and 0:9, were tolerant to Bacto-bile salts and sodium taurocholate. This result agrees with the report of Schiemann (1980). Chenodeoxycholate, deoxycholate and Bacto-bile salts #3 were highly inhibitory to the atypical strains of *Y. enterocolitica,* even at low concentrations. A selective medium developed recently by Soltesz et al. (1980) includes 1.6% deoxycholate. Since many of the food isolates of *Y. enterocolitica* belong to serotypes other than 0:3, 0:8 and 0:9, caution must be exercised in using high concentration of deoxycholate and Bacto-bile salts #3 in selective media for the isolation of *Y. enterocolitica* from foods. Bacto-oxgall and Bacto-bile salts showed slight or no inhibitory effect. The data from this investigation indicate that it would be prudent to use Bacto-oxgall or Bacto-bile salts in place of sodium deoxycholate and Bacto-bile salt #3 in selective media for the isolation of *Y. enterocolitica* from foods and environmental samples, since many of the food and environmental isolates do not belong to serotypes 0:3, 0:8, and 0:9. Serotypes other than 0:3, 0:8, 0:9 and 0:5, 27 generally do not cause gastrointestinal infections although they may cause extraintestinal infections (Bercovier et al. 1980).

Table 7—Effect of Bacto-bile salts on the recovery of Y. enterocolitica

	Percent recovery[a] 48 hr @ 25°C					
	Conc of Bacto-bile salts (g/100)					
Strain	0.5	1.0	1.5	2.0	2.5	3.0
0:3	89	80	85	84	77	78
0:8 (731707)	98	93	90	89	85	86
0:9	102	100	103	90	93	82
0:11, 24	127	105	90	83	68	64
0:12, 25	116	114	102	92	83	73
0:16	102	87	87	77	63	57
0:17	134	145	128	115	102	86

[a] Percent recovery = $\frac{\text{CFU/plate on test medium}}{\text{CFU/plate on control medium}} \times 100$

Table 8—Effect of Bacto-bile salt #3 on the recovery of Y. enterocolitica

	Percent recovery[a] 48 hr @ 25°C					
	Conc of Bacto-bile salts #3 (g/100 ml)					
Strain	0.5	1.0	1.5	2.0	2.5	3.0
0:3	101	87	75	64	62	44
0:8 (731707)	81	74	43	46	43	15
0:9	137	119	72	87	72	82
0:11, 24	29	11	11	9	5	1
0:12, 25	49	12	7	1	0	0
0:16	42	26	14	12	11	4
0:17	47	31	17	15	6	6

[a] Percent recovery = $\frac{\text{CFU/plate on test medium}}{\text{CFU/plate on control medium}} \times 100$

Table 9—Effect of different bile components incorporated into CIN[a] agar on the recovery of Y. enterocolitica

Medium	Count (CFU/plate) Strain 0:8 (731707)	0:8 (721621)	0:8 (288-77)	0:12, 25	0:17
Brain-Heart Infusion Agar (BHI) (control)	216.7 ± 5.8[b]	138 ± 7.6	157 ± 7.5	167 ± 7.1	149.3 ± 6.4
CIN	185.7 ± 13.6	145.3 ± 8.6	128.3 ± 5.5	107.3 ± 3.1	150.3 ± 11.4
CIN + 2% Sodium cholate (CIN + SC)	181.7 ± 6.4	119.3 ± 9.9	88 ± 9.2	93 ± 7.6	94 ± 11.8
CIN + 3% Sodium taurocholate (CIN + TC)	169.7 ± 5.5	100.3 ± 9.1	90.3 ± 7.5	71 ± 7.0	96.3 ± 6.6
CIN + 3% Bacto-bile salts (CIN + BS)	224 ± 8.2	130 ± 8.9	122.7 ± 9.3	129.0 ± 9.0	136 ± 9.2
CIN + 3% Bacto-bile salts #3 (CIN + BS #3)	188 ± 8.5	96.3 ± 10.1	81.3 ± 8.1	0	193 ± 5.5

Newman-Keul's range test for difference between media ($p < 0.05$). Values under the same line are not significantly different from each other.

BHI	CIN + BS	CIN	CIN + SC	CIN + TC	CIN + BS #3
165.8	148.3	143.4	115.3	105.5	77.0

[a] CIN Equals Cefsulodin-irgasan-novobiocin agar
[b] Mean ± standard deviation (3 plates)

Conjugated bile acids had less inhibitory effect than the deconjugated bile acids. Hill and Drasar (1968) reported that many human intestinal bacteria degraded bile salts. Ferrari et al. (1980) reported that 52 strains of *Bifidobacterium* deconjugated bile acids. The deconjugation of bile acids by *Y. enterocolitica* needs further investigation.

CIN agar is a selective differential medium developed recently for the isolation of *Y. enterocolitica* from foods. The regular formulation of CIN agar contains 0.2% Bacto-bile salts. After incorporating different bile components into CIN agar and evaluating their effects on the growth of *Y. enterocolitica,* Bacto-bile salts yielded optimal recovery of *Y. enterocolitica* from CIN agar plates. However, increasing the concentration of Bacto-bile salts in CIN agar did not inhibit the growth of competing pork organisms.

Gas chromatographic analysis of bile salt mixtures indicated that Bacto-bile salts were high in chenodeoxycholate and low in deoxycholate, but, Bacto-oxgall and Bacto-bile salts #3 were high in deoxycholate. Since chenodeoxycholate and deoxycholate are highly inhibitory to *Y. enterocolitica,* these compounds may be present in Bacto-bile salts and Bacto-oxgall in some complexed form that negates the inhibitory effect of such compounds. Therefore, the inhibitory effect of commercial bile salt mixtures cannot be explained only on the basis of their bile acid composition.

Table 10—Relative percentages of different bile acids in commercial bile salt mixtures

Bile acid	Bile salt mixture Bacto-bile Salts	Bacto-bile salts #3	Bacto-oxgall
Lithocholic	2.2	–[a]	–
Deoxycholic	1.1	41.7	44.7
Chenodeoxycholic	48.9	2.9	4.5
Cholic	0.4	53.0	48.8
Hyodeoxycholic	25.8	2.0	–
α-muricholic	21.6	0.6	–

[a] Not present

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Relationship Between Static Electrical Conductivity and Unfrozen Water Content in Food Products

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ABSTRACT

The static electrical conductivity measurements on frozen foods such as citrus juice and varieties of cheese showed that both conductivity and unfrozen water content in food products varied directly with temperature. In describing the log σ vs 1/T curve, where σ is the conductivity and T is the absolute temperature, it was found that the linear portion of the curves at low temperature was related to bound water content which was independent of temperature, whereas the non-linear portion in the temperature range between the freezing point and 10 or 35 degrees below the freezing point was related to the unfrozen free water content which was dependent on temperature.

INTRODUCTION

THE PHYSICAL STATE of water in foods influences their quality and stability. The water in a material exists both as bound and free. Bound water or the water that does not freeze (Meryman, 1966) is present in frozen foods: water in bread was not completely frozen until −57°C (Mannheim et al., 1957); a considerable portion of water in egg whites remained liquid at low temperature (Pichel, 1965); pulsed NMR studies of water in frog muscle suggested that the most strongly bound water, about 20% of the total water, did not freeze at temperatures until −80°C (Belton et al., 1972; 1973). Unfrozen water content in food product decreased with the decreasing temperature and increasing total water content (Dickerson, 1969). This decrease of unfrozen water content was significantly predicted from an expression based on freezing point depression of the content (Heldman, 1974).

Electrical conductivity will afford a meaningful term to investigate the physical state of water in frozen foods, because ice crystals have a by no means negligible static electrical conductivity; e.g., at −10°C this conductivity lay in the range $(1.0-1.4) \times 10^{-7}\ \Omega^{-1}\ m^{-1}$ (Bradley, 1957; Jaccard, 1959; Eigen et al., 1964; Durand et al., 1967). The conductivity in ice which is determined by the ionic state (Chan et al., 1965) is a function of temperature to an approximation.

Probably the relationship of static electrical conductivity to temperature results in that of the conductivity to unfrozen water content in food products. The measurements of static electrical conductivity appears to be an excellent way to verify those predictions by Heldman (1974). Moreover, this relationship between the conductivity and unfrozen water content for various products is a valuable contribution to existing literature on frozen foods.

The objective of this investigation is to establish the relationship between static electrical conductivity and unfrozen water content in food products; visual changes of the samples during freeze drying developed this relationship.

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EXPERIMENTAL

Sample

Food varieties such as fresh citrus juice, cottage cheese, and two kinds of cream cheese were used; their total water content lay in the range of 55.7–91.2%. Distilled water was used as the control.

Measurements of static electrical conductivity and temperature

The static electrical conductivity during thawing of the samples was measured. The conductivity change during freezing was estimated by that during thawing because of a super cooling tendency during freezing. One cubic centimeter of each sample was frozen in liquid nitrogen to avoid freeze denaturation of its solids content. As the temperature was raised from −196°C at the rate of 2–6°C/min, direct current in the samples was measured by the galvanometer with the accuracy about 0.4%; these samples were located between platinum electrodes of 1 cm^2 in area, and the static electrical field of 3–50 V/cm was supplied to them with the accuracy of 0.07–0.15%. The conductivity was calculated as the average value of 6–11 measurements of the current.

For temperature measurements, a copper-constantan or type T (Instrumental Soceity of America) thermocouple connected to the digital thermometer was located in the geometrical center of the samples. The measurements were performed with the accuracy about 0.1°C.

Freeze dehydration of food samples

Each food sample was freeze-dried with constant temperature. The temperature on its sublimating and evaporating interface was fixed at several values ranging from −35 to −10°C with the differentials of 0.2, 0.5, or 5.0°C; no heating apparatus was used to control this temperature. The pressure in the drying chamber was automatically controlled with both a variable leaking valve and an electric solenoid valve connected to the pressure meter of the chamber. The heat transfer coefficient at the drying interface varied so rapidly as the inner pressure increased or decreased that the drying temperature increased or decreased. The radiated thermal energy content through the sides of the chamber made of polished stainless steel of 3 mm in thickness, was sufficient to perform the dehydration.

To avoid an increase of temperature while exhausting the chamber which had a volume of 0.021 m^3, air in it was exchanged with water vapor so that a vacuum as low as 0.1 Pa was created within a few seconds. The cold surface of the trap between the chamber and the vacuum pump was cooled below −100°C directly by liquid nitrogen. Vacuum measurements were made by the Pirani gauge.

RESULTS & DISCUSSION

THE STATIC electrical conductivity of frozen food samples decreased with decreasing temperature. The conductivity of distilled water decreased by the factor of 10^4 at the freezing point due to its crystallization. The linear relationship between log σ and 1/T for pure ice was obtained below the freezing point; where σ is the conductivity and T is the absolute temperature. The conductivity of food samples varied in a different manner than that of pure ice below the freezing point (Fig. 1). The log σ vs 1/T curves of food samples had two portions: linear and non-linear (Fig. 2). The linear portion of the curve characterized the decreasing conductivity of ice at low temperature, whereas the change of conductivity of pure ice at the freezing point and the decreasing conductivity of food samples in the nonlinear

portion were associated with decreasing unfrozen free water content.

The higher conductivity of the food samples than that of distilled water or ice in the linear portion at low temperatures (Table 1) indicated that a by no means negligible amount of bound water existed in the samples. Several authors including Charm and Moody (1966) and Duckworth (1971) discussed that a considerable portion of water in food products was held in an immobilized form by the food product components and unfreezable at low temperature.

In many cases, unfrozen water content during freezing decreased with the decreasing temperature in the range between the freezing point and 10 or 20 degrees below the freezing point; these results conducted by Riedel (1949a, b; 1951; 1955; 1956; 1957a, b) using calorimetry and translated by Dickerson (1969) showed that the conductivity in the nonlinear portion decreased in the same manner as the unfrozen water content decreased with decreasing temperature. The large extent of the decreasing conductivity of distilled water at the freezing point and the positive correlation between the residuals of the nonlinear portion to the extrapolated linear portion and unfrozen free water content suggested that the decreasing conductivity in this nonlinear portion characterized the freezing of free water in the total water as shown in Fig. 3.

The following visual changes of the samples during freeze drying developed this relationship of the conductivity to unfrozen water content in the nonlinear portion: citrus juice dried at -27.5 ± 0.5°C (24–28 Pa) in the nonlinear portion was completely collapsed, having a dark

Table 1—Static electrical conductivity of food products at low temperature in the linear portion of the log σ vs 1/T curves.

Sample	Dissolved solids content, %	Freezing point temp, °C	Static elec. conductivity at −40°C, Ω^{-1} m^{-1}
Distilled water	0.0	0.0	1.0×10^{-11}
Citrus juice	8.8	−0.1	6.4×10^{-8}
Cottage cheese	20.2	−1.2	2.4×10^{-8}
Cream cheese A	41.3	−1.8	3.8×10^{-6}
Cream cheese B	44.3	−1.2	1.2×10^{-7}

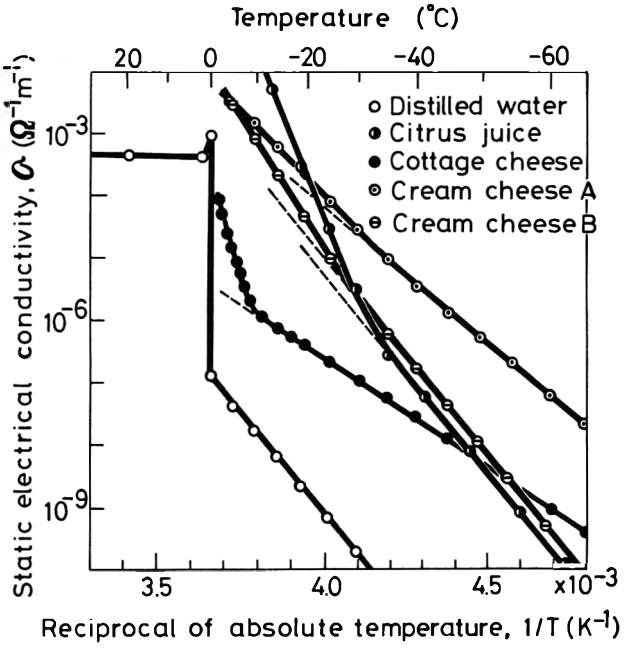

Fig. 1—Relationship between static electrical conductivity and temperature of ice and frozen food products.

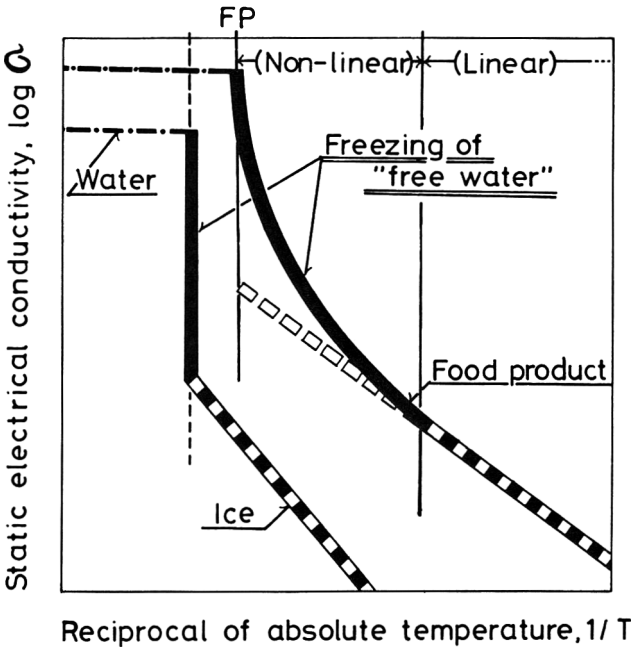

Fig. 2—Linear and nonlinear portions of the log σ vs 1/T curves of food products below their freezing point.

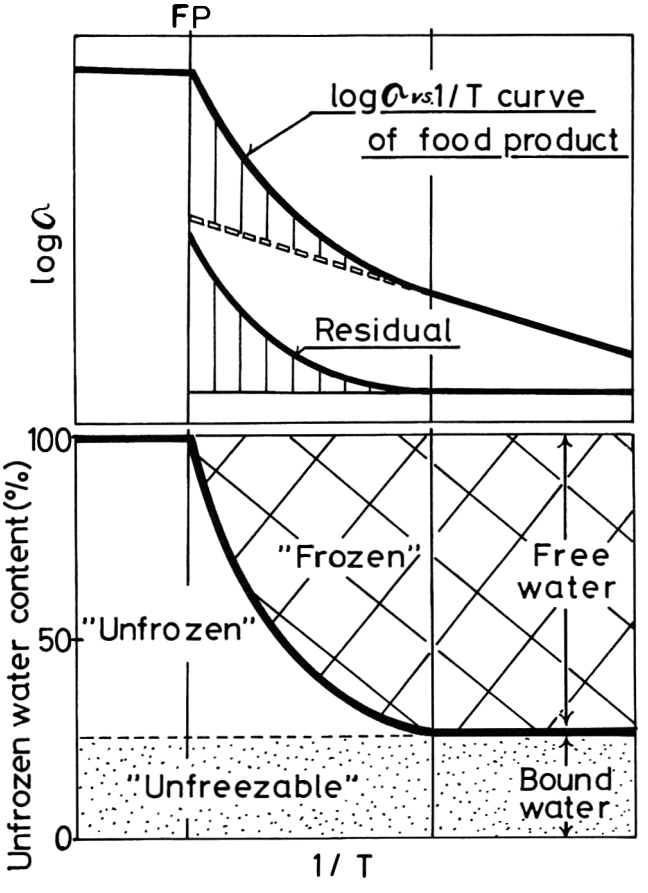

Fig. 3—Illustrative relationship between static electrical conductivity and unfrozen water content in food products.

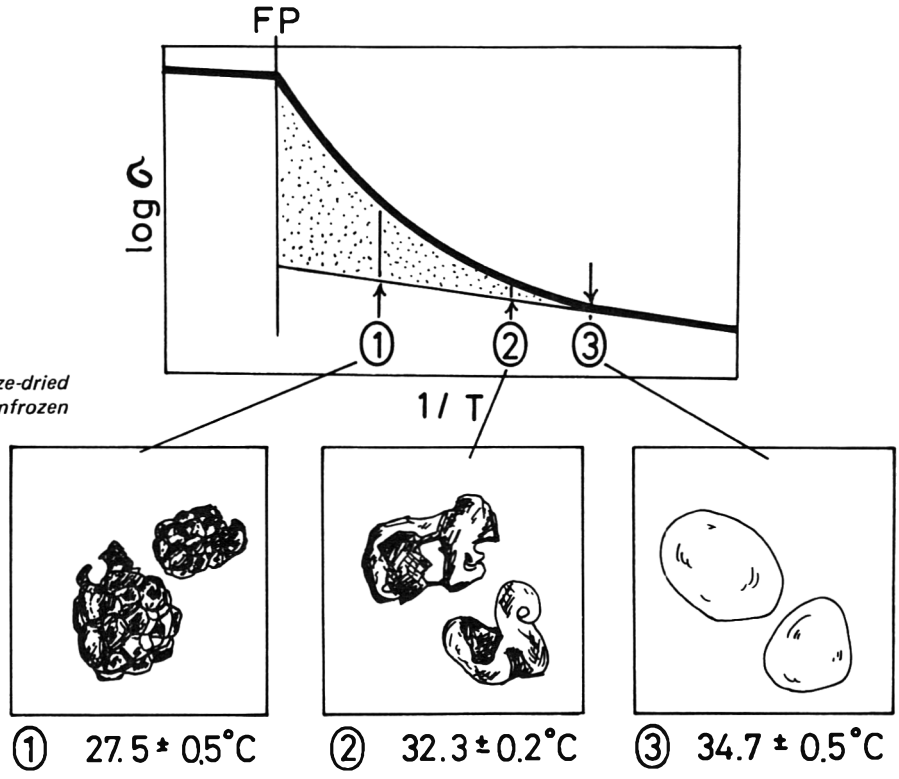

Fig. 4—Collapse tendency of citrus juice freeze-dried at constant temperatures in relation to the unfrozen free water content in it.

orange color and viscous characteristics like rubber; drying at −32.3 ± 0.2°C (15–16 Pa) caused slight collapse; that at the linear portion temperature of −34.7 ± 0.5°C (10–11 Pa), on the contrary, led to no collapse with no changes in appearance (Fig. 4). Because of the magnitude of the visual changes of dried material at nonlinear portion temperatures, citrus juice was selected as one of the food samples. The same realtionship between the structural collapse and freeze drying temperature was obtained through the dehydration of varieties of cheese: cottage cheese dehydrated at as high as −10.0 ± 0.5°C (186–213 Pa) had excellent sensory texture due to no collapse of the solute matrix, because this relatively high drying temperature was in the linear portion of the curve; that of cream cheese varieties at −35 ± 5°C (8–27 Pa) also resulted in excellent sensory preference; cream cheese varieties dried at −20 ± 5°C (33–106 Pa), as opposed to those at lower temperatures in the linear portion, had poor preference due to the shrinkage and oiling off in the solute matrix to a large extent together with less stability than those dried at −35 ± 5°C. Gershenson et al. (1981) concluded in the investigations on structural collapse during rehumidification of freeze-dried tomato juice that freeze-dried tomato juice exposed to a relative humidity of 53% resembled a highly viscous material rather than a solid with visual changes; on the other hand, samples at 7% RH showed no changes in appearance. In general, the unfrozen fraction of free water was evaporated, not sublimated, during the dehydration in the nonlinear portion temperature range so that high relative humidity was made in the matrix by this evaporation resulting in its collapse.

CONCLUSION

BOTH THE STATIC electrical conductivity of food products and their unfrozen water content varied directly with temperature. The linear portion of the log σ vs 1/T curves of the products suggested that a by no means negligible amount of bound water which was independent of temperature existed in them at low temperatures, whereas their nonlinear portion was related to the freezing of the fraction of free water.

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HPLC Analysis of Phytic Acid in Selected Foods and Biological Samples

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ABSTRACT

A rapid high-performance chromatographic (HPLC) method for phytic acid was developed and applied to wheat and rice bran, beans and feces. The method gives more reproducible results than those obtained by other methods tested and is more rapid than methods using ferric chloride precipitation. The phytic acid contents of bran, bean and feces samples were determined by three methods, including the new HPLC method. For most samples, the methods gave significantly different results.

INTRODUCTION

PHYTIC ACID [myo-inositol 1, 2, 3, 4, 5, 6 hexakis (dihydrogen phosphate)] has generally been determined by modification of the method of Heubner and Stadler (1914) which depends upon the ferric chloride precipitation of the phytic acid from extracts. Because these methods (Wheeler and Ferrel, 1971; Andrews and Bailey, 1932; Earley, 1944; Ellis et al., 1977) are time consuming and assume a certain ratio of iron to phytate phosphorus, other methods have been developed. Some methods use ion exchange to separate the phytic acid from other compounds (Caldwell and Black, 1958; Smith and Clark, 1951). Recently, HPLC has been used to quantitate phytic acid (Tangendjaja et al., 1980; Camire and Clydesdale, 1981). This paper describes an improved HPLC method for phytic acid and compares it to the classical method for measuring phytic acid in rice and wheat bran, beans, and feces.

MATERIALS & METHODS

Materials

Phytic acid was obtained as a 40% solution from ICN Pharmaceuticals, Inc., Plainview, NY. The phytic acid solution contained 43.0% phytic acid as determined by the method of Wheeler and Ferrel (1971). Potassium dihydrogen phosphate, sodium acetate, sodium hydroxide and trichloracetic acid (TCA) were analytical reagent grade. High purity water was used in making all mobile phases and extracting solvents. Wheat bran samples were taken from certified American Association of Cereal Chemists (AACC) lots prepared from hard red spring and soft white wheats. Samples of standard lots of California small white and California light red kidney beans were used. The rat feces were collected from gnotobiotic rats fed diets containing AACC soft white wheat bran. The human feces was collected from subjects consuming AACC soft white wheat bran.

Equipment

The HPLC system consisted of a pump (Constrimetric III, Laboratory Data Control, Riviera Beach, FL), injector valve (20 μl loop, Rheodyne, model 7125, Rheodyne Inc., Cotati, CA), column (Spherisorb ODS, 5 mm, 25 cm x 4.6 mm, Applied Sciences, Los Angeles, CA), differential refractive index (RI) detector (Model R401, Water Associates, Inc., Milford, MA) and a recorder (Model 855, E & K Scientific Products, Saratoga, CA). Mobile phase and extracts were filtered through nylon-66 membrane filters (0.2 μm pore size, Rainin Instrument Co., Woburn, MA). Mobile phase was degassed by applying vacuum to a flask in an ultrasonic cleaner (Model B-32, Branson Cleaning Equipment Co., Shelton, CT).

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Methods

Samples (1.0g) ground to pass a 20 mesh screen were weighed into 50 ml centrifuge tubes and 3% TCA solution (25.0 ml) was added. The mixture was mechanically shaken for 1/2 hr at 25°C. Samples were centrifuged at 48,000 x g for 15 min. The supernatant (1.0 ml) was diluted and adjusted to pH 6.0 by adding pH 6.0, 0.5M KH_2PO_4 (9.0 ml). The diluted sample was filtered through a 0.2 μm membrane and a 20 μl aliquot was injected onto the HPLC column.

The mobile phase (KH_2PO_4, 0.025M, pH 6.0) was pumped through the column at a rate of 2 ml/min giving a pressure between 2400 and 2600 psi. All operations were performed at room temperature. The peak height of the RI detector (IX attenuation) response was used to calculate concentration of phytic acid. A calibration curve was prepared from solutions containing 1.7, 3.4, 5.1, and 6.8 μg phytic acid per 20 μL. These solutions were prepared from the stock phytic acid (43.0%) solution by diluting with 3% TCA solution to concentrations of 0.085, 0.17, 0.255 and 0.34% phytic acid. The latter solutions were then diluted to final concentration with KH_2PO_4 (0.5M, pH 6.0).

The effect of pH on the phytic acid analysis was determined by using buffers of various pH levels for pH adjustment and dilution of extracts and as mobile phases. The buffers were formic acid-sodium formate (pH 2.6, 3.0), acetic acid-sodium acetate (pH 3.0, 4.0) and KH_2PO_4 (pH 6.0, 6.5). Buffers for dilution and pH adjustment were 0.5M. Mobile phases were 0.025 to 0.1M.

Three literature methods were compared with the method described herein. The methods of Camire and Clydesdale (1981) and Tangendjaja et al. (1980) used HPLC for quantifying the phytic acid. In the former method phytic acid was precipitated with ferric chloride and the sodium salt of the phytate formed. The sodium phytate was then subjected to HPLC for quantification. In the latter method, the extract is chromatographed (HPLC) without preliminary purification. The other method compared in this study was the Wheeler and Ferrel (1971) modification of the classical ferric chloride precipitation method.

In these studies, three separate extracts of each material were made. Aliquots of each extract were taken for analysis by the three methods. In the HPLC methods, three injections of each extract were made and the mean values calculated.

RESULTS & DISCUSSION

DIRECT ANALYSIS OF TCA extracts using the method of Tangendjaja et al. (1980) gave unreliable phytic acid values because the detector response (peak height) for repeated injections of standard solutions of phytic acid decreased by 25–40% over a period of 1 hr. This decreasing response which occurred even after prolonged equilibration of the HPLC system between injections could result from the deterioration of the column by the low pH (1.5) of the extract. (Spherisorb columns deteriorate at pH levels below 2). Also, conditions of the column could vary because the mobile phase (0.005M sodium acetate) could not sufficiently buffer the TCA extracts.

During the investigation on the effect of pH on the phytic acid analysis, it was found that the most consistent results were obtained at pH 6.0 using a KH_2PO_4 (0.025M) mobile phase. Under these conditions, a 99.96% correlation of peak height to phytic acid concentration was found. A

chromatogram typical for extracts and phytic acid standards and the standard curve is shown in Fig. 1. The regression equation calculated from standard curves prepared on different days were Y = −0.071 + 1.299 X and Y = −0.096 + 1.327 X indicating the similarity in day to day reproducibility. Based on these results, operating at pH 6.0 using 0.025M KH_2PO_4 mobile phase was adopted for the phytic acid assay.

Identification of phytic acid as the peak eluting in 1.1–1.2 min was established by the increase in peak height upon spiking the bran and bean samples with phytic acid before extraction. The recoveries of the added phytic acid were 97–100% for all samples tested. Further confirmation of the identity of the peak was made by hydrolyzing extracts and noting the decrease in peak height. Hydrolysis was accomplished by refluxing at pH 5.2 (de Boland et al., 1975). Refluxing for 6.5 hr and 33 hr in KH_2PO_4 (0.5M) caused the peak height at 1.1–1.2 min to be reduced by 42% and 100%, respectively. The absence of this peak after complete hydrolysis proves that it is only phytic acid. (The hydrolysis products of phytic acid were indicated by appearance of peaks later in the chromatograph.)

Phytic acid contents of various materials were determined by three methods (Table 1) and the data analyzed by analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960). The phytic acid values obtained by the new HPLC method and the Wheeler and Ferrel (1971) method differed except in the case of beans. The new method gave lower values for the human feces but higher values for the rat feces and and the bran samples. Also, the new HPLC method gave higher values, except for human feces, than the method of Camire and Clydesdale (1981). As yet, the differences in values have not been explained but may be attributed to problems inherent in the methods. The problems or disadvantages of earlier methods were discussed in the review by Cheryan (1980). He indicated that there was no published method which is totally satisfactory. The procedure reported herein provides an alternative method which has the following advantages: (1) results obtained by it are more reproducible than those obtained by the method of Tangendjaja et al. (1980) and (2) it is simpler and much more rapid than the methods of Wheeler and Ferrel (1971), Camire and Clydesdale (1981) and other methods which require precipitation of the phytic acid with ferric chloride.

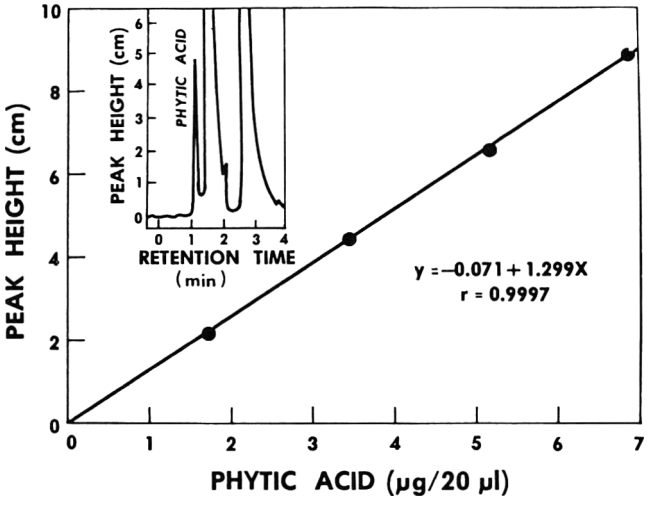

Fig. 1—Standard curve for calculation of phytic acid content. Each point is the mean of three injections. Insert shows a typical HPLC separation.

CONCLUSIONS

THE HPLC METHOD described herein gives consistent and reproducible values for phytic acid content of various food and biological samples. The excellent reproducibility and the rapidity of the method make it a useful method of analysis. Further study is required to determine the basis of difference observed in some samples between the precipitation and HPLC methods.

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Table 1—Phytic acid content of various materials as determined by three methods

Material	Phytic acid content[a] Wheeler & Ferrel Method (1971)	Camire & Clydesdale Method (1981)[b]	New HPLC Method[c]
		%, as is basis	
Brans:			
Hard red spring wheat	4.8a ± 0.05	5.4b ± 0.05	5.9c ± 0.05
Soft white wheat	4.2a ± 0.07	4.6b ± 0.11	4.9c ± 0.11
Stabilized rice	5.4a ± 0.06	7.3b ± 0.06	7.8c ± 0.07
Beans:			
California light red kidney	1.5a ± 0.05	1.2b ± 0.07	1.7a ± 0.07
California small white	0.9a ± 0.04	0.5b ± 0.04	0.9a ± 0.04
Feces (Freeze Dried):			
Rat	3.6a ± 0.09	4.1b ± 0.10	4.9c ± 0.10
Human	3.1a ± 0.08	2.3b ± 0.10	2.6b ± 0.10

[a] Means ± standard error of the mean. Means for a particular material not followed by the same letter are significantly different ($P < 0.05$).
[b] Sodium phytate (Sigma Chemical Co., St. Louis, MO) of 97% purity was used as a standard.
[c] Described in Methods section.

An Examination of the Minimal Water Activity for *Staphylococcus* aureus ATCC 6538 P Growth in Laboratory Media Adjusted with Less Conventional Solutes

GRACIELA VAAMONDE, JORGE CHIRIFE, and OSVALDO C. SCORZA

ABSTRACT

Present work investigated whether or not *Staphylococcus aureus* ATCC 6538 P growth may occur below a_w 0.86 in laboratory media adjusted by addition of less conventional a_w controlling solutes. Solutes examined included, l-proline, l-lysine, β-alanine, sorbitol, KCl, sodium lactate and polyethylene glycols. No growth was observed in any case at or slightly below a_w 0.86; thus Scott's (1953) general conclusion on the lowest limit for *S. aureus* growth remains unchallenged. Results obtained also showed that polyethylene glycols (M.W. 200 and 400) have a significant inhibitory effect on *S. aureus* ATCC 6538 P growth independent of a_w lowering.

INTRODUCTION

THE ABILITY OF *Staphylococcus aurues* to grow aerobically down to a_w 0.86 in laboratory media adjusted with different solutes, is well known (Scott, 1953; Troller, 1971; Lotter and Leistner, 1978). Scott (1953) initially noted that the minimal a_w which would allow staphylococcal growth was independent of the solutes employed to adjust the a_w of the medium. However, it was then observed that in some cases the bacterial response differs at a particular a_w when the latter is obtained with different solutes. In this way it is now more or less established that the a_w of the medium is not the only determining factor regulating the biological response of *S. aureus* but also the nature of the a_w controlling solute plays a role (Plitman et al., 1973; Pawsey and Davies, 1976; Christian, 1981). For instance, *S. aureus* has been reported to be more sensitive to glycerol than to sodium chloride (Marshall et al., 1971). Most of the published data yielding information about solute effects report the minimum a_w in various nutrient solutions at which growth was observed. The solutes most commonly tested have been sodium chloride, sucrose and glycerol, substances most likely to be encountered in high concentrations in conventional or some novel foods of depressed a_w. *S. aureus* is the bacterium having the greatest potential hazard to intermediate moisture foods (IMF). Knowledge of the lowest a_w permitting growth of *S. aureus* is thus of paramount importance when a food is to be preserved by lowering of a_w through addition of solutes. A number of technical reasons indicated that the possibilities of adjusting the a_w of IMF with most food additives in use today, are quite limited; as a consequence, unconventional additives have been explored looking for more adequate a_w controlling solutes (Benmergui et al., 1979). By this reason it is of interest to verify the validity of a_w 0.86 as the lowest limit for *S. aureus* growth even when a_w is adjusted using less usual solutes. Present work investigates whether or not *S. aureus* growth may occur in the close vicinity of 0.86 a_w (at or below) in laboratory media adjusted with less usual a_w controlling solutes.

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MATERIALS & METHODS

Culture

Staphylococcus aureus ATCC 6538 P was the test microorganism. A stock culture was prepared by inoculating a tube of nutrient agar (NA) incubating for 24 hr at 36°C and transferring at weekly intervals. The inocula for growth studies were prepared by transferring a loopful of the stock culture to a tube containing 7 ml of NA slant and incubating the tube for 24 hr at 36°C. The culture was diluted 1:1000 in sterile experimental medium described below and 2 ml were inoculated into 18 ml of sterile experimental medium to obtain approximately 10^4 cells per ml.

Media

Medium employed was Brain Heart Infusion (BHI) from Oxoid Ltd., England; water activity was adjusted to the desired value by adding one or the other of the following solutes: sodium chloride, potassium chloride, β-alanine, l-proline, l-lysine, sorbitol, sodium lactate, polyethylene glycol 200 (PEG-200), and polyethylene glycol 400 (PEG-400). Following dissolution of each solute the pH was restored to about 7.2–7.4 before sterilization by the addition of 5N NaOH or HCl. Media a_w-adjusted with sorbitol, l-proline, β-alanine and l-lysine, were sterilized by filtration in order to avoid undesirable thermally-induced changes; all other media were autoclaved at 121°C for 15 min. Autoclaving was made with precautions to avoid any loss of water by evaporation which could change a_w.

The a_w of the basal medium (BHI) is 0.993 (Christian and Waltho, 1962; Chirife et al., 1981a) and lower a_w levels were obtained by the addition of solute. The amount of each solute required to obtain a particular level of a_w was estimated from previous data on their a_w lowering behavior (Chirife et al., 1980a, b; Chirife and Ferro Fontán, 1980a, b). Water activity was experimentally determined on each different medium after sterilization and good agreement was always found between calculated and measured a_w values.

Materials

PEG-200, PEG-400, sodium lactate (70% w/w) and β-alanine were obtained from BDH Lab. Chem. (Poole, England); l-lysine from Lowens Lab. (Buenos Aires, Argentina); sodium chloride from May and Baker (Dagenham, England); potassium chloride from Mallinckrodt Chem. Works (U.S.A.); sorbitol from Pfanstiehl Lab. Inc. (Illinois, U.S.A.); l-proline from Fluka A.G. (Switzerland).

Growth inhibition studies

Growth studies were performed in 500 ml screwtop glass bottles containing 20 ml of inoculated solute added BHI, which were incubated at 30°C in a constant temperature oven.

Enumeration of cells

Cell numbers in the solute containing media were determined by the use of Plate Count Agar (PCA) pour plates of samples taken at selected times (24, 48, 72 hr, etc.) of incubation. The samples were serially diluted with 0.1% peptone before plating. The PCA plates were incubated at 37°C for 48 hr at which time the colonies were counted.

Determination of a_w

The a_w of the different solute containing media was determined using the "a_w-Wert Messer" manufactured by Firma LUFFT (Stuttgart, West Germany). In order to improve the reliability of the measurements the instrument was operated following the procedure of Chirife and Ferro Fontán (1980b). For this purpose the instrument

was carefully checked against different standard saturated salt solutions in the a_w range of interest to this study and a calibration curve was obtained. The calibration curve was checked every 48 hr. All a_w measurements were made at 25 ± 0.1°C in an air-circulating constant temperature cabinet.

RESULTS & DISCUSSION

TABLE 1 shows literature data on the minimal water activity which would allow *S. aureus* growth in laboratory media adjusted with different solutes; as already mentioned, minimal a_w supporting growth is around 0.86. However, Labuza et al. (1972) showed that pork slurry of an a_w as low as 0.84 (adjusted with glycerol) supported growth of *S. aureus*, and Tatini (1973) also reported slow growth of *S. aureus* in pork of an a_w of 0.83 (adjusted by adding NaCl). Troller (1976) confirming these low a_w limits reported that one out of various strains of *S. aureus* did grow in a protein hydrolizated medium supplemented with beef extract adjusted with NaCl to an a_w of 0.83.

In order to test the behavior of the strain used, the effect of NaCl (a well investigated a_w controlling solute) was first studied. Fig. 1 shows the influence of a_w adjusted by addition of NaCl on growth rate at 30°C of *S. aureus* ATCC 6538 P. In good agreement with a diversity of literature results, it is seen that reduction in a_w increases the lag period before growth, reduces specific growth rate and reduces the maximal number of cells; complete growth inhibition is observed at a_w 0.857. The same Fig. 1 shows that a_w 0.852 adjusted with KCl is inhibitory for the growth of *S. aureus*; the number of cells remained stationary and then decline slowly. Fig. 2 shows the inhibition of *S. aureus* growth in media adjusted with l-proline, β-alanine of l-lysine, to water activities around 0.86. It can be seen that in all three cases (l-proline a_w = 0.851, β-alanine a_w = 0.866, and l-lysine a_w = 0.863) complete inhibition of *S. aureus* growth in the whole incubation period occurs. Number of cells remained almost stationary (β-alanine) or declined slowly throughout the incubation period.

The results obtained with l-proline deserve some comments. Christian (1955) reported that l-proline added exogenously greatly stimulated the growth of *Salmonella oranienburg* in media of low a_w. Since then, various workers further characterized the phenomenon of growth stimulation of bacteria by l-proline in media of inhibitory a_w (Csonka, 1980; Britten and McClure, 1962). It is now known that when bacteria of a wide variety of species are stressed by a low a_w in the growth medium, a common response they exhibit is a pronounced elevation in the intracellular concentration of l-proline (Brown, 1976). In a number of cases in which the bacteria were grown in the absence of exogenously added l-proline this accumulation was due to the enhanced net rate of l-proline synthesis, but in others in which the cells were grown in a complex media it was not clear whether the increase in l-proline concentration were due to the stimulation of the synthesis or uptake.

It was speculated that when l-proline itself was the a_w controlling solute in the medium, a somewhat lower minimal a_w (below 0.86) for growth could be eventually observed. This was not the case, however, as shown in Fig. 2.

Fig. 3 shows the behaviour of *S. aureus* during incubation in media of a_w around 0.86 adjusted either with sorbitol, sodium lactate, PEG-200 and PEG-400. In all cases inhibition of growth is observed; however, the effect of the different a_w controlling solutes is different and deserves a comment. In medium adjusted at 0.862 a_w with sodium lactate, the number of cells remained almost stationary throughout the incubation period. In this regard, the behavior is similar to that displayed when NaCl, KCl, or the amino acids are the a_w controlling solutes. In medium adjusted with sorbitol (a_w = 0.866) the number of cells re-

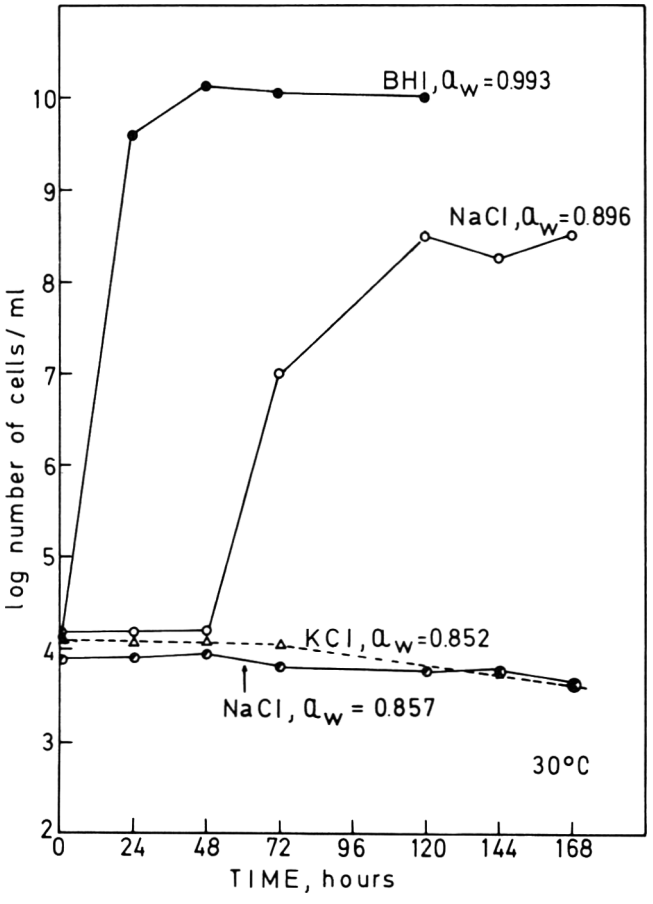

Fig. 1—Effect of a_w on growth of S. aureus ATCC 6538 P at 30°C in BHI containing sodium chloride or potassium chloride.

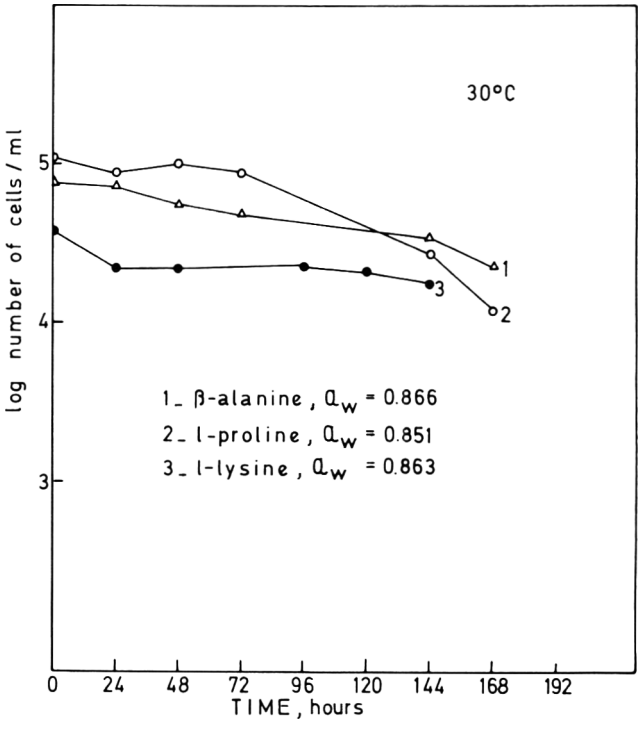

Fig. 2—Inhibition of S. aureus ATCC 6538 P at 30°C in BHI media with added different amino acids.

mained approximately stationary during the first 72 hr, but then a significant decrease was observed. *S. aureus* response to PEG-200 and PEG-400 was different. At 0.858 and 0.855 a_w, respectively, cell counts decreased continuously at a high rate and no counts (NC) were recorded after 48 hr (PEG-200) or 72 hr (PEG-400).

Fig. 4 shows further results on the influence of a_w adjusted by addition of PEG-200 on *S. aureus* growth. At 0.972 and 0.947 a_w there is an initial decrease in the number of cells but after 24 hr (a_w = 0.972) or 48 hr (a_w = 0.947) cell counts started to increase at a significant rate. At 0.922 and 0.904 a_w cell counts decreased continuously. Results with the PEG's resembled those obtained by Plitman et al. (1973) on the effects of aliphatic diols, (1,2-propanediol and 1,3 butanediol) on *S. aureus* growth in a_w lowered BHI broth. They reported that diols have a bactericidal effect which depends on the chemical structure of the humectant molecules. A more detailed study on the effect of polyethylene glycols on *S. aureus* growth is the subject of current investigation.

CONCLUSIONS

NO GROWTH of *S. aureus* ATCC 6538 P was observed in any of the media adjusted to around 0.86 a_w using several less conventional a_w controlling solutes. Thus, Scott's (1953) general conclusion on the lowest limit for *S. aureus* growth remains unchallenged. It is to be noted that only one strain was used in the present work and this one (ATCC 6538 P) is not a food isolate but rather is used primarily for germicide testing. However, as shown in Fig. 1, ATCC 6538 P response to a_w lowering with NaCl is in good agreement with a diversity of literature results using other food strains. Chirife et al. (1981b) recently studied the inhibition of *S. aureus* in sucrose solutions of varied a_w and found that the behavior of strain ATCC 6538 P was similar to that observed with other food strains. A more detailed study on the lowest limit of a_w for growth of *S. aureus* in laboratory media as well as in foods (using several strains) is being carried out.

As a side result of this investigation it is reported that PEG-200 and PEG-400 have a significant antimicrobial effect on *S. aureus* ATCC 6538 P independent of a_w lowering.

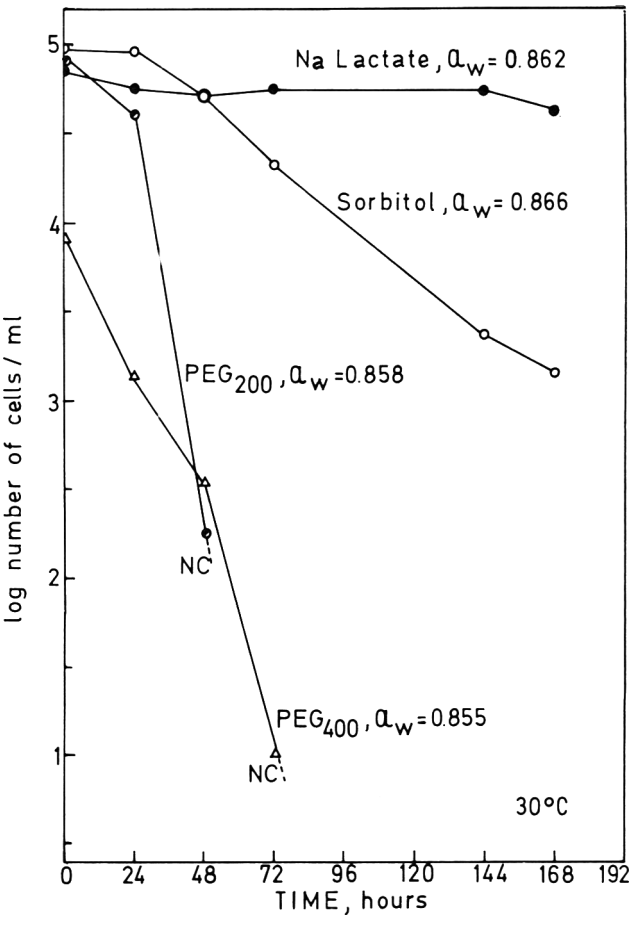

Fig. 3—Innibition of S. aureus ATCC 6538 P at 30°C in BHI media with added different solutes.

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Table 1—Limiting a_w values for inhibition or growth of S. aureus in liquid laboratory media djusted with different a_w controlling solutes

Strain[j]	Solute/s	Temperature (°C)	Limiting a_w	Reference
–	Glycerol	30	0.89[a]	Marshall et al. (1971)
196 E	Glycerol	25	0.87[b]	Kamman et al. (1978)
S-6	Glycerol	25	0.865[b]	Plitman et al. (1973)
196 E	1,2-Propanediol	25	0.96[b]	Kamman et al. (1978)
S-6	1,2-Propanediol	25	0.95[b]	Plitman et al. (1973)
196 E	1,3-Butanediol	25	0.96[b]	Kamman et al. (1978)
S-6	1,3-Butanediol	25	0.97[b]	Plitman et al. (1973)
M 7/1	NaCl:KCl:Na_2SO_4 (5:3:2 mol)	25	0.887–0.870[c]	Lotter and Leistner (1978)
100	NaCl:KCl:Na_2SO_4 (5:3:2 mol)	30	0.864–0.860[d]	Lotter and Leistner (1978)
100	NaCl:KCl:Na_2SO_4 (5:3:2 mol)	25	0.887–0.870	Lotter and Leistner (1978)
M 7/1	NaCl:KCl:Na_2SO_4 (5:3:2 mol)	30	0.864–0.860[d]	Lotter and Leistner (1978)
–	NaCl	37	0.860[a]	Measures (1975)
ATCC 6538 P	Sucrose	35	0.867–0.864[e]	Chirife et al. (1981)
FM 1	Sucrose	35	0.876–0.843[f]	Chirife et al. (1981)
Various	Sucrose	30	0.88–0.86[g]	Scott (1953)
Various	3.44m Sucrose + salts mixture[i]	30	0.86–0.84[h]	Scott (1953)
Various	4.07m Glucose + salts mixture[i]	30	0.88–0.86[g]	Scott (1953)
Various	0.1m Glucose + salts mixture[i]	30	0.88–0.86[g]	Scott (1953)
Various	NaCl:KCl:Na_2SO_4 (5:3:2 mol)	30	0.86–0.84[h]	Scott (1953)

[a] Limiting value for growth
[b] Limiting value inhibitory for growth
[c] Growth observed at 0.887 but not at 0.870 a_w
[d] Growth observed at 0.864 but not at 0.860 a_w
[e] Growth observed at 0.867 but not at 0.864 a_w
[f] Growth observed at 0.876 but not at 0.843 a_w
[g] Growth observed at 0.88 but not at 0.86 a_w
[h] Growth observed at 0.86 but not at 0.84 a_w
[i] NaCl:KCl:Na_2SO_4, 5:3:2 mol
[j] For specifications see original works

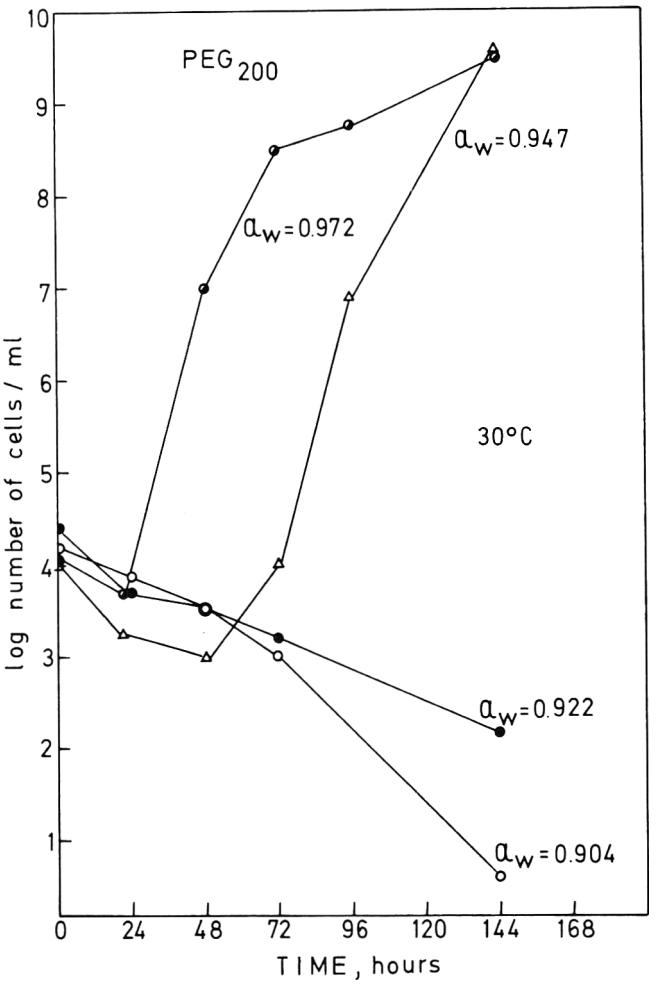

Fig. 4—Effect of a_W on growth of S. aureus ATCC 6538 P at 30°C in BHI medium adjusted with PEG 200.

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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.

Air Classification of Peas (*Pisum sativum*) Varying Widely in Protein Content

R. D. REICHERT

ABSTRACT

Four samples of field peas, ranging in protein content from 14.5–28.5% (dry, dehulled seed basis), were pin milled and air classified yielding protein concentrates and starch concentrates containing 33.6–60.2 and 3.8–11.3% protein, respectively. The protein content of the dehulled peas were negatively correlated with their starch content (49.7–59.8%), lipid content (3.0–4.1%) and cell wall material (CWM) content (7.1–9.6%). The % protein in all air classified fractions was positively correlated to % protein in the dehulled peas, whereas, lipid and CWM content was negatively correlated. Air classification at lower air flows (smaller cut sizes) resulted in protein and starch fractions containing higher levels of protein. Starch separation efficiency and protein separation efficiency generally increased with increasing protein content.

INTRODUCTION

AIR CLASSIFICATION is a means by which particles differing in density and mass are separated in a current of air. This technology has been widely exploited and its application to wheat, barley and malted barley, oats, triticale, rice, sorghum, potatoes and grain legumes has been reviewed by Vose (1978). Since field peas and horsebeans were reported to give the highest protein shift values, air classification technology appears well suited to grain legumes. In Canada, both field peas and Navy beans have been air classified commercially to produce protein concentrates which compete with imported soybean products. The starch concentrates have been utilized in a variety of industrial applications including potash ore refining, adhesives for corrugated board production and manufacture of pressure-sensitive microcapsule coatings for carbonless paper.

Studies of the chemical composition of air-classified fractions from grain legumes have been reported (Tyler et al., 1981; Patel et al., 1980; Sosulski and Youngs, 1979; Reichert and Youngs, 1978; Vose et al., 1976). However, few studies have been undertaken to investigate the effect of variability in chemical composition of the flour on the composition of the fractions. Wu and Stringfellow (1979) studied the air classification of three wheat flours containing 8.2–16.4% protein. They found that the protein content and, generally, the yield of the protein fractions was higher for wheat flours of higher protein content. Similar results have been obtained by others (Jones et al., 1959; Adair and Obermeyer, 1959). Stringfellow et al. (1976) found that a higher protein variety of triticale produced greater yields of the protein fractions which also had a higher proportion of protein. Tyler et al. (1981) air classified eight legume species and found that protein, starch, fat, ash and crude fiber of many of the air-classified fractions were correlated with levels of these constituents in the flour.

In Saskatchewan, a company producing protein and starch concentrates by air classification was hampered by variable and often low-protein peas. For example, in 1979 the protein content of the Trapper variety of peas (n=198) ranged from 13.3–27.1% (whole, dry basis) with a mean of 20.7% and standard deviation of 3.1. The detailed chemical composition of these variable protein content peas has been reported (Reichert and MacKenzie, 1982). The objective of this work was to study the air classification of four samples of these peas containing 14.5, 18.3, 24.2 and 28.5% (dry, dehulled basis). Correlation coefficients and regression equations relating initial % of pea protein to the % of the major chemical constituents present in the air-classified fractions were developed.

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MATERIALS & METHODS

Air classification

Air classification was accomplished in an Alpine air classifier Type 132 MP at two vane setting combinations. A schematic frontal view of the classifier is shown in Fig. 1. Heavier or more dense particles (e.g. starch granules) travel along the periphery of the classifying chamber. They are skimmed off by the knife edge and removed from the classifying chamber by the discharge auger. Lighter or less dense particles (eg. protein bodies) exit via the fine fraction outlet in a spiral air flow. The air flow is generated by an air turbine which draws air through the vanes and into the fine fraction outlet in a spiral fashion, and is regulated by adjusting the vane setting. A higher vane setting corresponds to a wider opening between guide vanes giving a greater air flow and a steeper entrance angle giving a shorter spiral path.

Processing

Smooth field peas (*Pisum sativum* L. cv. Trapper) containing 14.5, 18.3, 24.2 and 28.5% protein (dry, dehulled basis) were obtained from Saskatchewan farmers in 1979.

Peas were processed according to the scheme in Fig. 2. A Currier-type plate mill was used to dehull the peas followed by air aspiration to separate the cotyledons from the hulls. The dehulled peas (approximately 20 kg) were pin milled (about 190 kg/hr) in an

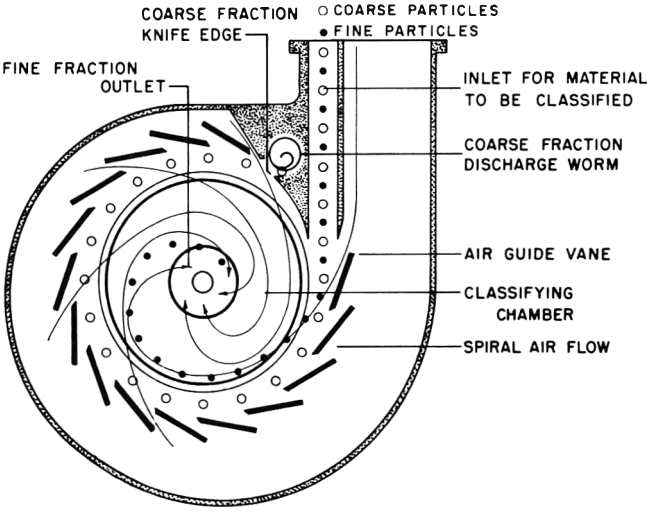

Fig. 1—Schematic frontal view of an Alpine air classifier type 132 MP (from Alpine bulletin 31 am).

Alpine pin mill model 250CW with counter-rotating pins operating at approximately 6,000 and 11,500 rpm, and the flour was separated into two equal lots. Pea flour samples (approximately 10 kg) were air classified at a rate of about 30 kg/hr at vane settings of 25 and 15 to yield protein (PI) and starch (SI) concentrates. The SI fractions were further purified by pin milling (about 160 kg/hr) and air classification (vane setting 20 or 15 at a rate of about 35 kg/hr) to yield lower protein content starch (SII) and protein (PII) concentrates.

To determine the reproducibility of the fractionation (yield data), the pin milling and air classification using vanes 25/20 was repeated for all four samples. For chemical analysis the replicates were thoroughly mixed.

Yields of fractions were based on the recovery of the starch concentrate since some of the protein concentrate is unavoidably lost in the bag house during the collection process.

Analytical methods

Protein (N X 6.25) was determined by the automated Kjel-Foss procedure [AACC (1969) method 46-08]. Moisture and ash contents were determined by standard AOAC (1975) methods.

Table 1—Correlation coefficients and linear regression equations (significant correlations only) of pea protein content on yield and constituents of air-classified protein and starch fractions (vanes 25/20)

	Correlation coefficient (r)	Regression equation, (X = seed protein content, range = 14.5–28.5%)
Yield of		
SI	−0.99***	= −0.524X + 83.0
SII	−0.97**	= −0.590X + 75.2
PI	0.99***	= 0.524X + 17.0
PII	0.37	———
Protein (%) in:		
SI	0.99***	= 0.325X + 1.92
SII	0.99***	= 0.101X + 2.30
PI	0.99***	= 1.40X + 20.3
PII	0.92*	= 1.10X + 19.4
Starch (%) in:		
Flour	−0.99***	= −0.687X + 69.7
SI	0.95**	= 0.200X + 70.9
SII	0.87	———
PI	−0.91*	= −0.168X + 10.1
PII	−0.40	———
Lipid (%) in:		
Flour	−0.99***	= −0.077X + 5.17
SI	−0.99***	= −0.034X + 2.52
SII	−0.97**	= −0.016X + 1.40
PI	−0.99***	= −0.273X + 13.5
PII	−0.99***	= −0.206X + 10.6
Cell wall material (%) in:		
Flour	−0.96**	= −0.162X + 11.7
SI	−0.91*	= −0.159X + 8.77
SII	−0.99***	= −0.226X + 7.72
PI	−0.99***	= −0.341X + 22.6
PII	−0.93*	= −0.324X + 28.2
Ash (%) in:		
Flour	−0.85	———
SI	−0.84	———
SII	−0.93*	= −0.045X + 2.14
PI	−0.92*	= −0.139X + 9.11
PII	−0.83	———
Sugars (%) in:		
Flour	−0.81	———
SI	−0.78	———
SII	−0.76	———
PI	−0.94*	= −0.202X + 17.4
PII	−0.66	———

*, ** and *** Significant at the 10%, 5% and 1% level of significance, respectively.

Starch was assayed by using a modification of the dual enzyme semi-micro method of Banks et al. (1970) using α-amylase and amyloglucosidase (Tenase and Diazyme L-100, respectively; Miles Laboratories, Inc.,Elkhart, IN). The chromogen used in conjunction with the glucose oxidase-peroxidase system was 2,2′-azino di-3(-ethyl benzthiazoline sulfonic acid) from the Sigma Chemical Company, St. Louis, MO.

Lipid content was determined by summing the neutral and polar lipid fractions. Neutral lipids were determined by refluxing 5g of pea flour in 50 ml hexane. The hexane was separated from the flour and the flour was thoroughly washed with clean hexane. The dried extract was considered neutral lipid. The hexane-extracted flour was further extracted (50 ml) with chloroform:methanol (2:1 v/v) and treated as above to obtain the polar lipids.

Cell wall material was determined by multiplying the neutral detergent fiber content (AACC method 32-20) by 2.26 (Reichert, 1981).

Total sugar present in the 80% aqueous methanol extract of the flour was determined by the phenol-sulfuric acid method of Dubois et al. (1956).

All analyses are reported on a dry weight basis.

Starch and protein separation efficiency

The starch separation efficiency (SSE) was calculated as the percentage of starch which was recovered in the starch fraction (SII). The protein separation efficiency was determined by calculating the percentage of the total flour protein recovered in the SII fraction and subtracting this value from 100% (Tyler et al., 1981).

RESULTS & DISCUSSION

THE CONCENTRATION of many of the major chemical constituents present in the air-classified fractions was predictable from the initial % protein in the pea sample. The correlation coefficients (r) and regression equations for the pea samples fractionated at vanes 25/20 are given in Table 1 and discussed in later sections. Since only four samples were used, the statistical significance of correlations was tested at the 1, 5 and 10% level of significance. Minimum r values required for significance at these levels are 0.990, 0.950 and 0.900, respectively, (degrees of freedom = 2).

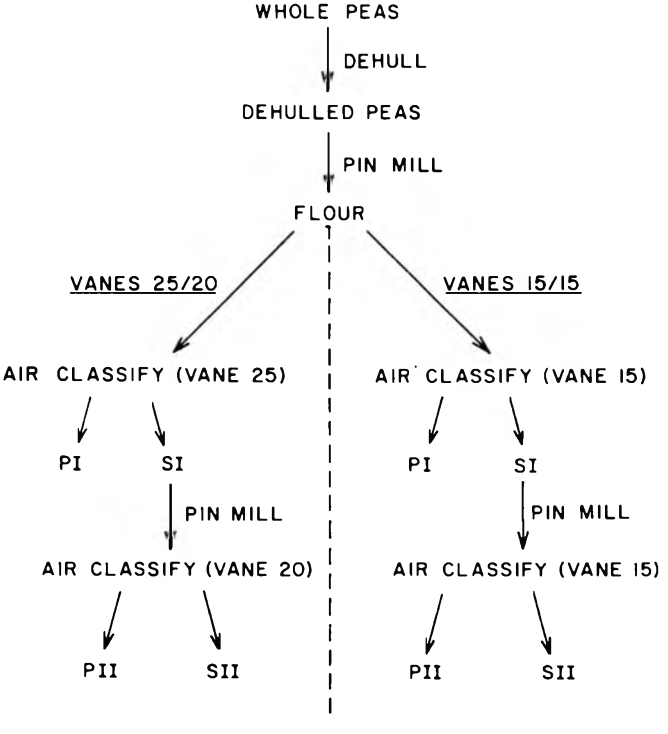

Fig. 2—Flowchart of the process used to obtain protein (PI, PII) and starch concentrates (SI, SII) by air classification at two sets of vane conditions.

Statistically significant (10% level or higher) relationships are designated by their regression lines on Fig. 3–10; nonsignificant relationships are designated by simply joining individual points on these graphs.

Significant correlations between yield of the PI, SI and SII fractions and protein content of the peas were obtained from the air classification at vanes 25/20 (Fig. 3). A relatively constant yield of the fractions irrespective of protein content was produced when the fractionation was carried out at vane 15 as shown for the PI fraction in Fig. 3. An increased yield of the PI fraction is expected using higher protein content peas. However, it is apparent that this is only achieved when the airflow in the classifier is sufficiently large enough to permit all of the freely dispersed protein to be blown into the protein fractions (as for vanes 25/20).

Reproducibility of the yields of fractions obtained from air classification using vanes 25/20 was very good. The mean coefficient of variation (CV) of the duplicates run at each of the four protein levels was 1.6% and 2.5% for SI and SII fractions, respectively. The mean CV values for PI and PII (3.6 and 17.0%, respectively) were higher since the yields of these fractions were much lower than those of the starch fractions.

Protein contents of the air classified fractions prepared at vanes 25/20 ranged from 40.9–60.2% for the PI and 3.8–5.2% for the SII (Fig. 4). The protein contents of the SI, SII, PI and PII fractions were predictable from, and positively correlated to, the protein content of the pea flour (Table 1). In commercial production the advantage of using high protein content peas is that concentrates with a very high protein content can be produced. However, concommittant production of starch fractions with higher protein levels cannot be avoided.

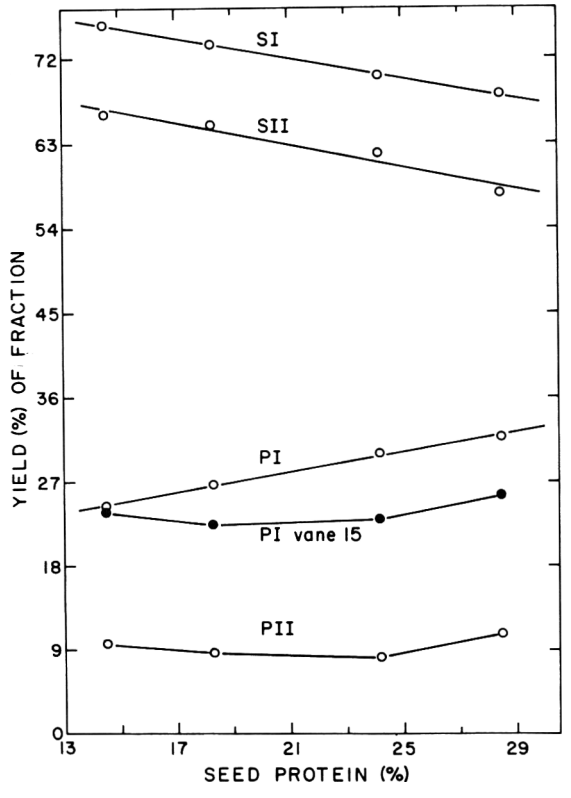

Fig. 3–Yield of fractions obtained from air classification of dehulled peas containing various levels of protein. All results are for air classification using vanes 25/20 except for $PI_{vane\ 15}$ (•). The sum of the yields of PI, PII and SII as well as the sum of PI and SI equal 100%.

Lower vane settings increased the protein content of the PI fraction prepared from peas containing more than 16% protein (Fig. 5). The improvement in protein content becomes greater as the seed protein content increases. The use of lower vane settings, however, also results in the production of starch concentrates containing substantially more protein.

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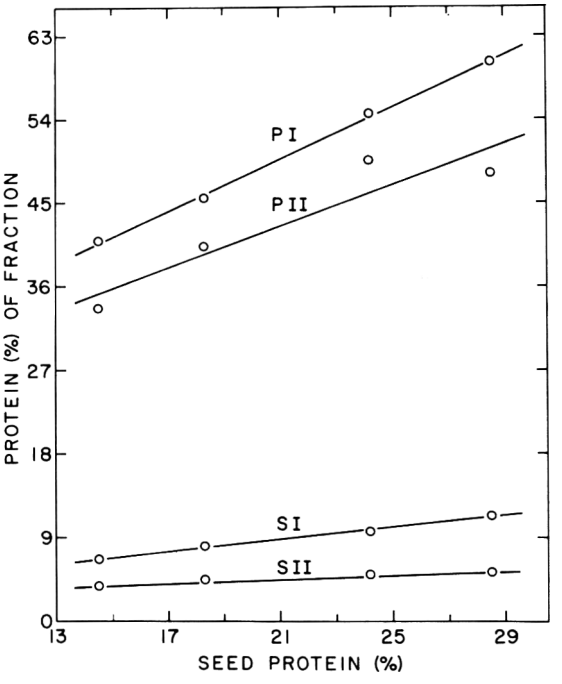

Fig. 4–Protein content of air-classified fractions (vanes 25/20) prepared from dehulled peas varying in protein content.

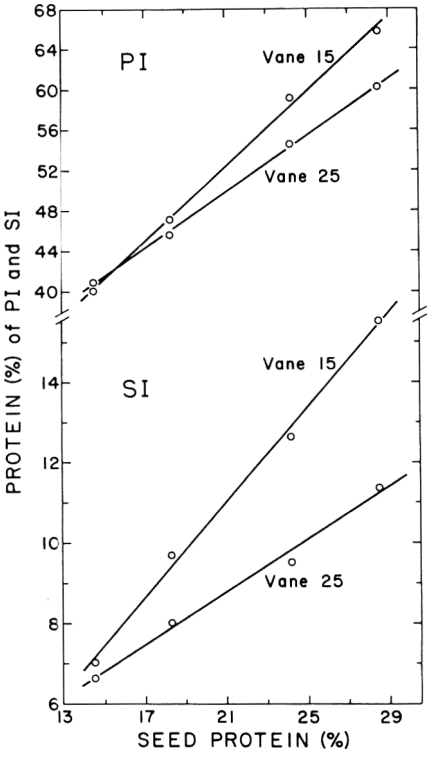

Fig. 5–Effect of air classifier vane setting on the protein content of the PI and SI fractions prepared from dehulled peas varying in protein content.

Starch content of the peas ranged from 49.7–59.8% and was negatively correlated with seed protein content (Fig. 6 and Table 1). However, the starch content of the PI, SI and SII fractions only varied over a limited range as a function of protein content. Starch contents in the PII fraction were more variable and not correlated with seed protein content. The SII fractions contained more starch than the SI fractions indicating that the remilling and reclassification of the SI fractions improved their purity. The starch contents of the PI and PII fractions prepared using vanes 15/15 were somewhat lower than those prepared using vanes 25/20. Starch contents of the SI and SII fractions for both sets of air classification conditions were within the same range.

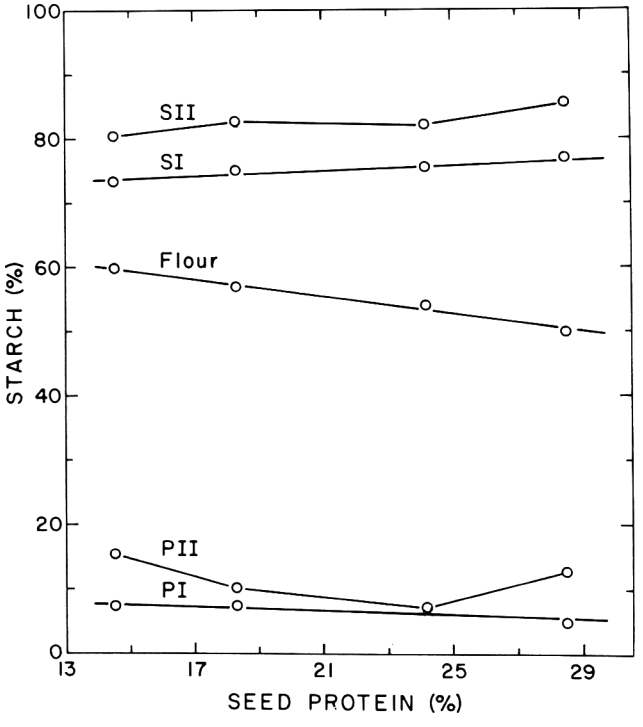

Fig. 6—Starch content of the flour and air-classified fractions (vanes 25/20) prepared from dehulled peas varying in protein content.

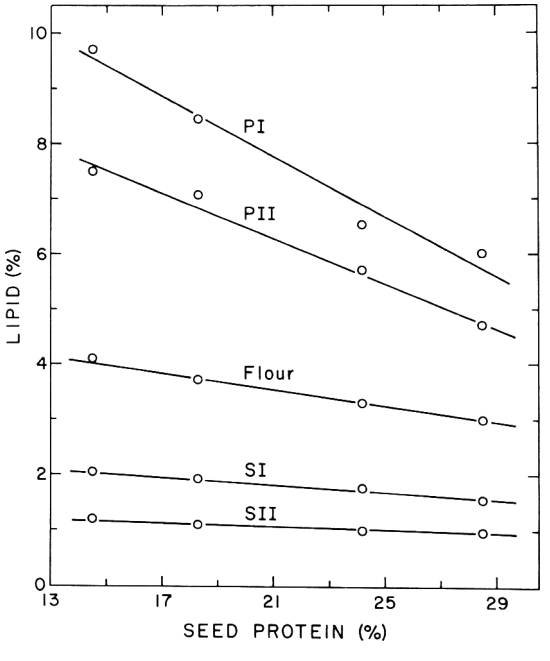

Fig. 7—Lipid content of the flour and air-classified fractions (vanes 25/20) prepared from dehulled peas varying in protein content.

Lipid content of the peas varied over a small range (3.0–4.1%) and was negatively correlated with pea protein content (Fig. 7). This range was amplified in the air-classified (vanes 25/20) protein fractions (6.0–9.7% for PI) and reduced in the starch fractions (1.0–1.2% for SII). Lipid content of all air-classified fractions was negatively correlated to pea protein content (Table 1). It has been suggested that protein bodies in air-dry peas are coated with a complex of convoluted structure, apparently rich in lipid (Swift and Buttrose, 1973). This presumably accounts for the fractionation of the lipid largely into the protein fraction on air classification.

Cell wall material (CWM) content of the pea flours showed a negative correlation with protein content over a small (7.1–9.6%) range (Fig 8). The CWM was largely concentrated into the protein fractions on air classification. The PII fraction contained higher levels of CWM (19.0–24.2%) than the PI fraction (12.6–17.6%). This suggests that more than one pass through the pin mill is required to reduce the particle size of the CWM so that it may be effectively removed from the starch. Even after two fractionations the SII fractions still contained 1.5–4.5% CWM. The CWM content of all fractions was negatively correlated with pea protein content (Table 1). The CWM content of the fractions generated at vanes 15/15 were similar to those in Fig. 8.

Ash (Fig. 9) and sugar contents (Fig. 10) were similarly fractionated. The concentration of both constituents was much higher in the protein concentrates than in the starch concentrates. The sugar and ash content of the PI fraction was negatively correlated with pea protein content although neither constituent in the flour was similarly correlated (Table 1). This may be explained in part, by the effect of increasing yield of the PI fraction with increasing pea

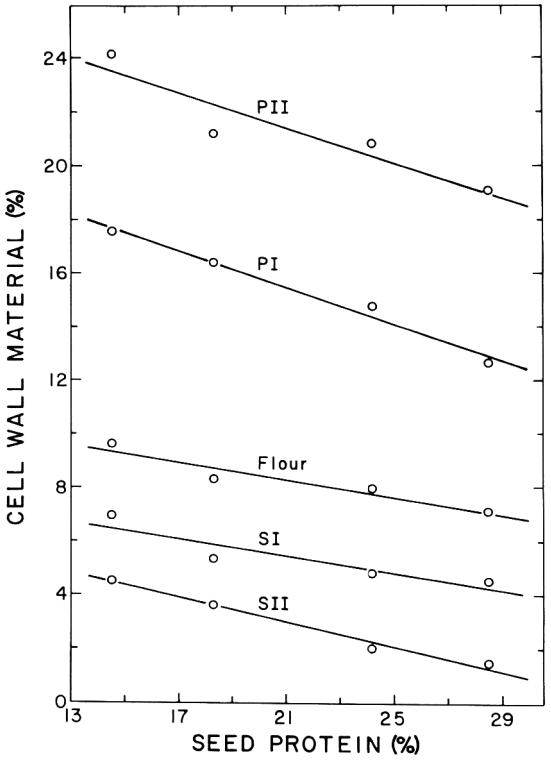

Fig. 8—Cell wall material content of the flour and air-classified fractions (vanes 25/20) prepared from dehulled peas varying in protein content.

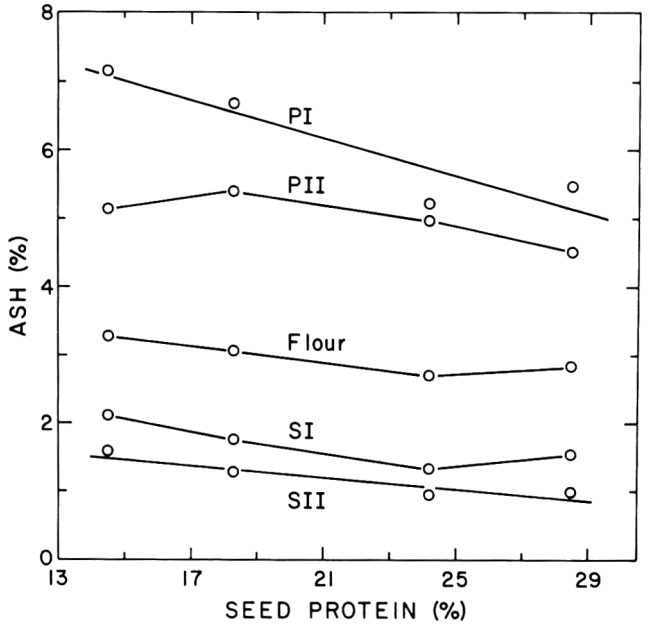

Fig. 9—Ash content of the flour and air-classified fractions (vanes 25/20) prepared from dehulled peas varying in protein content.

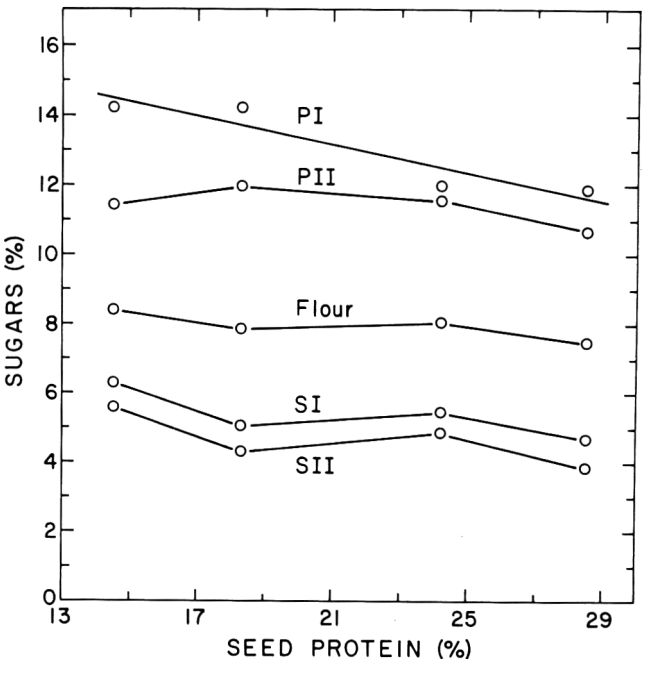

Fig. 10—Sugar content (soluble in 80% aqueous methanol) of the flour and air-classified fractions (vanes 25/20) prepared from dehulled peas varying in protein content.

protein content. The same quantity of a constituent fractionated into a larger weight of PI (as for the high protein content pea) appears less concentrated.

The majority of the chemical constituents in the four flour samples and 16 air-classified fractions produced at vanes 25/20 were accounted for by starch, protein, lipid, CWM, ash and sugar. The sum of these constituents was between 95.7–101.7 (mean = 98.5 and standard deviation = 1.5) for all samples. This summation represents a slight overestimation of the total since the small quantity of protein (p), ash (a) and lipid (l) in the CWM is actually counted twice. The formula $CWM_{p,a,l\ free} = CWM - (0.0696)(CWM)$ can be applied to the points on Fig. 8 to obtain corrected CWM values (Reichert, 1981). Taking into account this correction the mean sum of constituents was 97.8% (SD = 1.4).

Starch separation efficiency (SSE) and protein separation efficiency (PSE) of the higher protein content peas (18.3% or more) fractionated at vanes 15/15 were similar to values reported by Tyler et al. (1981) who used a vane setting of 12 (Table 2). The SSE values for vanes 25/20 tended to be lower than for vanes 15/15, whereas the PSE values showed the opposite trend. A higher air flow in the fractionation using vanes 25/20 results in the loss of some starch (small or broken granules) and, therefore, accounts for lower SSE values. However, the higher air flow also tends to sweep out the protein more completely into the fine fraction resulting in higher PSE values for the fractionations at vanes 25/20.

The SSE and PSE values for vanes 25/20 were positively correlated with pea protein content (r = 0.90 and 0.99, respectively). For vanes 15/15 the SSE and PSE values showed somewhat of the same trend, however, the correlations with pea protein content were not significant. The efficiency of air classifying lower protein content peas was probably reduced by the higher concentrations of lipid and CWM present in these peas (Fig. 7 and 8, respectively). This argument was substantiated by the observation that a gummy film deposited on the surface of the vanes and the knife during the air classification of the lowest protein content pea sample. The film was found to contain 30.1% lipid and 42.9% protein. Higher concentrations of CWM in the cotyledons of the lower protein content peas probably made the disintegration of the cells into discrete protein and starch particles by pin milling more difficult. This would have the effect of increasing the number of agglomerated particles, which are not amenable to efficient separation.

Table 2—Starch separation efficiency (SSE) and protein separation efficiency (PSE) of peas varying in protein content

Protein (%)	Vanes 25/20[a] SSE (%)	Vanes 25/20[a] PSE (%)	Vanes 15/15 SSE (%)	Vanes 15/15 PSE (%)
14.5	88.7	82.6	94.1	80.4
18.3	94.7	85.6	98.3	83.4
24.2	94.4	87.6	102.3	84.8
28.5	98.9	89.6	101.1	79.4

[a] The vane setting of the air classifier regulates the air flow in the classifying chamber.

CONCLUSION

THIS INVESTIGATION has demonstrated that the composition of a single cultivar of peas shows marked variation, and predictably affects the yield and chemical composition of fractions produced by air classification. To insure that uniform products are produced by this technology it is suggested that only peas within a narrow range of protein content be used. This can be achieved by separate binning and mixing of farmers samples. To compensate for some variablility in pea protein content the vane setting of the air classifier can be adjusted accordingly. In a commercial operation, however, this is time consuming and somewhat unpredictable unless a thorough study of the effect of vane setting has been undertaken.

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Germination and Heat Resistance of *Bacillus cereus* Spores from Strains Associated with Diarrheal and Emetic Food-Borne Illnesses

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ABSTRACT

Bacillus cereus has been implicated as the cause of both diarrheal and emetic forms of food-borne illness. Spores of eight strains of *B. cereus*, representing diarrheal, emetic and atoxigenic origins, were examined for heat resistance and germination responses. No correlation was observed between heat resistance at 85° or 90°C and origin of the strain. Germination of spores in Trypticase soy broth at 30°C, measured by loss of heat resistance, was more extensive for diarrheal strains than for emetic strains. These data should be useful in evaluating potential hazards from *B. cereus* in foods.

INTRODUCTION

Bacillus cereus has been implicated in two types of food-borne illness—one is characterized by symptoms that include vomiting and are similar to those encountered in staphylococcal intoxications, the other by diarrheal symptoms similar to those produced by *Clostridium perfringens* (Gilbert, 1979; Goepfert et al., 1972). The two syndromes are thought to be caused by different toxins (Turnbull et al., 1979). The emetic response has been associated almost exclusively with the consumption of rice, while a wide variety of foods, including meat and vegetable dishes, soups and dairy products have been implicated in diarrheal outbreaks (Gilbert, 1979).

Few investigators have studied differences between the emetic and diarrheal types of *B. cereus*. Raevouri et al. (1977) found no difference in antibiotic sensitivity, fatty acid composition and vascular permeability factor between diarrheal and emetic types. They did note that the emetic strains tested were incapable of mannose or salicin fermentation, while 40% and 80% of the diarrheal strains, respectively, had these abilities. Gilbert and Parry (1977) found *B. cereus* serotype 1 to be frequently associated with emetic outbreaks whereas many different serotypes were isolated from diarrheal outbreaks.

Bacillus cereus is widely distributed in foods not associated with food-borne illness outbreaks and has been isolated from dried food products (Kim and Goepfert, 1971), cereal and legumes (Blakey and Priest, 1980), a variety of oriental foods including rice (Schiemann, 1978), and spices (Powers et al., 1976). Despite this broad distribution, the reported incidence of *B. cereus* outbreaks in the United States for 1978 comprised only 3.9% of total food-borne illness outbreaks of known cause (Center for Disease Control, 1981). The low levels detected may be due to lack of awareness of the problem, to the use of selective media which will not detect the presence of the organism in suspect foods, and/or to lack of information on the fundamental properties of the organism.

The objectives of this work were to characterize the heat resistance of spores of eight strains of *B. cereus* representing diarrheal, emetic and atoxigenic strains, to observe germination responses of these strains and to detect possible differences that may exist between the diarrheal and emetic types.

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EXPERIMENTAL

Test organism

Eight strains of *B. cereus* were acquired, including strains associated with human food-borne illness and representative of both emetic and diarrheal syndromes. Several strains, including vomiting-type strains F4810/72, F4165/75 and F4552/75, diarrheal-types F4433/73 and F2769/77 and atoxigenic strain F3484/77, were obtained from the Food Hygiene Laboratory in London, England. Diarrheal-type strain B4ac was obtained from the Food and Drug Administration and a reference strain T (also called F1248) was obtained from the culture collection of the Dept. of Food Science and Nutrition, Univ. of Minnesota.

Spore suspension preparation

Spore suspensions of each strain were prepared on fortified nutrient agar (FNA) containing beef extract, 3g; peptone, 5g; NaCl, 8g; agar, 20g; glucose, 0.1g; $CaCl_2 \cdot 2H_2O$, 0.08g; $(NH_4)_2SO_4$, 0.08g; $MnCl_2 \cdot 4H_2O$, 0.008g; modified G_b mineral solution ($MnSO_4 \cdot H_2O$, 1g; $MgSO_4$, 4g; $CuSO_4 \cdot 5H_2O$, 0.1g; $ZnSO_4 \cdot 7H_2O$, 0.1g; $FeSO_4 \cdot 7H_2O$, 0.01g, brought to 1000 ml), 50 ml; and distilled water, 950 ml, all adjusted to pH 7.0. Forty FNA plates were surface inoculated with an overnight suspension of *B. cereus* grown in Trypticase soy broth (TSB, BBL) at 30°C in a 125 ml Erlenmeyer flask on a shaker at 200 rpm. The inoculated FNA was initially incubated upright at 30°C for 24 hr, then inverted for an additional 24 hr at 30°C. The plates were then held at 4°C for 24 hr.

Growth on each plate was suspended in 10 ml of cold sterile distilled water by scraping the surface with a bent glass rod. Following filtration through six layers of cheesecloth, suspensions were centrifuged at least eight times for 20 min starting at 653 x *g* and increased to 200 x *g*. Between each centrifugation the supernatant fluid was discarded and pellets were resuspended in ca. 100 ml of cold sterile distilled water. During this procedure, pellets were observed for any stratification, and any stratum containing predominantly vegetative cells (as detected by microscopic examination under phase contrast) was discarded. Final pellets containing >98% refractile spores were stored in sterile distilled water at 4 ± 2°C at a concentration of ca 10^9 spores/ml for five months or longer. In subsequent studies, no difference in heat resistance was noted for spores stored as long as 18 months. A 1:10 dilution of the spore suspensions, made with sterile distilled water containing 3 mm glass beads to break clumps, was used for working stock suspensions.

Heat inactivation

A population of ca 10^7 spores/ml was achieved by inoculating 0.9 ml of 25 mM sodium phosphate buffer, pH 7.0, in 14 x 100 mm screw-capped test tubes with 0.1 ml of a spore suspension. Tubes were placed in a waterbath heated by a Haake model E52 heater to ca 85° or 90°C. The temperature of the waterbath and an uninoculated tube were monitored with an Omega digital thermometer, and when the tube temperature reached the water bath temperature timing of the study commenced. Generally this time interval was less than 2 min. Sample temperatures were controlled to ± 0.2°C.

At predetermined times, two to four replicate samples were removed and immediately plunged into an ice bath. Survivors were enumerated using Trypticase soy agar (TSA, BBL) pour plates incubated at 30°C for 24 ± 2 hr following the procedures of Gilliland et al. (1976). Six to eleven sampling times were used in each trial and from three to six trials were conducted for each strain. D-values were determined from the negative reciprocal of the slope of the

least squares regression line of individual trials using the linear portions of the survivor curves plotted on a semilog scale (Snedecor and Cochran, 1967; Cerf, 1977).

Germination procedure

Germination in TSB at 30°C was measured by loss of heat resistance in duplicate or triplicate trials for each strain. Spores in distilled water were heat activated at 70°C for 15 min prior to the inoculation of TSB, previously equilibrated at 30°C. The initial population in TSB was ca. 10^7 spores/ml. Samples were taken every 15 min for 2 to 4 hr. At each sampling, a 3 ml aliquot was removed; 1 ml was immediately diluted and plated and the remaining 2 ml were heat shocked for 15 min at 70°C prior to plating. The plating medium used for germination studies was mannitol egg yolk polymyxin agar (MYP, Mossel et al., 1967) with one modification: 50% egg yolk instead of the suggested 20% egg yolk was used. All plates were incubated for 18–24 hr at 30°C prior to enumeration of colonies. The percentage of germinated spores at a given sampling time was calculated using the following equation:

$$\% \text{ germination} = \left[1 - \frac{\text{heated population}}{\text{unheated population}}\right] \times 100$$

The maximal extent of germination was defined to be the geometric mean of the percentage of germinated spores for samples taken during the stable period which followed an initial decrease in number. Analysis of variance was conducted on the geometric means of the heat resistant fraction of stable period samples (Snedecor and Cochran, 1967), and differences between means were tested using the Bonferroni significant difference method (Bailey, 1977).

RESULTS & DISCUSSION

THE D-VALUES predicted for 85°C and z-values determined by least squares regression analysis of thermal death time curves are presented in Table 1. D-values ranged from 106.0 min to 32.1 min at 85°C. A broad spread of heat resistance of *B. cereus* spores has been reported in the literature. Parry and Gilbert (1980) reported a range of $D_{95°C}$-values from 2.5–36.2 min for strains isolated from emetic food-borne illness outbreaks, and Gilbert et al. (1974) published $D_{95°C}$-values from 5.0–36.0 min for illness related and nonrelated strains. Bradshaw et al. (1975) recovered two highly resistant strains from underprocessed canned soup with calculated $D_{95°C}$ of 256.7 and 5122.3 min. For comparative purposes, the calculated $D_{95°C}$ determined during this work ranged from 1.2–20.2 min.

Strains T, F4552/75 and F4433/73 demonstrated subpopulations (tails) with greater heat resistance than the majority populations of these supensions. Although these tails represented approximately 1% or less of the population, the more resistant fraction is important to consider in the evaluation of cooking processes due to an increased potential for the survival of these spores.

The z-values for the eight strains ranged from 6.8–13.9 Celsius degrees, with an average of 9.2 Celsius degrees. Bradshaw et al. (1975) reported z-values of 7.9–9.9 Celsius degrees for two *B. cereus* isolates, and Gilbert et al. (1974) found z-values ranging from 6.7–8.3 Celsius degrees.

Fig. 1 presents D-values normalized to 85° and 90°C for all strains studied, and demonstrates that no overall pattern of heat resistance related to strain type appears to exist. Tails and shoulders were evident in the survivor curves of emetic, diarrheal and nonfood related strains, and a wide range of heat resistances was observed. Emetic strain F4810/72 demonstrated a heat resistance pattern nearly identical to that of diarrheal strain B4ac. The majority population of diarrheal strain F4433/73 had low heat resistance similar to that of F2769/77; however, the tail population was the second most resistant population of tested strains related to food-borne illness.

Parry and Gilbert (1980) reported differences between the serotypes of *B. cereus* isolated from cooked rice asso-

Table 1—Heat resistance of B. cereus spores heated in 25 mM sodium phosphate buffer, pH 7.0, and plated on Trypticase soy agar

Type	Strain	Predicted $D_{85°C}$ (min)	z(C°)
Reference	T (majority)	33.8	9.7
	(tail—ca. 1%)	72.3	10.8
Atoxigenic	F3484/77	77.1	10.9
Emetic	F4810/72	74.7	8.9
	F4165/75	106.0	13.9
	F4552/75 (majority)	50.1	8.1
	(tail—ca. 1%)[a]	–	–
Diarrheal	F4433/73 (majority)	34.4	6.9
	(tail—ca. 0.1%)	103.5	6.8
	F2769/77	32.1	7.7
	B4ac	75.1	7.9

[a] Tail evident at 90°C but not at 80°C. $D_{90°C}$ = 30.3 min.

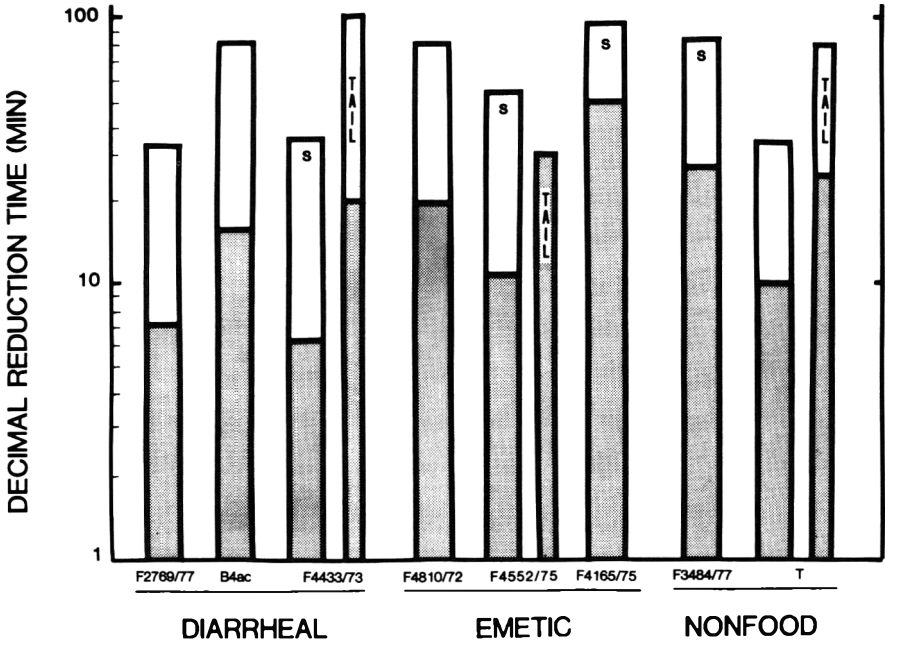

Fig. 1—Heat resistance of B. cereus spores at 85° (unshaded) and 90°C (shaded) in 25 mM sodium phosphate buffer, pH 7.0 and plated on Tryticase soy agar — "s" indicates that shoulders were present on survivor curves. "Tail" refers to more resistant subpopulation of < 1% (see text).

ciated with the emetic syndrome and those isolated from uncooked rice. The cooking procedure was thought to be the selective agent for isolation of type 1, which has been frequently implicated in the emetic syndrome, due to an apparent increased heat resistance of this serotype. Strains F4810/72 and F4165/75 are type 1 strains, with $D_{95°C}$-values calculated from our data of 5.6 and 20.2 min, respectively. Parry and Gilbert reported a $D_{95°C}$ of 9.5 min for strain F4810/72, and a range of 2.9–36.2 min for all type 1 strains studied. A majority of type 1 strains had $D_{95°C}$-value greater than 20 min. These workers did not include diarrheal strains in this study and therefore inference on cooking selection of diarrheal strains was not examined.

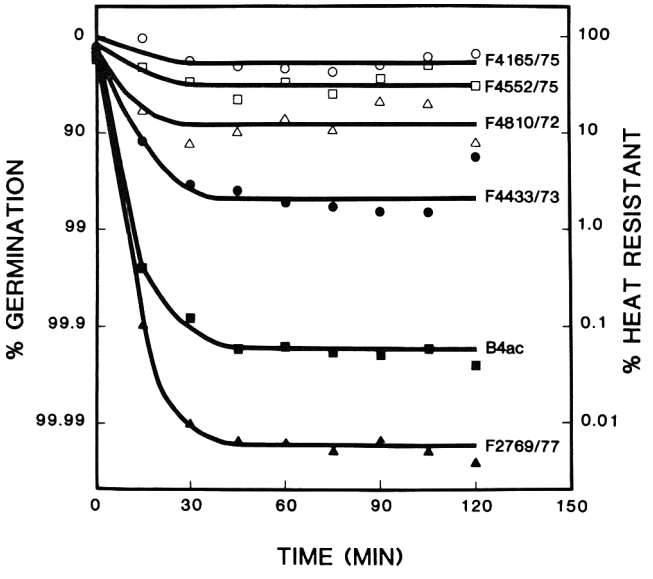

Fig. 2—Germination, measured by loss of heat resistance to 70°C for 15 min, of B. cereus spores from strains associated with diarrheal (closed symbols) and emetic (open symbols) strains in Trypticase soy broth at 30°C.

Data from representative germination trials of strains related to food-borne illness outbreaks are presented in Fig. 2. Faster initial germination rates were observed for strains with greater maximal germination. Maximal germination was achieved within 30 min for poor germinating strains and within 45 min for strains capable of extensive germination. All strains appeared to reach a stable maximum within 2 hr.

Values on maximal germination of spores, geometrically averaged over replicate trials, are presented in Fig. 3. Bars with the same lower case letter are not significantly different by the Bonferroni significant difference test ($p < 0.05$). Greater than 98% of the spores of diarrheal strains germinated whereas less than 87% of the spores of emetic strains were capable of germination under these conditions. The atoxigenic strain F3484/77 demonstrated the lowest germination, 28.9%, and 99.97% of the spores of reference strain T germinated. No significant difference in maximal germination was noted between the emetic strains tested. All diarrheal strains tested differed from emetic strains ($p < 0.05$) in terms of maximal germination.

This difference between germination capabilities of diarrheal and emetic strains has not been previously reported; however, Stadhouders et al. (1980) observed so called fast- and slow-germinating spores of *B. cereus* in pasteurized milk. These slow-germinators exhibited a high heat resistance and required a more extensive heat shock to stimulate growth. In preliminary studies, an 80°C heat shock appeared to increase the extent of germination of strain F4165/75, but did not seem to affect the low germination in spores of strains F3484/77 and F4810/72 (data not presented).

Spores of low level germinating strains are capable of more extensive germination on MYP, which is demonstrated by differences between unheated and heated counts in the germination studies. A most probable number determination using TSB recovered numbers greater than or equal to those determined on MYP for enumeration of survivors in heat resistance studies. This indicated that extensive germination of emetic strains in TSB required a long incubation because of very slow rates of germination.

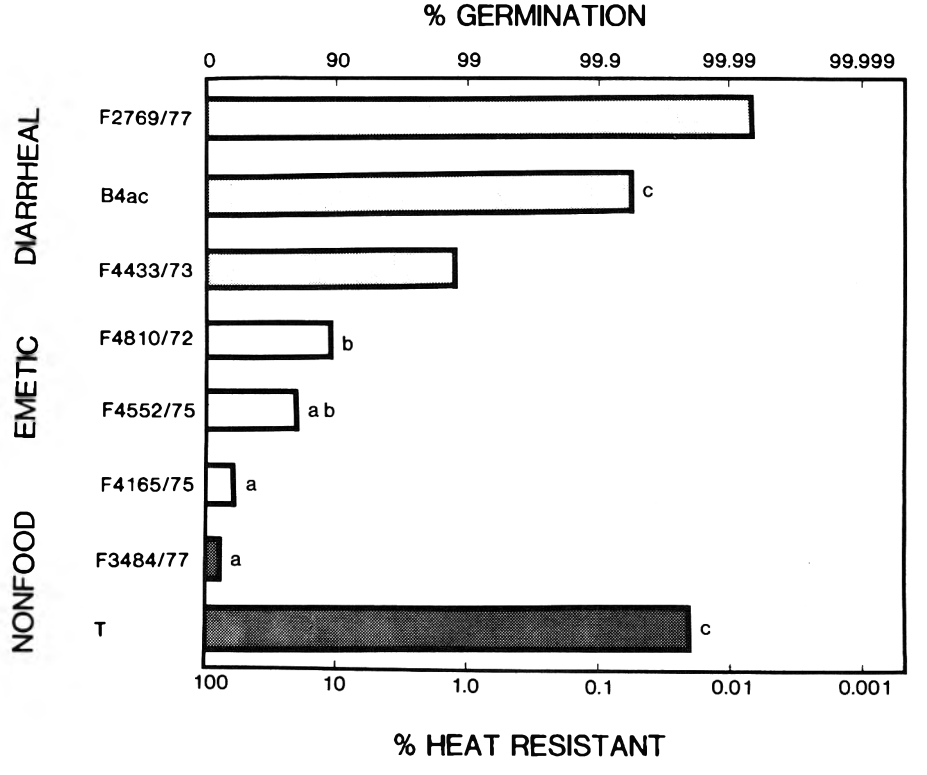

Fig. 3—Maximal germination of B. cereus spores in Trypticase soy broth within 4 hr at 30°C, measured by loss of resistance to 70°C for 15 min. Matching lower case letters indicate no significant difference between maximal extents of germination using the Bonferroni difference test ($p < 0.05$).

One could speculate that the fast strains may germinate extensively at the initial temperatures of the heating processes, with these germinated spores being easily inactivated during the cooking process. The slow germinators on the other hand, would remain in the dormant heat resistant state and the potential of survival would be increased. The germination rates reported in this work are those for heat activated spores, and nonactivated spores will be more commonly encountered in raw foods. Germination rates may be slower for these nonactivated spores. Survival during cooking would depend upon the extent of germination before, and during heating. The significance of rate and extent of germination in relation to subsequent toxin formation is unknown. Furthermore, these germination differences have been demonstrated among only six strains representing the two types of food-borne illness. Evaluation of a greater number of strains is required to confirm the findings and firmly establish this relationship which may aid in the elucidation of the occurrence and cause of the two syndromes of *B. cereus* food-borne illness.

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Effect of Drip Irrigation of Pecan Quality and Relationship of Selected Quality Parameters

E. K. HEATON, J. W. DANIELL, and L. C. MOON

ABSTRACT

The effect of four drip irrigation rates 0, 22.7, 45.4, and 68.1 L/hr/tree (LHT) on several quality parameters of 'Stuart' pecans were investigated along with values calculated from inshell weights, volumes, and kernel weights. Irrigation significantly increased kernel yield/tree, nut weight, diameter, kernel specific gravity, fill of nut, oil content, and appearance rating. Values calculated by computer analyses from weights and volumes were useful for evaluating the quality of nuts from the different irrigation treatments. This technique should be useful for researchers engaged in quality evaluations of inshell pecans. Nut size and % fill, and kernel color are the best parameters to estimate quality and value of pecans before actual shelling and processing. Additionally processing costs are partially dependent on nut size (no./kg) and kernel yield.

INTRODUCTION

THE PARAMETERS most frequently associated with quality of the pecan nut (*Carya illioensis*) are nut size, kernel development or filling, kernel color, flavor, and shelling characteristics. It is generally accepted that both size and degree of filling within cultivars are influenced by climatic conditions and cultural practices throughout the growing season. One of the main concerns is an ample supply of soil moisture. Magness (1955) summarized the effects of water deficiency on pecan nut quality and placed them in five groupings. If a shortage of water occurs: (a) between bloom and shell hardening the nuts will be small; (b) during shell hardening the shell will be thinner than normal; (c) during kernel development stage following a normal supply in early stages the nuts will be normal size with poorly developed kernels; (d) during kernel development following a short supply at the time of shell formation, nuts will be small and well filled or overfilled; (e) during the entire growing season the nuts will be small and poorly filled. Other abnormal variations in weather may also be associated with erratic pecan crops. An excess of water increases the problem of disease and insect control. The relation of normal and extreme variations in rainfall, temperature, and growing seasons between 1940 and 1962 were explored by Hunter (1963). He concluded that extremes in weather were related to either high or low production the following year.

In farm level experiments, Goodyear and Wetherbee (1970) reported that irrigation increased production of total pecan kernels per acre by 30% over nonirrigation in the southeast. This confirmed the earlier work of Romberg (1960) that nut yield was increased by 32% in the southwest. Daniell (1978) reported on drip irrigation of 'Stuart' trees over a 4-yr period which included 3 yr with below normal rainfall (70–85 cm) during the growing season and 1 yr when rainfall was 50 cm. His data showed sizable increases in yield of total nuts per acre for irrigated trees during normal as well as dry years.

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In processing pecans, nut size, degree of filling and shelling characteristics are highly important considerations since they substantially influence the time and energy required for processing. One cracking machine can crack about 80 nuts per min. Since nuts are cracked individually, less time is required to crack an equal weight of large as contrasted to small nuts.

Evaluating pecans of different cultivars, or from various cultural treatments is a laborous and time consuming task that involves quantitating several quality parameters because no single measurement takes into account all those parameters involved. Sitton (1944) suggested that specific gravity might be a good measure of nut filling. However, this parameter omits nut size and the proportion of kernel to shell. Smith et al. (1948) claimed that percent kernel was a satisfactory means for comparing filling of nuts of a single cultivar grown with different cultural treatments. A method for calculating a value representing % fill, i.e., the amount of internal volume of nut occupied by kernel was proposed by Romberg (1952). His analysis was based on nut specific gravity, nut volume, shell specific gravity, and kernel specific gravity. Six quality parameters (volume, weight, specific gravity, diameter (by 1/6 inch), % kernel, kernel weight, and calculated fill) were used by Amling et al. (1975) for evaluating pecan varieties and selections.

The purpose of this research was to determine the effect of drip irrigation treatments on quality of pecan nut produced on mature "Stuart' trees and to determine the relationship of the various quality parameters to each other.

MATERIALS & METHODS

PECANS were harvested from mature 'Sutart' trees with a spacing of about 16.3 trees per acre. Four drip irrigation treatments using 0, 22.7, 45.5, and 68.1 L/hr/tree were employed which resulted in 0, 13.79, 27.59, and 41.38 ha-cm/ha being applied during growing seasons. Each treatment consisted of nine tree replicates. Daily application of water was based on 70% evaporation from a class A pan (Daniell, 1981). Total rainfall during the year was 93.44 cm which was 28.56 cm below normal rainfall; the rainfall during the growing season was 39.73 cm which was 30.10 cm below normal. Nuts were harvested and brought to the Food Science Laboratory for storage at 0°C, 65% rh until nut moisture equilibrated with the storage atmosphere before evaluations were made.

Nut size was estimated by three general methods: Count by weight (Number/kg or lb); diameter (usually separated by 1/16 in, 1.59 mm); or volume (cm^3 or in^3). The first two procedures are used throughout the industry, with the USDA standards for grades (1976) listing five size classifications based on number of nuts per pound.

Diameter

The nuts within each sample were separated according to minimum diameter by passing them over a series of slots that were progressively smaller by 1/16 in. (1.59 mm) within the range of 17/16 (27.0 mm) to 9/16 in. (14.3 mm).

Volume, specific gravity and buoyancy

These values were determined by using mustard seed displacement instead of water displacement as reported by other researchers Romberg (1960) and Amling et al. (1975). A rectangular loaf pan with outside dimensions of 7-3/8 x 3-5/8 x 2-1/4 in. (18.7 x 9.2 x

5.7 cm) and a capacity of 871 cm^3 was used. To establish the specific gravity of seed, the pan was filled to overflowing with seed, then leveled off with the edge of a ruler and weighed. To obtain the volume, the seeds were measured in a graduate cylinder. This step was repeated several times to establish the specific gravity of the seed. To obtain data for estimating parameters listed below, a layer of seed about 1 cm deep was placed in the bottom of the pan. Samples (100g) of nuts were placed on the seed so they did not touch the pan or other nuts. The pan was again filled to overflowing with seeds and leveled off as described above and weighed. After nuts were separated from seed by pouring over a No. 4 screen they were cracked and the kernels were recovered and weighed. Several quality parameters were easily estimated by computer analysis of data from nut, nut and seed, and kernel weights using the following equations.

1. Ds = Ws/Vs
2. Wsd = Ws – [(Wns) – Wn]
3. Vn = Wsd/Ds
4. Dn = Wn/Vn
5. B = Vn – Wn
6. Dk = Wk/Vn
7. Dy = Dn – Dk
8. % Kernel = Wk/Wn
9. % Fill = Dk/0.83 – 0.8 (Dy)*

*Derived from data of Romberg (1952).

B = buoyancy
D = specific gravity
d = displaced
k = kernels
n = nuts
s = mustard seed
V = volume-cc
W = weight-g
y = nut shells

Moisture was determined using a moisture register Model G 8R and the appropriate detection module for the moisture range. Samples consisting of about 40 g of kernels were blended in an 8-oz (249 ml) blender cup for 10 sec at low speed with a Waring Blendor. A 55-ml portion was used for moisture determination.

Oil content was obtained using a Goldfish extractor. A 4-g portion of ground and dried kernels was extracted for 24 hr with 50 ml of petroleum ether (Skelly F).

Gardner color values were determined using the same sample portion as moisture. The pressed disks from the mositure register were read on a Gardner Color Difference meter model C-4 (L) set against a maize standard (SBC-35) #801, L = 76.6, a = 1.1, b = 24.2).

Evaluations of sensory quality attributes (appearance, color, aroma, texture and falvor) were obtained by submitting samples to a 10-member panel experienced in judging pecan quality. Scoring sessions were conducted in a taste testing room (10 booths) having white surfaces and controlled lighting. Four samples (10g chopped), consisting of one rep (randomly chosen) from each of the four treatments were numbered 1–4 and placed in 2-oz (56-g) capacity paper cups and arranged on plates so that each judge received samples in a different order. A 9-point scale (9 = excellent; 5 = borderline; 1 = very poor) was used for judging each quality attribute.

Data were subjected to ANOVA and General Linear Model Analyses (Helwig and Council, 1979).

RESULTS & DISCUSSION

DATA IN TABLES 1 to 3 include means for the main treatment effects of four irrigation rates on several quality parameters of the nuts. On the basis of the data obtained it is apparent that highest quality was attained when pecan trees had sufficient and continuous supply of moisture throughout the growing season. The shortage of water that occurred in the nonirrigated check and the lowest rate of irrigation resulted in nuts that were small, often abnormally shaped, and with under developed shells.

Size

Although the nuts from the highest irrigation rates were smaller than in the previous 2 yr (unpublished) by about 24%, size and uniformity of size were closely related to irrigation. Nuts were larger and more uniform from the two highest irrigation levels (Table 1). Based on nut size in Table 1, cracking time for equal weights of nuts would be 25.4% longer for the nonirrigated control compared to the highest irrigation.

Processors size pecans by diamter to obtain a more uniform cracking and a greater yield of unbroken half kernels. variations in diameter, weight and volume that were associated with irrigation treatments appear in Table 2. The greater increases in weight and volume of nuts from the higher levels of irrigation were greater than increases in diameter confirming that these two values together furnish more information about nut quality than does diameter alone. By considering nut diameter and volume a reasonable estimate of the general shape of nuts can be made. Nuts from the nonirrigated and low irrigation rate were shorter in relation tc diameter than those receiving intermediate and high rates.

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Table 1–Size and uniformity of 'Stuart' pecans grown with four irrigation rates in 1980

Treatment	Nuts/kg	Percent of nuts by diameter (inches) (mm)					
		15/16(23.8)	14/16(22.2)	13/16(20.6)	12/16(19.1)	11/16(17.5)	10/16(15.9)
Non-irrigated	185	2.01	20.75	24.10	47.52	2.81	2.81
22.7 L/hr/tree	174	0.41	32.28	28.30	34.20	4.67	0.14
45.5 L/hr/tree	142	1.83	84.53	13.64	0	0	0
68.1 L/hr/tree	138	6.53	88.44	5.03	0	0	0

Table 2–Effect of four levels of drip irrigation applied to mature 'Stuart' pecan trees on six quality parameters of inshell nuts

Quality parameters	Nonirrigated Check-O	Rate of water applied (L/hr/tree)[a]			Error M.S.
		22.7	45.4	68.1	
Kernel yield (kg/tree)	24.50 a	26.00 a	36.10 b	40.00 b	–
Nut Diameter (mm)	20.10 a	20.70 a	22.10 b	22.30 b	0.278
Weight (g/nut)	5.16 a	5.60 b	7.03 c	7.23 c	88.490
Volume (cm^3/nut)	6.18 a	6.51 a	8.27 b	8.62 b	136.482
Buoyancy	1.14 ab	0.92 a	1.25 ab	1.39 b	14.821
Nut sp gr	0.83 a	0.86 a	0.85 a	0.84 a	0.002
Kernel sp gr (g kernel/cm^3 nut)	0.40 a	0.42 b	0.41 ab	0.41 ab	–
Shell sp gr (g shell/cm^3 nut)	0.44 a	0.44 a	0.44 a	0.43 a	9.600

[a] One L/hr. The time that the irrigation system was operated was based on 70% evaporation from a class A pan (Daniell, 1981). Within each line, numbers that are followed by the same letters are nonsignificant at P = < 0.05.
ANOVA Model used was of the form Quality Parameter = Treatment
Degree of freedoms for the error term = 32

Specific gravity

Nut specific gravity (g/cm^3 nut) consists of the portion due to weight of kernels, and the portion due to weight of shells. Specific gravity of shells and kernels separately are used to calculate % fill. Significant differences in kernel specific gravity were observed for each irrigation treatment. It increased as water was increased to 45.4 L/hr/tree then decreased at the high level. Nut specific gravity and shell specific gravity were not significantly changed by irrigation (Table 2). The higher nut specific gravity at the two intermediate irrigation levels confirms that an excess as well as shortage of water results in decreased filling (Table 2). The relationship of kernel specific gravity to shell specific gravity is linear according to data of Romberg (1952).

Kernel content

The percent (w/w) of sound mature kernels in conjunction with their color grade, and size is widely used for estimating kernel yield and value of inshell pecans. Based on data (Table 2), % kernel was equal to kernel specific gravity for estimating total kernel yield in 'Stuart' pecans. However, in blends (mixtures) of cultivars which usually occurs with mechanically harvested pecans there are large variations in the proportions of kernels to shells due to inherent cultivar differences. Therefore when cultivars are mixed, nut and kernel specific gravity have no more value as an estimate of kernel yield than % kernels. However, for evaluating cultural treatments where single cultivars are used more information about the extent of kernel development is obtained by calculating % fill from shell and kernel specific gravity than from % kernels.

Kernel moisture

Kernel moisture was used to adjust % kernels to a uniform basis of 4.5% moisture, and to confirm that the nuts had equilibrated with the storage environment. Irrigation treatments did not effect equilibrium moisture content.

Percent fill

An estimate of the degree to which the interior volume of the nut shell is filled with kernel is obtained from the specific gravities of kernels and shells by Eq (9). Thus, % fill takes into account the space within the shell that is available for kernel. Percent fill values were significantly higher at the two intermediate levels of irrigation, confirming that a deficiency or excess of water decreased filling (Table 2).

Oil content

This value is considered by many to be a highly dependable assessment of kernel filling and plumpness. Oil was signficantly increased by irrigation, however, there were no significant differences among levels (Table 3).

Buoyancy

An estimate of the space within the nut shell that is not occupied by kernel or soft inner shell is obtained from buoyancy. These values (Table 2) differed significantly, but were not consistent with irrigation treatments.

Color

Hunter L-values, a measure of visual lightness of kernel skins were not significantly different among the various treatments, although they were higher indicating brighter nut meats at highest irrigation rate.

Sensory panel

Scores for appearance and color were significantly higher for kernels from harvested trees receiving the two highest irrigation rates, although no significance was shown for aroma, texture or flavor among the treatments (Table 3). The main reason for lower scores for appearance and color in kernels from low water treatments compared with higher water treatments was that the nuts from the nonirrigated control and low rate were smaller than in the higher water treatments.

Irrigation treatments were significantly correlated in a positive and linear manner with average diameter of nuts, nut weight, nut volume, and kernel weight (Table 4).

Data in Table 5 include the averages and ranges of values for the various quality parameters considered in this study. The linear correlation matrix for the same values appear in Table 6. These correlations were mainly associated with volume, weight and diameter and values calculated from them.

Interrelation of quality measurements

Linear correlation coefficients for 13 quality parameters were determined to indicate the degree of association of objective measurements to each other (Table 6). The strong ($P = < 0.01$) positive correlations between nut weight and four other parameters (diameter, volume, kernel weight, and bouyancy) showed that when weight changed (increased or decreased) a corresponding change occurred in the other parameters. Pecan nut weight consists of kernel

Table 3—Effect of four levels of drip irrigation applied to mature 'Stuart' pecan trees on quality of nut kernels[a]

Quality Parameters	Nonirrigated Check-O	Rate of water applied (L/hr/tree) 22.7	45.4	68.1	Error M.S.
Kernel wt (g/nut)	2.63 a	2.74 a	3.42 b	3.50 b	25.289
Filling (%)	82.90 a	89.20 b	92.80 b	84.10 a	27.915
Kernel content (%)	47.60 a	49.00 a	48.70 a	48.83 a	4.600
Oil content (%)	74.10 a	75.50 b	75.50 b	75.30 b	1.053
Moisture content (%)	3.61 a	3.58 a	3.67 a	3.47 a	0.129
Color (L-value)	48.50 a	48.40 a	48.70 a	50.00 a	3.381
Appearance	6.39 a	6.67 a	7.07 b	7.22 b	1.464
Color	6.56 a	6.64 a	7.09 b	7.14 b	1.671
Aroma	7.06 a	7.07 a	7.14 a	7.18 a	1.325
Texture	7.37 a	7.41 a	7.56 a	7.57 a	0.490
Flavor	7.40 a	7.46 a	7.49 a	7.61 a	0.604

[a] Within each line numbers that are followed by the same letters are non-significant at $P = <0.05$.
ANOVA Model used was of the form Quality Parameter = Treatment
Degree of freedoms for the error terms — 32 except for sensory factors = 348

weight (high value) and shell weight (low value). Within a single cultivar shell specific gravity (sp gr) is more constant than kernel sp gr. Therefore differences in weight of nuts of similar size reflect differences in kernel. This is confirmed by the correlations between % kernel and kernel sp gr (+); and shell sp gr (−). Among various cultivars, nut size and shell thickness vary greatly with kernel content ranging from 35–65%. For industry use, data must be expanded to include other cultivars with different characteristics. No significant correlations were shown between Gardner color and other quality parameters.

These data fail to take into account two important considerations in estimating value, namely color variability and defects.

The magnitude of the associations of the several quality parameters are shown by regression coefficients (Table 7). It was apparent that nut volume was affected to a greater extent than nut diameter by variations in nut weight. From the linear regression coefficients (Table 8) it is apparent that kernel weight is mainly related to size and volume. These data show that volume changed more than diameter as kernel weight changed.

Table 4–Linear correlation coefficients for irrigation treatments with quality parameters of 'Stuart' pecans (1980 crop)

Quality parameter	Correlation coefficients[a]
Average size	0.698**
Nut weight	0.625**
Nut volume	0.595**
Kernel weight	0.599**
Oil	0.372*
Color	0.331*

[a] Significance; **P < 0.01; *P < 0.05; Coefficients for other parameters were not significant.

Table 5–Average and range of values for various quality parameters in 'Stuart' pecans (means are from 36 samples taken from 36 trees)

Quality parameters	Average	Low	High
Diameter	13.4	11.8	14.5
Weight	6.3	3.9	8.7
Volume	7.5	4.2	10.5
Kernel weight	3.1	1.7	4.2
Buoyancy	1.2	0.3	2.1
Nut sp gr	0.845	0.764	0.926
Kernel sp gr	0.409	0.355	0.484
Shell sp gr	0.436	0.386	0.512
% fill	87.3	76.8	98.3
% kernel	49.3	44.7	55.4
% oil	75.1	71.4	77.6
% moisture	3.6	2.3	4.5
Color	29.6	26.2	33.5

CONCLUSION

OBJECTIVE QUALITY MEASUREMENTS and subjective taste panel evaluations made on 'Stuart' pecans grown under four irrigation treatments in an abnormally dry and hot season showed that a deficiency or excess of moisture influenced quality.

Information on eight quality parameters of pecans calculated by computer analysis from weights taken on inshell nuts and meats showed that specific detailed information on quality can be estimated quickly.

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Table 7–Effect of nut weight on those quality parameters that showed significant correlation coefficients with nut weight of 'Stuart' pecans[a]

Quality Parameter	Intercept	Coef.	Std. error	F-value	R	C.V.
Size	9.742	0.582	0.0035	275.51**	0.8901	1.868
Volume	0.073	1.184	0.0483	599.83**	0.9464	4.607
Buoyancy	0.073	0.184	0.0483	14.49**	0.2989	29.483

[a] Estimate: y = M + bw where y = quality parameter, M = intercept, b = coefficient, w = nut weight.
**P = 0.01.

Table 8–Effect of kernel weight on quality parameters with significant correlation coefficients with kernel weight in 'Stuart' pecans[a]

Quality Parameter	Intercept	Coef.	Std. error	F-value	R	C.V.
Size	9.988	1.120	0.0073	235.68**	0.8739	2.001
Volume	5.455	2.267	0.1153	386.57**	0.9192	5.656
Buoyancy	0.696	0.359	0.0937	14.70**	0.3019	29.421
% kernel	0.422	0.001	0.0005	6.62*	0.1629	4.077

[a] Model estimated was y = M + bw, where y = quality parameter; M = intercept, b = coefficient, W = kernel weight.
**P = 0.01.

Table 6–Simple correlation coefficient matrix of quality parameters in 'Stuart' pecans

	Diameter	Weight	Volume	Kernel weight	Buoyancy	Nut sp gr
Diameter	1.000					
Weight	0.943**	1.000				
Volume	0.914**	0.973**	1.000			
Kernel weight	0.935**	0.982**	0.959**	1.000		
Buoyancy	0.503**	0.547**	0.726**	0.549**	1.000	
Nut sp gr	0.047	0.042	–0.186	0.017	–0.795**	1.000

	Kernel sp gr	Shell sp gr	% Fill	% kernel	% oil	% Moisture	Color
Kernel sp. gr.	1.000						
Shell sp. gr.	0.095	1.000					
% fill	0.586**	0.433**	1.000				
% kernel	0.617**	–0.724**	0.069	1.000			
% oil	–0.030	–0.192	0.148	0.128	1.000		
% moisture	0.393	–0.102	0.070	0.110	0.187	1.000	
Color	0.134	–0.068	0.007	0.140	0.130	0.609**	1.000

**Significant P = <0.01

Adsorption of Bile Acids by Components of Alfalfa and Wheat Bran in Vitro

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ABSTRACT

Adsorption of 2.5 mM and 5.0 mM cholic acid and deoxycholic acid by plant materials high in dietary fiber (alfalfa and wheat bran) and fractions derived from sequential extraction of plant material to remove various components was measured. Results tend to support the hydrophobic nature of adsorption and indicate a lack of macromolecular interactions between dietary fiber and bile acid micelles. Lignin appears to be an extremely important component in the interaction of dietary fiber with bile acids, but holocellulose also plays a significant role. Fractionation of dietary fiber sources may provide a method for study of the effects of specific components of dietary fiber which avoids some of the harsh isola- procedures.

INTRODUCTION

SOME DIETARY FIBER containing foods and some isolated components of dietary fiber have the ability to adsorb bile acids and bile salts (Eastwood and Hamilton, 1968; Kritchevsky and Story, 1974; Story and Kritchevsky, 1976; Eastwood et al., 1976; Vahouny et al., 1980). This adsorption has been theorized to be the cause of increased fecal bile acid excretion and, in turn, decreased levels of serum and/or tissue cholesterol (Story, 1980). The component(s) of dietary fiber responsible for this adsorption has not been determined but early work has yielded some clues. Through sequential extraction of "dry grain," Eastwood and Hamilton (1968) found that bile salt adsorption was present after extraction of many carbohydrate components of dietary fiber but was eliminated upon removal of lignin, leaving only holocellulose (cellulose and hemicellulose). Isolated lignin has also been shown to be active in adsorbing bile acids (Story and Kritchevsky, 1976; Kay et al., 1979). Other isolated components have been shown to adsorb bile acids in vitro (Nagyvary et al., 1980) and to increase bile acid excretion in vivo (Kay and Truswell, 1980: Kelley et al., 1981) indicating the complexity of the interaction of dietary fiber with bile acids and their subsequent effects on sterol balance.

This study was designed to examine the adsorptive capacity of alfalfa and wheat bran for cholic and deoxycholic acids in vitro and investigate the effects of sequential removal of various components on this adsorption. The suggestion that saponins are required for bile acid adsorption was also investigated.

EXPERIMENTAL

Plant materials

Alfalfa (*Medicago sativa* var. *Apollo*) was collected at the Purdue University Agronomy Farm and stored frozen until being lyopholized (Vritis Co., Gardinier, NY) and pulverized through a 1 mm mesh screen (Wiley Mill, A.H. Thomas, Co., Philadelphia, PA). Wheat bran was from the standard wheat bran preparation of the American Association of Cereal Chemists (St. Paul, MN) and was pulverized as above.

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Extraction procedures

In Experiment 1, alfalfa and wheat bran were subjected to sequential Soxhlet extractions with petroleum ether (30–60°C), acetone, and 95% ethanol with the marc being thoroughly dried between extraction steps (Robinson, 1963). The remaining material was extracted three times with 50% ethanol (10 ml/g at 70°C for 3 hr with stirring to remove saponins and then three times with hot water (10 ml/g) at 90°C for 3 hr with stirring. The remaining material was then delignified with sodium chlorite (Wise et al., 1946). A sample of the marc from each of the extraction steps was dried and stored for subsequent determination of its bile acid adsorption capacity. All soluble fractions were discarded.

In Experiment 2, alfalfa was extracted exhaustively with boiling ethanol-water (4:1, 10 ml/g) and the residue from this extraction was extracted with cold water (10 ml/g) for 24 hr with stirring. The marc was divided at this point and two methods of pectin extraction used. The first involved two extractions with 0.5% ammonium oxalate solution (10 ml/g) mixed at 80–90°C for 3 hr and thorough washing with deionized water (Aspinal and Fanshawe, 1961). The second involved three extractions with 2% disodium ethylenediamine tetraacetic acid (EDTA; 10 mg/g) at 70°C for 3 hr with stirring (Aspinal et al., 1968). Both these residues were then delignified as above (Wise et al., 1946). Again, dried samples of each marc were stored for bile acid adsorption measurements. Table 1

Table 1—A partial list of substances removed by extraction procedures

Extraction process	Substances removed
Experiment 1	
Petroleum ether	hydrocarbons, terpenes, fats, etc.
Acetone	some alkaloids, coumarins, phenols, etc.
95% Ethanol	alkaloids, some saponins, carbohydrates, etc.
50% Ethanol	glycosides, saponins, proteins, carbohydrates, etc.
Hot water	pectins, starch, gums, proteins, mucilages, other carbohydrates, etc.
Sodium chlorite	lignin
Residue	holocellulose (cellulose, hemicellulose)
Experiment 2:	
Ethanol (4:1)	glycosides, some saponins, carbohydrates, etc.
Cold water	glycosides, organic acids, proteins, saponins, carbohydrates, etc.
Hot water and 0.5% ammonium oxalate or 2% EDTA	pectins
Sodium chlorite	lignin
Residue	holocellulose (cellulose, hemicellulose)

summarizes the major substances removed by each extraction procedure.

Bile acid adsorption

The bile acid adsorption capacity of each of the above fractions was measured using a combination of the methods of Eastwood et al. (1976) and Story and Kritchevsky (1976). Solutions of cholic and deoxycholic acids (Calbiochem-Behring Corp. LaJolla, CA) with [^{3}H(G)] cholic acid or [^{3}H(G)] deoxycholic acid (New England Nuclear, Boston, MA) were prepared in phosphate buffer (pH 8.0) at concentrations of 2.5 and 5.0 mM (specific activities of 20 and 10μ Ci/m mol, respectively). Triplicate samples (100 mg) of the residue from each extraction were incubated with 3.5 ml of the bile acid solution in screwcap culture tubes for 8 hr at room temperature. Tubes were then centrifuged for 30 min at 2100 x g (Damon/IEC DPR-6000, Scientific Products, McGaw, Park, IL) and 0.1 ml samples assayed for radioactivity by liquid scintillation spectrometry (Prias-PL, Packard Instruments, Downers Grove, IL). The supernatant was then removed and replaced by the same volume of phosphate buffer (pH 8.0) and incubated for 8 hr, centrifuged, and a sample removed for assay of radioactivity as before. This washing procedure was used to determine the influence of changes in water-holding capacity induced by extraction procedures on bile acid adsorption. Preliminary studies indicated that subsequent washes removed very little additional bile acid. Supernatant radioactivity is a measure of unbound substrate allowing calculation of bound bile acids by subtraction. Similarly, adsorption after washing can be calculated using the amount of activity remaining after removal of supernatant as the total available bile acid.

RESULTS

Experiment 1

As can be seen in Fig. 1 and 2, the hydrophobic nature of the adsorption of bile acids is reconfirmed by the higher level of adsorption of deoxycholate than cholate by alfalfa (Eastwood and Hamilton, 1968; Story and Kritchevsky, 1976). In addition, (Fig. 1–3) little difference in adsorption was observed between the 2.5 and 5.0 mM concentrations of either bile acid indicating negligible micellar interactions with either alfalfa or wheat bran.

Adsorption of both bile acids at either concentration was affected similarly by the fractionation procedures. Adsorption of 2.5 mM cholic and deoxycholic acids (Fig. 1) was not decreased by removal of lipid soluble substances and increased slightly upon extraction with 95% ethanol. Little change resulted from treatment with 50% ethanol or hot water but a significant decrease in adsorption of both bile acids resulted from delignification ($p < 0.05$). However, the holocellulose retained a significant capacity to adsorb deoxycholate at this concentration. Increases over control adsorption probably arise from a concentration of adsorbing components as a result of extraction of inactive materials, since a similar amount of all materials was used for adsorption measurements.

No significant differences in adsorption were observed when 5.0 mM cholate and deoxycholate were used (Fig. 2) except the capacity of holocellulose for 5.0 mM cholate which is higher than that for 2.5 mM. This difference may have been due to some adsorption of micelles but was not observed in any other fraction with either bile acid.

The adsorption of deoxycholate at 2.5 and 5.0 mM by wheat bran and its fractions is shown in Fig. 3. The level of adsorption is lower at 5.0 mM but approximately the same at 2.5 mM as that observed for alfalfa. This is unlike differences in adsorption reported from similar measurements done at a lower pH (Kirtchevsky and Story, 1974; Story and Kritchevsky, 1976). Like alfalfa, little change in capacity is observed with removal of lipid soluble materials. A significant increase occurred after extraction with 50% ethanol at both deoxycholate concentrations as a result of removal of non-adsorbing components. Treatment with hot water resulted in a decrease to control levels, possibly indicating some involvement of polysaccharides such as pectins and gums. Other methodology has indicated an ability of these polysaccharides to adsorb bile acids (Nagyvary et al., 1980). Delignification with sodium cholorite reduced adsorption significantly but significant levels of adsorption were also observed with wheat bran holocellulose.

A significant quantity of the bile acids adsorbed to both alfalfa and wheat bran were apparently reversibly bound to the plant material as evidenced by reduction in adsorbed

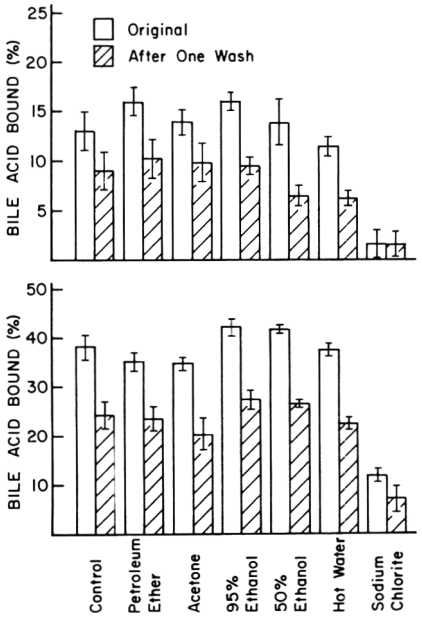

Fig. 1—Adsorption of 2.5 mM cholic acid (top) or deoxycholic acid (bottom) by alfalfa and its fractions from Experiment 1. (Mean ± standard deviation)

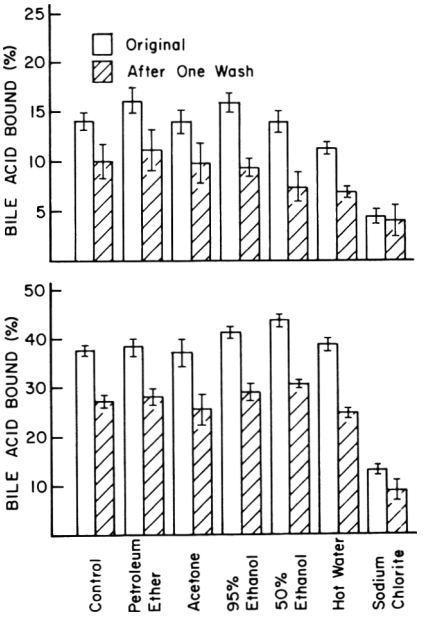

Fig. 2—Adsorption of 5.0 mM cholic acid (top) or deoxycholic acid (bottom) by alfalfa and its fractions from Experiment 1. (Mean ± standard deviation)

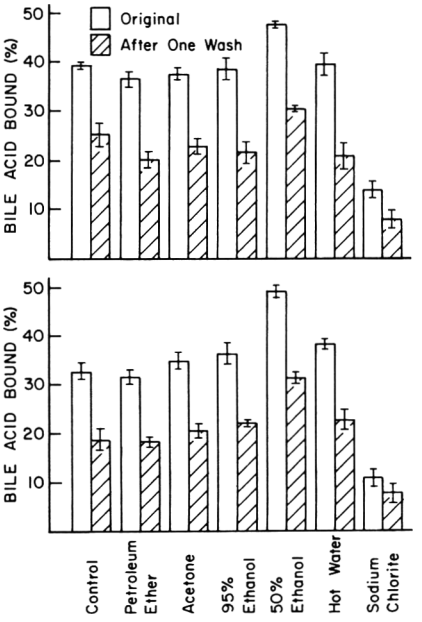

Fig. 3—Adsorption of 2.5 mM (top) and 5.0 mM (bottom) deoxycholic acid by wheat bran and its fractions from Experiment 1. (Mean ± standard deviation)

bile acid after the washing procedure. Loss of adsorbed bile acid as a result of the higher water holding capacity of wheat bran. Cholic acid adsorption to alfalfa was made less permanent by removal of saponins as evidenced by the higher loss upon washing when saponins were removed. Hollocellulose exhibited very little reduction in adsorbed bile acid as a result of washing. Water adsorbed by the dietary fiber sources with bile acid in solution would be readily diluted by the added buffer and the bile acids which were apparently adsorbed during the first incubation would no longer be determined as adsorbed. Some exchange or loss of adsorbed bile acids would also occur during the washing process. Holocellulose has apparently lost the structural characteristics responsible for water holding as a result of the extensive extraction procedures and thus has little bile acid to be lost during the washing procedure.

Experiment 2

Adsorption of cholate by alfalfa was again lower than adsorption of deoxycholate at both 2.5 and 5.0 mM (Fig. 4–7). The concentration of deoxycholate did not influence adsorption but more cholate was adsorbed at 5.0 mM than at 2.5 mM by alfalfa in both extraction schemes of Experiment 2.

No change in adsorption capacity was observed as a result of the ethanol-water or cold water extraction of alfalfa. Neither of the alternate methods of pectin extraction (hot water, ammonium oxalate: EDTA) caused any significant reduction in adsorption. Delignification again reduced adsorption significantly for both bile acids at both concentrations.

The apparent reversible portion of bile acid adsorption was significant at both concentrations of deoxycholate, with exception of the residue after delignification which had no apparent loss of adsorbed bile acid. The reduction in adsorbed cholate at the 2.5 mM concentration was not significant after any of the extraction procedures. At 5.0 mM, a reduction was observed with all the fractions except holocellulose.

DISCUSSION

THESE DATA CLARIFY several points concerning the bile acid adsorption phenomenon of dietary fiber. Fat soluble components do not appear to be involved in bile acid adsorption and their hypocholesteremic effects (Malinow et al., 1977) are apparently not due to direct interaction with bile acids. Similar studies which indicated a requirement for saponins in bile acid adsorption (Oakenful and Fenwick, 1978) were carried out at pH 6.7, conditions which would confound adsorption with decreased solubility of the bile acid. Many materials which have very low saponin content (e.g. wheat bran in these studies) adsorb appreciable quantities of bile acids and bile salts (Eastwood and Hamilton, 1968; Kirtchevsky and Story, 1974; Story and Kirtchevsky, 1976; Eastwood et al., 1976; Vahouny et al., 1980).

Removal of the water soluble components of dietary fiber (pectins, gums, mucilages, etc.) did not alter the adsorption of bile acids except in the case of wheat bran (Fig. 3). As yet unidentified hot water soluble components of wheat bran may possess bile acid adsorption capacity. Evidence that pectin interacts with bile acids suggests that these changes are related primarily to the loss of these dietary fiber components.

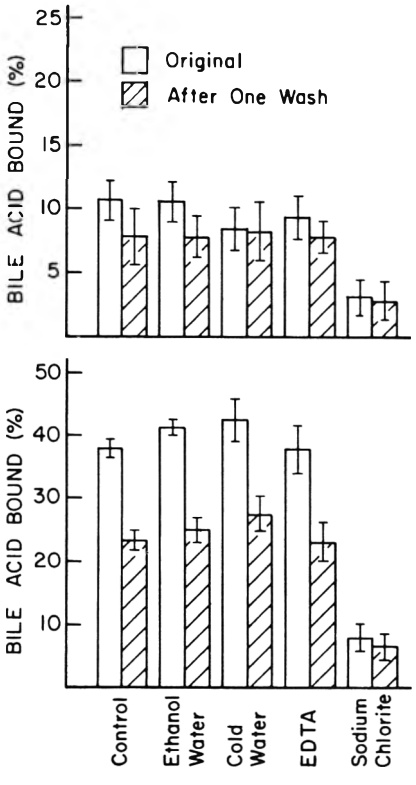

Fig. 4—Adsorption of 2.5 mM cholic acid (top) and deoxycholic acid by alfalfa and its fractions from Experiment 2 using hot water and ammonium oxalate to extract pectins and gums. (Means ± standard deviation)

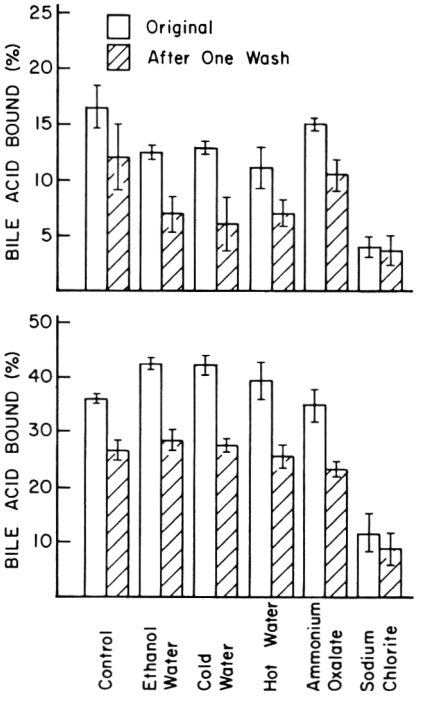

Fig. 5—Adsorption of 5.0 mM cholic acid (top) and deoxycholic acid by alfalfa and its fractions from Experiment 2 using hot water and ammonium oxalate to extract pectins and gums. (Mean ± standard deviation)

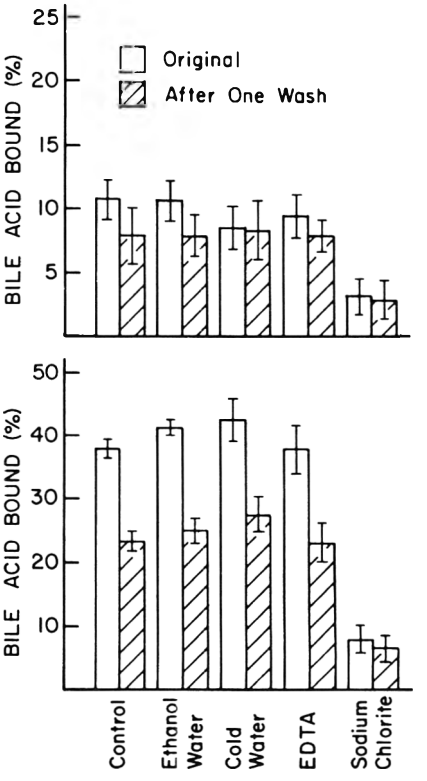

Fig. 6—Adsorption of 2.5 mM cholic acid (top) and deoxycholic acid by alfalfa and its fractions from Experiment 2 using EDTA to extract pectins and gums. (Mean ± standard deviation)

In both experiments, the greatest decrease in adsorption was caused by removal of lignin. Reports of adsorption of bile acids by lignin isolated in several ways (chemical and physical) as well as earlier evidence that delignification resulted in a loss of adsorption (Eastwood and Hamilton, 1968; Story and Kritchevsky, 1976; Kay et al., 1979) suggest a central role for lignin in the adsorption process. However, the observation that holocellulose retained bile acid adsorption capability indicates that this component may also be involved. Isolated cellulose has little capacity for bile acids or salts (Kritchevsky and Story, 1974; Story and Kritchevsky, 1976; Vahouny et al., 1980) but little is known concerning the interaction of hemicellulose with bile acids due to a lack of data using appropriate hemicellulose isolation techniques. Based on current knowledge it would appear that hemicellulose may display in vitro interaction with bile acids. It should be noted that delignification is the most destructive of the procedures applied to the plant materials and causes changes in the structural interrelations of the components which may alter adsorption capacity.

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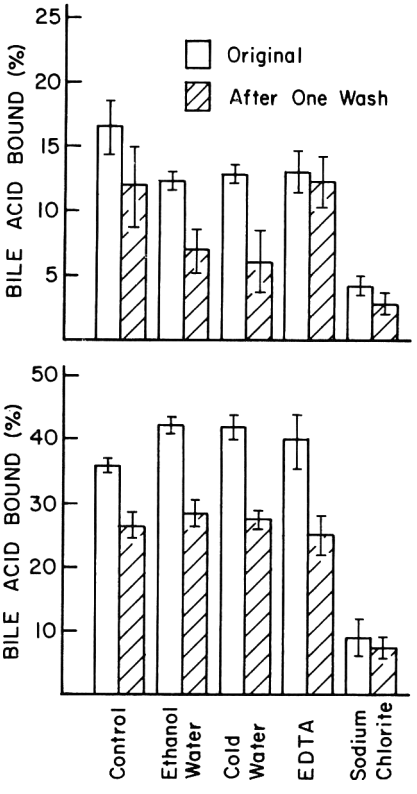

Fig. 7—Adsorption of 5.0 mM cholic acid (top) and deoxycholic acid by alfalfa and its fractions from Experiment 2 using EDTA to extract pectins and gums. (Mean ± standard deviation)

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EFFECT OF DRIP IRRIGATION ON PECAN QUALITY . . . From page 1275

This method of quality evaluation should be very useful for researchers engaged in cultural practice and production type research, and have application for assessing value of nuts in shelling and processing, since processing costs are partially dependent on nut size (no./kg) kernel shell out yield.

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Phytate Removal from Soy Protein Isolates Using Ion Exchange Processing Treatments

J. R. BROOKS and C. V. MORR

ABSTRACT

A combination cation/anion exchange process was developed to remove 96–97% of the phytate and 97–99% of the Ca and Mg from soy protein isolates. Application of either of the ion exchange treatments alone failed to effectively remove phytate from soy protein extracts and also failed to convert the remaining phytate to a dialyzable form. Effective removal of phytate was only achieved by the combination ion exchange treatment. Consideration of these findings led to a proposed mechanism for the sequential disruption of the protein-Ca/Mg-phytate complex by the ion exchange treatment.

INTRODUCTION

PHYTATE, the hexaphosphate salt of myo-inositol (Erdman, 1979) is the principal storage form of P in the soybean (Okubo et al., 1975). Phytate possesses eight negatively charged phosphoric acid residues per molecule between pH 6.3 and 9.7 and six negative groups between pH 1.8 and 6.3 (Crean and Haisman, 1963; de Rham and Jost, 1979). As a result of this polyvalent nature, it is strongly associated with soy proteins above their isoelectric point (pH $\geqslant$ 4.5), especially in the presence of Ca^{++} and Mg^{++} ions (Hartman, 1979). Phytate is also associated with soy proteins at pH values below their isoelectric point by electrostatic interactions involving their cationic lysyl, histidyl, arginyl and terminal amino groups (Okubo et al., 1975, 1976).

Since commercial soy protein products normally contain 2–3% phytate, there is some concern that they may adversely alter the bioavailability of Fe and other trace minerals as well as that of the proteins per se. This subject has been reported by numerous workers (Erdman, 1979). Additionally, it is highly likely that removal of phytate from commercial soy protein products would drastically alter their basic physicochemical and functional properties.

Research efforts to remove phytate from soy proteins include' (a) phytase enzymatic treatments with and without ultrafiltration (Okubo et al., 1975, 1976); (b) alkaline extraction at pH 11–12 (Hartman, 1979); (c) ultrafiltration (Omasaiye and Cheryan, 1979; Ford et al., 1978); and (d) dialysis and anion exchange treatments (Smith and Rackis, 1957). The highest degree of phytate removal reported by the above researchers was in the range of 90%.

This paper describes a two-stage ion exchange processing technique for effectively removing Ca^{++}, Mg^{++}, phytate and total P from soy protein extracts.

MATERIALS & METHODS

LOW TEMPERATURE, hexane extracted defatted soy flakes with high protein solubility were obtained from Ralston Purina Company (St. Louis, MO). Ion exchange resins were Rexyn 101 cation exchanger and Rexyn 202 anion exchanger, both purchased from Fisher Scientific Company. Dialysis tubing was Spectrapore No. 1 (32 mm flat diameter with a 6,000–8,000 MW cutoff) from Fisher Scientific Company. Sodium phytate was purchased from Sigma Chemical Company and 2.4-diaminophenol dihydrochloride was purchased from Eastman Kodak Company. Demineralized distilled water was prepared by passing glass distilled water through a Barnstead Model BD-2 demineralizer.

Soy protein extraction and ion exchange treatments

Defatted soy flakes were twice extracted with dilute NaOH solution at pH 8.5–9.0 as shown in Fig. 1. The alkaline extracts were then subjected to various cation and anion exchange and dialysis [treatments (Fig. 1 and 2) to remove cations, phytate and other polyvalent ions prior to freeze drying. Resin bed dimensions were 5 cm i.d. x 32 cm length (cation) and 5 cm i.d. x 35 cm length (anion). Extracts were passed through the ion exchange columns at a flow rate of approximately 20 ml/min. One hundred ml fractions of effluent were generally collected for analysis (Fig. 1) in Trial One and 300 ml fractions were collected for Trial Two (Fig. 2). For this latter experiment the 300 ml effluent fractions were divided into two parts. One part was freeze-dried directly and the other part was dialyzed against four liters of distilled water for 72 hr at room temperature (23–24°C) with bath changes after 24 and 48 hr dialysis.

Ion exchange column regeneration

The anion exchange column was regenerated by passing 10 bed volumes of regenerant solution containing 1% NaOH and 10% NaCl with a minimum contact time of 30 min. The column was then rinsed with sufficient 10% NaCl solution to drop the pH to $\cong$ 8, which usually required in the order of five bed volumes of rinse solution. Finally, the column was backflushed with distilled water to remove excess NaCl and to repack the column.

The cation exchange column was regenerated and placed in the Na^+ form by passing two bed volumes of 10% NaCl solution at a minimum contact time of 30 min. The column was then backflushed with distilled water as above. Regeneration of new cation

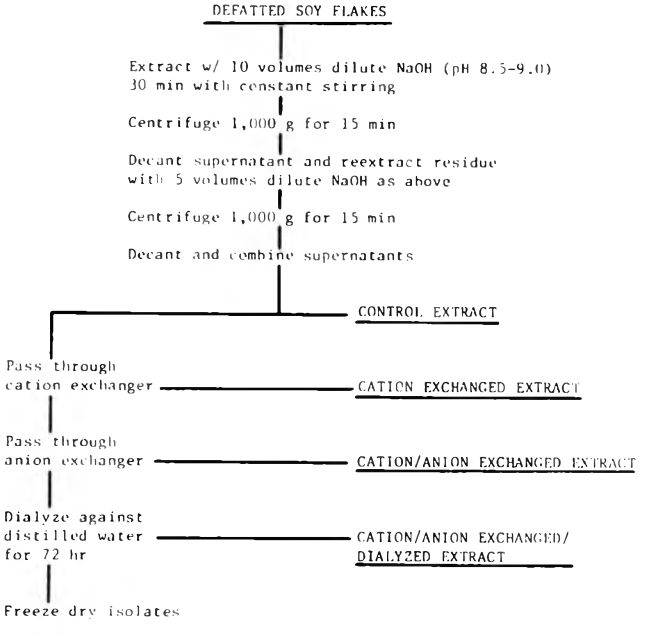

Fig. 1—Procedure for producing combination ion exchanged soy protein extracts and isolates (Trial One).

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resin (H^+ form) was accomplished by treating with two bed volumes of 1% NaOH and 10% NaCl solution, followed by three bed volumes of 10% NaCl solution and backflushing as above.

All columns were occasionally treated with 1% formaldehyde solutions to prevent microbial contamination and related problems (Kunin, 1973).

Analytical methods

Freeze-dried isolates were oven dried at 102–104°C for 24 hr to determine moisture content prior to chemical analysis. Protein determination was by micro Kjeldahl (AOAC, 1970) with the following modifications: a mixture of 10 parts K_2SO_4 and one part $CuSO_4$ was used as catalyst and a 50% NaOH solution was used for neutralizing the H_2SO_4 digest in order to distill the ammonia. Protein values were calculated from N data using a conversion factor of 5.70.

Total P was determined by the method of Allen (1940). The molybdenum blue complex was assayed at 640 nm using a Coleman Jr. II Spectrophotometer.

Phytic acid was determined by an adaptation of the method of Earley (1944) with suitable modification from Makower (1970) and Wheeler and Ferrel (1971). A finely ground sample of isolate containing 0.18-7.0 mg phytate was weighed into a 125 ml Erlenmeyer flask. The sample was extracted with 50 ml of 1.2% HCl for 2 hr on a shaker bath at room temperature (23–24°C). The extract was filtered through Whatman No. 41 filter paper and an aliquot containing sufficient P to meet the requirements of Allen's method was transferred to a clear centrifuge tube. Sufficient $FeCl_3$ solution containing 2 mg Fe/ml, made up in 0.6% HCl/5% Na_2SO_4 solution, was then added to each tube to provide twice the theoretical Fe requirement, e.g., eight atoms Fe per six phosphoric acid residues. The tubes were subsequently heated for 1 hr in a boiling water bath (Wheeler and Ferrel, 1971) to provide complete phytate precipitation. A clear yellow supernatant was obtained in all tubes following heating, which confirmed the presence of excess Fe. Tubes were cooled in an ice bath and centrifuged at 30,900 x *g* (max force) for 20 min. The supernatant fracton was carefully decanted and the phytate precipitate was retained and washed by adding 20–25 ml of 0.6% HCl/5% Na_2SO_4 solution, reheated as above for 5–10 min, cooled and recentrifuged. The supernatnat was carefully decanted and the precipitate was dispersed in distilled water and quantitatively transferred to a 100 ml micro-Kjeldahl digestion flask. The precipitate was digested and analyzed for total P by the method of Allen (1940). The percentage of phytate was calcualted on the basis that it contains 28.2% P (de Boland et al., 1975).

Ca and Mg were determined by flame photometry using a Hitachi 170–50 Atomic Absorption Spectrophotometer equipped with a 508–0143 burner chamber and a 208–0106 three slot burner. Samples were wet digested in a 2:1 mixture (v/v) of nitric and perchloric acids, diluted to appropriate volume with 0.15% strontium chloride solution and readings were made at 422.7 nm for Ca and at 285.2 nm for Mg. The strontium chloride solution was used as diluant to prevent the depressive effects of certain ions in the solution (Dinnin, 1960).

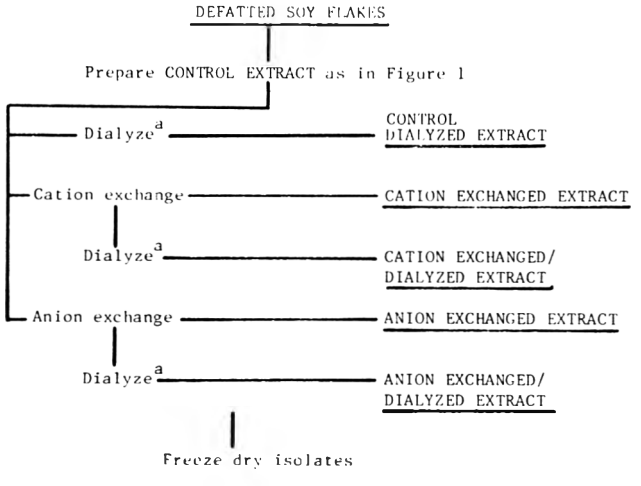

Fig. 2–Procedure for producing cation and anion exchanged soy protein extracts and isolates. [a]dialysis against distilled water for 72 hr as in MATERIALS & METHODS (Trial Two).

RESULTS & DISCUSSION

TWO TRIALS were conducted in which defatted soy flakes extracts were subjected to a series of ion exchange and dialysis treatments (Fig. 1 and 2) to remove Ca, Mg, phytate and other polyvalent ions. The dialysis step was used to remove other nonprotein components, e.g., sugars, flavor compounds, pigments, enzyme inhibitors and others, without resorting to the conventional acid precipitation steps. Thus, the dialysis treatment was essential for increasing the protein content from 60–65% (nondialyzed extracts) to ≅ 86% on a dry basis (Table 1) for dialyzed extracts.

As expected, the cation exchange treatment alone (Trial One) effectively removed both Ca and Mg from the extracts and their isolates, but removed only about 30% of the phytate and had no apparent effect upon their P contents. The combination cation and anion exchange treatment removed 96–97% of the phytate and most of the residual Ca and Mg. Dialysis of cation/anion exchanged extracts unexpectedly resulted in a slight regain of both Ca and Mg and also in P. A major portion of this apparent mineral regain was undoubtedly due to removal of nonprotein/nonmineral components listed above during the dialysis which had the effect of concentrating the protein and protein associated minerals on a dry basis. The dialysis treatment resulted in a 1.4:1 increase in protein content of the dry isolate, a 1.1:1 increase in P, but a 7:1 and 3.5:1 increase in Ca and Mg content, respectively, on a dry basis. Thus, dialysis was found to consistently produce an increase in both of these important cations, although the mechanism for this effect is unknown.

The further reduction of Ca and Mg content by anion exchange treatment of cation exchanged extract suggests that these ions are probably associated with the protein and phytate in a form that does not permit their removal by the initial cation exchange treatment. Thus, it is necessary to use both the cation and anion exchange treatments, and in this particular order, to obtain effective removal of Ca, Mg and phytate from soy protein extracts. To test this hypothesis further, Trial Two was conducted in which extracts were subjected to cation or anion exchange treatments separately rather than in combination as in Trial One. The results of these experiments are in Table 1.

None of the single ion exchange treatments was effective for removing Ca, Mg or Phytate. These findings further confirm that the combination cation/anion exchange treatment is necessary for effective removal of cations and phytate from soy protein extracts. This latter process (as in Trial One) resulted in complete phytate removal.

Based upon the above findings, the following mechanism is proposed for the ion exchange removal of phytate from soy extracts. The Ca, Mg and phytate are complexed with the proteins in the soy extract. The cation exchange treatment removes most of the available Ca and Mg from the complex, thereby converting the proteins and phytate to their Na forms which remain nondialyzable at this stage, e.g., they retain an effective molecular weight in excess of 6,000–8,000. This latter finding appears to be at variance with reports of Okubo et al. (1975), who found that EDTA disrupts the protein-phytate complex in alkaline solutions, thus rendering the phytate dialyzable. Subsequent removal of the above dissociated phytate molecules by the anion exchange treatment results in concomitant removal of small amounts of additional Ca and Mg not removed during the initial cation exchange treatment. This difficult to remove Ca and Mg may have been located within the interior of the protein-phytate complex or otherwise unaccessible to the cation exchange treatment.

–Continued on next page

Table 1—Chemical composition of soy protein isolates as influenced by ion exchange processing[a]

Soy protein isolate	Protein		Phytate		Phosphorus		Calcium		Magnesium	
	%, Dry Basis									
	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg
Trial One[b]										
Control isolate	58.9–59.8	59.2	2.00–2.05	2.03	0.867–0.885	0.876	0.180–0.200	0.190	0.207–0.211	0.209
Cation exchanged isolate	60.0–60.7	60.3	1.32–1.35	1.33	0.884–0.891	0.888	0.021–0.037	0.029	0.00138–0.00153	0.00145
Cation/anion exchanged isolate	68.4–73.7	71.1	<0.011[c]	<0.011[c]	0.084–0.092	0.087	0.0023–0.0098	0.0056	0.00146–0.00187	0.00161
Cation/anion exchanged/dialyzed isolate	85.9–87.2	86.6	<0.011[c]	<0.011[c]	0.090–0.096	0.092	0.028–0.044	0.039	0.00551–0.00586	0.00571
Trial Two[d]										
Control isolate	59.3–60.7	60.0	2.04–2.06	2.05	0.847–0.849	0.848	0.180–0.200	0.190	0.204–0.209	0.207
Control dialyzed isolate	83.5–84.5	84.2	2.65–2.71	2.68	0.945–0.979	0.961	0.300–0.310	0.310	0.208–0.230	0.222
Cation exchanged isolate	60.0–60.5	60.2	1.88–1.92	1.90	0.857–0.886	0.876	0.032–0.036	0.034	0.00590–0.00613	0.00600
Cation exchanged/dialyzed isolate	85.6–88.0	86.5	1.90–1.92	1.91	0.760–0.931	0.859	0.067–0.083	0.075	0.0164–0.0168	0.0165
Anion exchanged isolate	65.0–65.7	65.2	1.81–1.83	1.82	0.694–0.704	0.699	0.210–0.250	0.230	0.197–0.210	0.205
Anion exchanged/dialyzed isolate	84.4–86.9	85.7	1.95	1.95	0.733–0.777	0.756	0.250–0.310	0.290	0.206–0.209	0.208

[a] Data are based on triplicate determinations for a single trial
[b] Procedure as in Fig. 1
[c] Data from subsequent trials range from 0.062 to 0.064% which corresponds to a 96–97 phytate removal
[d] Procedure as in Fig. 2

The following experiment was conducted to determine the extent of soy protein bound to the ion exchange columns and thereby lost from the process stream. An excess volume of soy extract was sequentially passed through the cation/anion exchange columns in order to totally saturate their binding sites with phytate. For example, 250 ml of pH 8.5 soy extract was passed through 20 ml bed volume cation and anion exchange columns, each at a flow rate of about 5 ml/min, e.g., about 2.8 ml/cm^2 cross-sectional area/min. Both ion exchange columns were then sequentially rinsed with two bed volumes of distilled water to remove influent solution and this rinse solution was discarded. the columns were then regenerated by sequentially passing two bed volumes of 10% NaOH, one bed volume distilled water, five bed volumes of 10% NaCl and two bed volumes of distilled water. All regenerant solutions were combined, dialyzed against distilled water and concentrated to a final volume of 25 ml by vacuum evaporation. Aliquots of the final regenerant concentrate as well as of the soy extract were analyzed for total nitrogen content by micro-Kjeldahl. Results indicated that about 0.77% of the original protein in the soy extract was actually bound and lost from the process stream.

Additional work is underway to investigate the possibility that such ion exchange processing treatments may result in denaturation of the proteins with subsequent alterations in their basic physico-chemical properties that would diminish their functionality for food applications uses. Preliminary results do not substantiate detectable protein denaturation by ion exchange processing treatments described in this paper. On the contrary, ion exchange treated soy protein isolates, following freeze drying, are more soluble at pH 4.5 and at pH values above and below the isoelectric point than are control soy protein isolates, which is in general agreement with findings of Smith and Rackis (1957). However, more definitive experiments are needed to determine the possible alterations in the structure and solution properties of the soy protein molecules, their complexes and subunits that may result from ion exchange processing to remove major portions of their phytate contents.

In conclusion, the combination cation/anion exchange process investigated in the present study effectively removes phytate from soy extracts and its relatively simple and rapid operational characteristics should make it attractive for use by the soy protein processing industry. Although it was not used in this study, mixed-bed ion exchange reaction technology should be an even more acceptable approach for preparing phytate-free soy protein extracts and should further simplify the processing schedule.

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Accumulation of Selected Trace Elements in Hydroponically Grown Soybeans and Distribution of the Elements in Processed Soybean Fractions

S. E. LEVINE, C. M. WEAVER, and A. W. KIRLEIS

ABSTRACT

Soybeans grown hydroponically labeled with ^{59}Fe or two levels of a ^{51}Cr-^{65}Zn mixture were analyzed for the distribution of these radionuclides in mature edible and nonedible parts. The concentration of ^{59}Fe was significantly higher in the hulls than in other plant parts unlike for ^{51}Cr and ^{65}Zn. The seeds produced were then processed into a defatted soybean meal in order to investigate the effects of processing on the content of iron, zinc, and chromium. No detectable change in total Zn content and no significant change in ^{51}Cr content were evident during processing. However, an overall 9% gain of total Fe content was observed as a result of processing probably due to Fe contamination by processing equipment.

INTRODUCTION

INCREASED CONSUMPTION of refined and processed foods in the U.S. has probably increased the susceptibility of the population to trace element deficiencies. The essentiality for trace elements in the diet has prompted searches for good food sources. The trace minerals Fe, Zn, and Cr have been implicated as being at least marginally deficient in the United States and other countries probably due to inadequate consumption and/or poor mineral bioavailability (Mertz, 1980; Kumpulainen et al., 1979; Klevay et al., 1979; Underwood, 1978; Hambidge, 1974; and Hambidge et al., 1972).

Soybeans, an important crop in the United States and a staple crop in many countries, contribute substantial amounts of trace elements to the diet. The United States is one of the leading exporters of soybeans producing 49.4 million metric tons of soybeans in 1980–81, and this figure is expected to increase (Herren, 1981). In the same year, 11.4 million metric tons of soybean meal were processed.

Knowledge of the distribution of trace elements in edible and nonedible plant parts may be used to predict losses during processing as a direct result of removing fractions rich in micronutrients. Distribution of mineral elements depend in part on the time of their application relative to the growth cycle of the plant and how easily each element is mobilized to reproductive parts. Generally, iron is relatively immobile once it has arrived via the xylem to living tissues. Therefore, leaves typically contain more Fe than any other plant parts. However, seeds do accumulate significant amounts of Fe. Legume seeds accumulate more Fe than cereals, but cereals and grains typically contain more Zn and Cr than do legume seeds (Schroeder, 1971). Distribution of Zn among plant parts varies with species (Rao et al., 1977; Singh and Steenberg, 1974). Zinc accumulation was reported to be greater in the stems than in the leaves of soybeans and the roots contained considerably less Zn than the tops (Rao et al., 1977). The beans were not analyzed. Chromium generally appears to accumulate in plant roots and is poorly translocated to the tops (Garcia et al., 1979; Lahouti and Peterson, 1979; Myttenaere and Mousny, 1974; Huffman and Allaway, 1973a, b; Turner and Rust, 1971; Lyon et al., 1969). In plant tops, most of the Cr remains in the leaves and stems and little Cr is transported to the seeds (Cary et al., 1977; Huffman and Allaway, 1973a).

A Cr-Zn interaction has been reported in both animals and plants. Oral Cr markedly depressed whole body ^{65}Zn absorption in Zn-deficient rats (Hahn and Evans, 1975). The presence of ^{51}Cr in the nutrient solution of hydroponically grown bush beans and oats resulted in a suppressed accumulation of ^{65}Zn (Schmitt and Weaver, 1980; Rynearson, 1981). In addition, ^{51}Cr depressed the yields of bush beans. Contrastingly, when soybeans were grown in soils containing up to 60 ppm Cr or hydroponically in nutrient solutions containing up to 60 ppm Cr or hydroponically in nutrient solutions containing up to 5 ppm Cr, Zn accumulation by roots and tops were not affected (Turner and Rust, 1971). However, the 7 to 9 day old plants received the Cr treatments for only 5 days before they were harvested for analysis.

This study employed radioactive tracers of Fe, Zn, and Cr to more accurately follow accumulation of these minerals by soybean plants and to assess the effect of each individual processing operation during preparation of a defatted meal on mineral element concentration. Specifically, the objectives were (a) to determine the accumulation and distribution of these nuclides in edible and nonedible tissues, (b) to determine the effect of application rate on distribution of ^{51}Cr and ^{65}Zn, and (c) to investigate the effects of ^{51}Cr added to the hydroponic nutrient solution on subsequent crop yields and the accumulation of ^{65}Zn by soybeans grown to maturity. In addition, the distribution of ^{59}Fe, ^{51}Cr, and ^{65}Zn and of total Fe and Zn in hull, defatted meal, and oil fractions of the mature soybeans are shown and the points at which mineral contamination or losses can occur during a typical soybean processing operation are reported here.

EXPERIMENTAL

Growth conditions

Soybeans (*Glycine max* L. merr 'Century') were grown in a hydroponic system constructed inside a greenhouse where day temperatures were approximately 27°C. Supplemental lighting supplied 146 microeinsteins/m^2 (cloudy day) with a 14 hour light period. The hydroponicum consisted of either 5 cm diameter polyvinylchloride pipes or fiberglass line trough in which nutrient solution (85 liters per trough or set of pipes – each of which contained approximately 50 plants) was circulated. The composition of the nutrient solution (pH = 5.5) was: 0.75 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.855 mM EDTA, 1.15 mM K_2PO_4, 1.25 mM NH_4NO_3, 2.145 mM KOH, 115 μM H_3BO_3, 50 μM $Na_2MoO_4 \cdot 2H_2O$, 8 μM $CuSO_4 \cdot 5H_2O$, 0.345 μM Co $(NO_3)_2 \cdot 6H_2O$, 45 μM $MnCl_2 \cdot 4H_2O$, 45 μM $FeSO_4 \cdot 7H_2O$, 510 μM $MgSO_4 \cdot 7H_2O$, 115 μM $ZnSO_4 \cdot 7H_2O$.

Soybeans were germinated in cellulose BR-8 grow-blocks (Famco, Inc., Medina, OH) and grown in unlabeled nutrient solution for 10 days. From this time on, 1 trough remained unlabeled and served as a control, and the remaining troughs incorporated the appropriate radioactive nutrient solution. The radionuclides, $^{51}CrCl_3$ (351 mCi/mg Cr) $^{65}ZnCl_2$ (23 mCi/mg Zn), and $^{59}FeCl_3$ (10.7 mCi/mg Fe) were obtained from New England Nuclear (Boston, MA

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02118). Plants were grown in nutrient solutions representing different treatments and containing the following radiolabels: ^{59}Fe (2.15×10^6 cpm/L of nutrient solution); 1X level ^{65}Zn (7.82×10^5 cpm/L); 1X level ^{51}Cr (2.41×10^5 cpm/L) plus 1X level ^{65}Zn (7.82×10^5 cpm/L); and 9X level ^{51}Cr (2.18×10^6 cpm/L) plus 3X level ^{65}Zn (2.3×10^6 cpm/L). Since previously the presence of ^{51}Cr had been shown to depress accumulation of ^{65}Zn in other plant species (Schmitt and Weaver, 1980; Rynearson, 1981); when the level of ^{51}Cr was increased here, the level of ^{65}Zn was also increased to insure that measurable quantities of ^{65}Zn reached the seeds. The final nutrient solutions contained approximately 7.45 ppb Zn, 2.5 ppm Fe, and those with added ^{57}Cr contained <0.045 ppb Cr. Losses in nutrient solution due to evapotranspiration were automatically replaced by a level sensing mechanism. Plants were exposed to the nuclides throughout their 4.5 month growing period so that their uptake into the plant would be optimized whether maximal uptake normally occurs in the vegetative or reproductive phase of the plant growth cycle.

Distribution analyses

At maturity 12 to 18 plants from each radionuclude treatment were harvested, and subsequently separated into edible dehulled seeds (hereafter designated "beans") and nonedible (stems and leaves, pods, and hulls) parts and analyzed for radioactivity by gamma counting techniques. For the mineral element distribution analysis, soybean seeds were then hand dissected into hulls and beans.

Soybean processing procedures

The soybean seeds (beans with intact hulls) from 25 to 40 plants from each treatment were pooled before being separated into individual hulls, defatted soybean meal, and oil fractions, according to the flow diagram shown in Fig. 1. Twenty-four hours prior to heat conditioning, the moisture content of the soybeans was adjusted to 12.5% with ultra-high purity water. In order to improve the separation of hulls and beans, the soybeans were heat conditioned for 7 min at 150°C and held in a sealed container at room temperature for at least 24 hr before cracking was begun. A modified pair of rolls from a Tag-Heppenstall moisture meter, style GR (C.J. Tagliabue Mfg. Co., Brooklyn, NY) was used to crack the preconditioned soybeans. The cracking operation produced a mixture of free hulls and pieces of the broken beans. The fine bean particles were removed by screening through a stainless steel sieve with a 0.787 mm opening. The remaining free hulls along with some small, light bean pieces were then separated from the large bean pieces by aspiration on a Burrows Model 1836 series seed blower. The resultant mixture of hulls and small bean pieces were separated by screening on a 5.6 mm round hole screen. The bean fractions were combined before being ground on a Model 4 Wiley mill fitted with a 1.0 mm round hole screen, and the oil was then extracted with hexane in a Soxhlet apparatus for 16 hr. The oil-extracted meal was air dried and autoclaved at 15 psi for 15–20 min to remove residual hexane in the meal and to inactivate trypsin inhibitor. The hexane was removed from the oil-hexane mixture in a rotoevaporator and the residual solvent remaining in the oil was removed in a vacuum oven (70°C and ≤ 25 mm Hg).

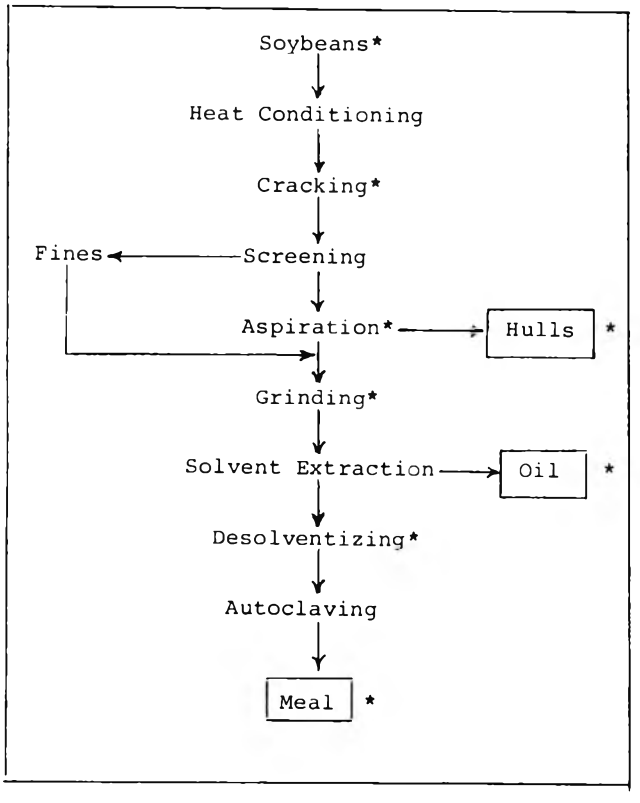

Fig. 1—Flow chart for soybean processing procedure. Asterisks denote sampling of products at different points for assay of radionuclides or stable mineral elements.

Nonradioactive mineral analysis

Three to six samples (approximately 2g each) from each stage of processing (Fig. 1) of the unlabeled plants were analyzed for Fe, Zn, and Cr by atomic absorption spectroscopy using a Perkin Elmer Atomic Absorption Spectrophotometer, Model 360, following a nitric-perchloric digestion (Weaver et al., 1981). All reagents used for ashing were redistilled in glass containers and ultra-high purity water was used for diluting purposes throughout. Analyses of the three elements were confirmed by also determining the recovery of pure compounds added to alternate samples.

Radionuclide assays

Ground plant tissues and soybean fractionated samples were weighed (approximately 1g each except for approximately 0.2g for hulls), dried in a vacuum oven (70°C and ≤ 25 mm Hg) for 18–20 hr, and then assayed for radioactivity by gamma ray spectroscopy using a 5 cm NaI (TI) crystal detector in a Beckman 4000 counting system. Samples which had been dually labeled with both ^{65}Zn and ^{51}Cr were assayed first by counting the activity of the ^{65}Zn only, with a window setting of 200–600 and then the activity of ^{51}Cr only with a window setting of 110–200. There appeared to be no significant interference of the ^{51}Cr activity in the ^{65}Zn window. However, interference from some of the ^{65}Zn activity was resolved from the ^{51}Cr photopeak by solving the following equation:

$$^{51}Cr(cpm) \text{ only} = N_A - f_{^{65}Zn} N_B$$

where N_A = Net counts per minute total, minus background, in Window A(^{51}Cr); N_B = Net counts per minute total, minus background, in Window B(^{65}Zn); $f_{^{65}Zn}$ = Fraction of counts that ^{65}Zn activity contribtes to Window A as determined with radionuclude solutions. The ^{59}Fe was counted integrally to include the entire gamma spectrum. All samples were counted to 10,000 counts, and the amount of radioactivity associated with each sample was recorded as counts per min after making corrections for background activity, radioactive decay, and fluctuations in counting efficiencies due to daily changes in the gamma counter.

Statistical analysis

Statistical differences in the distribution of radioactivity by treatment and plant parts and for the effect of processing on mineral content were determined by a one-way analysis of variance and a Duncan's multiple range test at alpha levels of 0.01, 0.05 and 0.10.

RESULTS

Distribution of radioactive mineral elements

The accumulation of ^{59}Fe, ^{51}Cr, and ^{65}Zn isotopes and their percentage distribution in each soybean plant tissue are presented in Table 1. The bean accumulated approximately 23% of the total ^{59}Fe found in the above-ground tissues of the plant. The concentration (cpm/g) of ^{59}Fe in the beans, however, was not significantly ($P > 0.05$) different from the pods or combined leaves and stems. The hulls however, contained a significantly higher ($P < 0.05$) concentration (cpm/g) of ^{59}Fe than other plant parts.

Although the significance is not indicated within rows in Table 1, the resultant concentration of ^{51}Cr in the leaves and stems, pods, and bean significantly increased ($P < 0.05$) with the increasing levels of ^{51}Cr and ^{65}Zn added to the nutrient solution. The mean percentage distribution of ^{51}Cr in the edible portion (beans) was significantly ($P < 0.01$) less in the $9X^{51}Cr$ treatment (4.3%) than in the $1X^{51}Cr$ treatment (14.6%) which also reflects the reduced percentage edible weight yield in the higher ^{51}Cr treatment (Table 2). The leaves and stems accumulated a greater percentage of the mineral in the higher ^{51}Cr treatments.

The concentration of ^{65}Zn (cpm/g) increased significantly ($P < 0.05$) in the leaves and stems, bean, and hull with the increased level (3X) of ^{65}Zn (Table 1). Also, more of the nuclide (percentage wise) was distributed into the combined leaves and stems in the $3X^{65}Zn$ treatment than in the $1X^{65}Zn$ applications. The activity (cpm/g) of ^{65}Zn increased in plants labeled with the $9X^{51}Cr$-$3X^{65}Zn$ treatment when compared to the lower application rate of ^{65}Zn and ^{51}Cr. However, since the edible yields decreased with increasing ^{65}Zn and ^{51}Cr application (yields not given), a decrease in percentage of the total ^{65}Zn accumulated by the edible portion of plants labeled with the higher application rate of ^{65}Zn and ^{51}Cr resulted (Table 1).

Chromium-zinc radionuclide interactions

Comparisons between soybean plants singly labeled with $1X^{65}Zn$ and those dually labeled with $1X^{65}Zn$ and $1X^{51}Cr$ treatments showed no significant differences ($P > 0.05$) in the accumulation of ^{65}Zn between the corresponding plant parts (Table 1). The presence of ^{51}Cr also did not significantly ($P > 0.05$) affect the % edible weight yields (Table 2) or dry weight yields (not given) at either level of ^{51}Cr when compared to plants only exposed to ^{65}Zn. However, plants exposed to the higher level of ^{51}Cr did have a signifiantly ($P < 0.05$) reduced % edible weight yield and plants exposed to either level of ^{51}Cr had significantly ($P < 0.05$) suppressed edible dry weight yields when compared to unlabeled plants. No significant differences ($P > 0.05$) were found in percent edible yields (or in weights on a dry basis) between plants exposed to unlabeled solution and those exposed to either ^{59}Fe or ^{65}Zn (Table 2).

Effects of processing on product mineral element concentration

Tracing the changes in concentration of both stable and radioactive mineral elements in products derived as a result of processing can help determine the specific steps in processing which significant mineral element gains or losses can occur. The concentration of Fe, Zn, and Cr at various steps of processing whole soybeans into a defatted meal and the resultant percentage gains and losses of these minerals as effected by processing are given in Table 3. In this paper, a loss of mineral element refers to a reduction in concentration as can occur upon removal of a seed component rather than an unaccountable quantity of the mineral. Concentration of the minerals in hydroponically grown soybeans are higher than for field grown beans due to the high availability of the mineral elements to the plant from the nutrient solution. Percentage changes due to processing were calculated by comparing the total quantity of the specific element or nuclide introduced to a processing step with that quantity which remained after the processing step shown in Fig. 1.

Significant ($P < 0.01$) losses of Fe occurred after the hulls were separated from the beans. The percentage losses of total stable Fe (−20.8%) and radioactive Fe (−16.2%) in the dehulling stage of processing were similar. Although the percent loss of ^{59}Fe was not significant at an alpha level of 0.05, it was a significant loss at an alpha level of 0.10. Nonradioactive mineral and radionuclude analyses showed that these apparent percentage losses of Fe thus resulted from removal of the hulls ($P < 0.05$). The hulls themselves accounted for 18.0% of the total Fe and 20.8% of ^{59}Fe of the whole soybean (Table 4). Significant ($P < 0.05$) gains in stable Fe (+15.9%) but not ^{59}Fe were found during the cracking of the whole beans on corrugated rollers and during defatting ($P < 0.01$) of the meal (+24.3%). The gain in stable Fe upon defatting was not due to an increase in concentration as a result of removing the oil since calculations were based on total Fe of the sample before defatting compared to the total Fe after the defatting step. The increase may have been caused by contamination from the Soxhlet extraction thimble which contains approximately 35 ppm of Fe. The oil recovered here did not contain detectable quantities of ^{59}Fe (Table 4).

Since the effect of processing on ^{65}Zn levels were similar for single and dual labeled plants, only the results for the single labeled plants are reported (Table 3). Significant losses of stable Zn (−8.9%; $P < 0.01$) and ^{65}Zn (−14.9%; $P < 0.05$) occurred as a result of hull removal ($P < 0.05$). The hulls had accounted for 8.0% of the total Zn and 14.2% of the ^{65}Zn content of the whole soybeans (Table 4). The apparent gain in Zn content from processing on the rollers was not significant.

The effect of processing on ^{51}Cr showed no significant ($P > 0.05$) changes in this radioactive mineral element (Table 3) because the hulls and the oil recovered contained no detectable quantities of ^{51}Cr (Table 4). Although the component parts only totaled approximately 40% of the ^{51}Cr in the whole beans, the concentration of ^{51}Cr in the defatted meal was not significantly ($P > 0.05$) different than that of the whole beans due to the large standard deviations. The large standard deviations for determinations of levels of ^{51}Cr were primarily due to the interference of

Table 1—Percent distribution and radioactive concentration (cpm/g) of ^{59}Fe, ^{65}Zn, and ^{51}Cr by plant tissues in soybeans (d.w.b.)***

	RADIONUCLIDE LABELING											
Nuclide measured	^{59}Fe		^{65}Zn		^{65}Zn		^{65}Zn		^{51}Cr		^{51}Cr	
Treatment	^{59}Fe		^{65}Zn		in $1X^{51}Cr/1X^{65}Zn$		in $9X^{51}Cr/3X^{65}Zn$		in $1X^{51}Cr/1X^{65}Zn$		in $9X^{51}Cr/3X^{65}Zn$	
Plant Tissue	cpm/g $\bar{x} \pm S$	% Nuclide $\bar{x} \pm S$	cpm/g $\bar{x} \pm S$	% Nuclide $\bar{x} \pm S$	cpm/g $\bar{x} \pm S$	% Nuclide $\bar{x} \pm S$	cpm/g $\bar{x} \pm S$	% Nuclide $\bar{x} \pm S$	cpm/g $\bar{x} \pm S$	% Nuclide $\bar{x} \pm S$	cpm/g $\bar{x} \pm S$	% Nuclide $\bar{x} \pm S$
Bean (dehulled)	25212 ± 803^{ab}	22.5 ± 10.2^{a}	8855 ± 597^{ab}	14.4 ± 11.4^{a}	9634 ± 104^{a}	17.2 ± 8.3^{a}	24400 ± 350^{ab}	11.1 ± 7.8^{a}	192 ± 31^{a}	14.6 ± 14.2^{a}	439 ± 88^{a}	4.3 ± 3.9^{a}
Hull	93525 ± 3310^{c}	10.7 ± 4.7^{b}	10964 ± 356^{ab}	2.4 ± 2.0^{b}	14566 ± 2545^{a}	4.2 ± 2.3^{b}	37448 ± 3851^{b}	3.0 ± 2.2^{b}	ND***^{a}	0^{b}	ND***^{a}	0^{b}
Pod	12714 ± 3662^{a}	9.8 ± 5.4^{b}	7654 ± 3371^{a}	13.9 ± 8.8^{a}	8909 ± 12440^{a}	13.3 ± 13.9^{a}	13221 ± 5040^{a}	6.7 ± 5.0^{c}	225 ± 115^{a}	14.5 ± 17.8^{a}	484 ± 132^{e}	5.1 ± 3.6^{a}
Combined Leaves and Stems	38135 ± 24943^{b}	57.1 ± 18.6^{c}	15034 ± 12260^{b}	69.3 ± 18.2^{b}	12171 ± 8372^{a}	65.3 ± 19.4^{c}	43299 ± 25474^{b}	79.3 ± 11.8^{d}	507 ± 230^{b}	70.9 ± 29.9^{c}	2643 ± 1653^{b}	90.6 ± 6.3^{c}

* The % Nuclide is based on the percentage cpm in the particular plant tissues out of the total cpm in all the above-ground portion of the plant only.

** Means and standard deviations of cpm and % nuclide for pods and leaves and stems were based on replicates of 12 to 18 plants. Seeds from all 12 to 18 plants were pooled and three aliquots were separating into dehulled beans and hulls, the remaining seeds were used for the processing study.

*** Not detectable: The cpm/g was less than background cpm.

The numbers within a column having different letters are significantly different at the 5% level according to Duncan's multiple range test.

^{65}Zn gammas in the ^{51}Cr photopeak region. When the activity ratio of ^{65}Zn to ^{51}Cr is not excessive, radioactivity due to each radionuclide can be resolved. However, the half-life of ^{51}Cr is shorter (27.8 days) than that of ^{65}Zn (244.9 days) and by the time processed fractions could be analyzed and because of radioactive decay, the relative activities of ^{65}Zn to ^{51}Cr were too high for resolution. However, it is unlikely that an analysis of soybeans labeled only with ^{51}Cr might reveal alterations due to processing that were not apparent in the dual labeled beans used in this study, provided the presence of ^{65}Zn did not interfere with detection of small amounts of ^{51}Cr in the hulls. Levels of stable Cr found in soybeans are difficult to detect because levels are essentially at the detection level of traditional atomic absorption spectroscopy, and therefore, these results are not reported here.

Table 2—Percent weight yields (d.w.b.) of edible and nonedible above-ground soybean plant tissues by treatment

Treatment	Edible (%) $\bar{x} \pm S$	Nonedible* (%) $\bar{x} \pm S$
Control	20.9 ± 4.8^{ab}	79.1 ± 4.8^{ab}
^{59}Fe	24.7 ± 5.8^{a}	75.3 ± 5.8^{a}
$1X^{65}Zn$	17.2 ± 7.2^{bc}	82.8 ± 7.2^{bc}
$1X^{51}Cr/1X^{65}Zn$	18.7 ± 5.7^{b}	81.3 ± 5.7^{b}
$9X^{51}Cr/3X^{65}Zn$	13.0 ± 6.8^{c}	87.0 ± 6.8^{c}

* "Nonedible" soybean parts include leaves and stems, pods, and hulls, whereas, "edible" denotes dehulled beans.
The numbers within a column having different letters are significantly different at the 5% level according to Duncan's multiple range test.

DISCUSSION & SUMMARY

Distribution of Fe, Cr, and Zn

Increasing application rates of Cr and Zn increased the mineral element concentrations in a number of plant species (Cary et al, 1977; Singh and Lang, 1976; Singh and Steenberg, 1974; Huffman and Allaway, 1973b; Carroll and Loneragan, 1969). This same effect has also been shown for soybeans in this work and also by other researchers (Ham and Dowdy, 1978; Rao et al., 1977; Turner and Rust, 1971). Rao et al. (1977) reported that Zn accumulation by plant tissue varied with the soybean genotype. They also verified that uptake and percentage distribution of radioactive ^{65}Zn mimics that of total stable Zn.

Since a greater percentage of ^{59}Fe was translocated to the beans than for ^{51}Cr or ^{65}Zn (Table 1), it may be inferred that Fe was more available or mobile than either Cr or Zn. Ham and Dowdy (1978) studied the influence of sewage sludge soil amendment on the uptake of several minerals in soybean plant parts. They found relatively high concentrations of Fe, Zn, and Cr in the vegetative plant parts of mature soybeans and concluded that none of these three minerals are readily mobilized to the reproductive plant parts. In their study, percentage distributions of these elements among the plant parts were not given so relative mobilities could not be determined.

Although legumes have been reported to accumulate more Fe but less Zn and Cr than cereals, oats grown simultaneously in the same nuclide treatments as the soybeans used in this study (Rynearson, 1981) accumulated 2, 65, and 19 times greater concentrations of Fe, Zn, and Cr, respectively, in the groats of the oat plants than in the seeds of the soybeans. Similarly, a substantially greater accumulation of Zn in edible parts of corn was reported by

Table 3—Established concentrations (d.w.b.) and percent concentration changes of stable Fe, Zn and radioactive ^{59}Fe, ^{65}ZN and ^{51}Cr in succeeding products during processing of soybean products at different stages

Mineral	Soybeans Raw $\bar{x} \pm S$	After Cracking Rollers $\bar{x} \pm S$	% Change[a]	Screened Beans After Aspiration $\bar{x} \pm S$	% Change[a]	Ground Full-Fat Meal $\bar{x} \pm S$	% Change[a]	Defatted Meal $\bar{x} \pm S$	% Change[a]	Final Autoclaved Defatted Meal $\bar{x} \pm S$	% Change[a]
Stable mineral elements	(μg/g)	(μg/g)		(μg/g)		(μg/g)		(μg/g)		(μg/g)	
Fe	88.9 ± 4.1	99.7 ± 8.3	+15.9**	85.3 ± 2.6	−20.8*	82.7 ± 11.0	−5.8	130.1 ± 9.5	+24.3*	128.8 ± 6.8	−3.2
Zn	56.8 ± 9.0	66.3 ± 2.7	+16.3	63.9 ± 2.6	− 8.9*	65.1 ± 6.1	+0.1	80.3 ± 3.5	−0.8	79.1 ± 4.5	−2.7
Radioactive mineral elements	(cpm/g)	(cpm/g)		(cpm/g)		(cpm/g)		(cpm/g)		(cpm/g)	
^{59}Fe	24199 ± 1848	23356 ± 2426	−4.9	21000 ± 924	−16.2	20961 ± 300	+6.8	26385 ± 257	+1.1	27076 ± 449	+1.8
^{65}Zn	8883 ± 694	9492 ± 667	+5.9	8685 ± 202	−14.9**	9073 ± 104	+3.4	11072 ± 84	−1.3	11151 ± 1487	−0.5
^{51}Cr	489 ± 85	367 ± 114	−25.6	368 ± 82	− 6.0	262 ± 34	−29.0	285 ± 102	−16.1	258 ± 92	−9.7

[a] Changes were significant at the 1%(*) and 5% levels (**).

Table 4—Distribution of Fe, Zn, and Cr concentration (d.w.b.) in soybeans and derived fractions

Seeds of fraction	Weight Distrib. (%)	Fe: Stable Fe (ppm)	Fe: % Fe of total in whole bean	Fe: ^{59}Fe (cpm/g)	Fe: % ^{59}Fe of total in whole bean	Zn: Stable Zn (ppm)	Zn: % Zn of total in whole bean	Zn: ^{65}Zn (cpm/g)	Zn: % ^{65}Zn of total in whole bean	Cr: ^{51}Cr (cpm/g)	Cr: % ^{51}Cr of total in whole bean
Whole	100	90	100.0	24000	100.0	60	100.0	9000	100.0	490	100.0
Hulls	6	270	18.0	83000	20.8	80	8.0	16000	14.2	ND**	ND**
Defatted Meal	76	130	109.8	27000	85.5	80	101.3	11000	92.9	260	40.3
Oil	18	—*	—*	ND**	ND**	—*	—*	ND**	ND**	ND**	ND**

*Not measured.
**Not detectable.

Garcia et al. (1977) than was accumulated in soybeans. Kernels of corn accumulated 27.6% total plant ^{65}Zn representing 37% of the total ^{65}Zn accumulated by above ground parts as compared to 14% found in soybeans (Table 1). Approximately 32% of the total ^{59}Fe in the seed was found in the hand dissected hulls (derived from Table 1). The distribution of ^{59}Fe in the hull fraction resulting from processing was only 20.8% of the total seed ^{59}Fe (Table 4) probably due to the presence of small pieces of clinging cotyledon which contained a lower concentration of ^{59}Fe. A much greater portion of ^{59}Fe was found in the hull than was found for either ^{57}Cr (0%) or ^{65}Zn (14%; single label, hand dissected).

Chromium-zinc interaction

The presence of ^{51}Cr, at levels used in this study, did not suppress ^{65}Zn accumulation in soybean plants. Turner and Rust (1971) also did not find a suppression of Zn accumulation in young soybean plants by Cr for 5 day treatments up to levels of 5.0 ppm Cr in the nutrient solution. However, suppression of ^{65}Zn accumulation has been reported in bush beans exposed to levels of ^{51}Cr in the nutrient solution higher than those levels used in the present study (Schmitt and Weaver, 1980) and also in oats exposed to levels identical to those used in the present study (Rynearson, 1981). Accumulation of ^{65}Zn was reduced by 83% in the edible parts of bush beans and by 80% in oat groats.

Although the presence of ^{51}Cr was significantly ($P < 0.05$) associated with a suppression of the dry weight seed yields of soybeans when compared to the control plants (54% for both $1X^{51}Cr$ and the $9X^{51}Cr$ treatments, yields not given), the yields were not significantly different from plants singly labeled with ^{65}Zn ($P > 0.05$). Plants exposed to ^{65}Zn alone exhibited a yield of 15% less than control plants, but this decrease was not significant ($P > 0.05$). When Cr salts (55kg/ha) were added to soils, Ham and Dowdy (1978) found decreased soybean yields. Turner and Rust (1971) also reported a 17% decrease in weight of soybean tops with additions of 0.50 ppm of Cr added to the nutrient solutions and an 11% decrease in weight with additions of 5 ppm to soil. However, Rynearson (1981) did not find a suppression of oat groat yields in the presence of ^{51}Cr added to the nutrient solution at levels used in the present study. The levels of ^{51}Cr used in the study reported here was, therefore, not noticeably toxic to oats or soybeans.

Alterations in mineral content by processing

Processing of soybeans into a defatted meal resulted in a 14.5% loss of ^{59}Fe and a 7.1% loss of endogenous ^{65}Zn. Total Fe and Zn content increased or remained unchanged suggesting that minerals were picked up through contamination from the processing equipment. The ^{51}Cr content of soybeans was not significantly ($P > 0.05$) reduced by processing which is comparable to the effect of milling of barley on Cr content (Weaver et al., 1981). Removal of the hulls accounted for losses of endogenous Zn and Fe. None of the minerals were detectable in soybean oil. Increases in total Fe and Zn occurred when the beans were cracked on the metal rolls. Weaver et al. (1981) reported a significant ($P < 0.05$) increase in Fe, but not for Zn during roller milling of barley.

The increases of Fe and Zn during processing of soybeans into a defatted meal do not necessarily reflect a nutritional contribution to the diet since the minerals may not be biologically available in the soy meal. Studies on the bioavailability of these minerals in soybeans are presently being conducted in our laboratory.

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Effect of Heat Treatment on Sorption Isotherms and Solubility of Flour and Protein Isolates from Bean *Phaseolus vulgaris*

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ABSTRACT

The effect of heating at 50, 70 or 90°C and low moisture contents on water sorption behavior (above about 0.65 water activity) and nitrogen solubility of bean flour and protein isolates, was investigated. The results suggested that relative water sorption changes, in the range studied, may not be the most adequate indicators of nitrogen solubility changes in "dry"/"moist" heated protein products. This is so because heated samples may undergo very important changes in nitrogen solubility while having experienced relatively little modifications in water sorption capacity.

INTRODUCTION

MANY OF THE IMPORTANT functional properties of food proteins relate to water-protein interactions since the first step in imparting their desired functional property to the food system is their interaction with water to rehydrate, swell and/or solubilize them. Thus, the nature of the protein-water (and also protein-protein) interactions is critically important in determining whether the proteins will function in the food system to which they are added (Chou and Morr, 1979).

Solubility and water vapor sorption capacity are two functional properties of food proteins which are related to their interaction with water. Solubility behavior provides a good index of the potential (and limitations) of applications of proteins and also gives information of processing procedures and in determining the effects of heat treatments which might affect potential applications (Wu and Inglett, 1974). Water sorption is defined as the water sorbed by a dried protein after equilibration against an atmosphere of a given (and constant) relative humidity. Water sorption, water binding and "bound water" have been sometimes used interchangeably in the literature (Kinsella 1976; Hagenmaier 1972, Hermansson 1977), although there is some controversy regarding the exact equivalence of the various terms.

Hermansson (1977) noted that few studies have been made of the relationships between sorption of water and solubility, both functional properties depending on water-protein interactions. Fennema (1977) reviewed the interrelationships among water binding (or "bound" water), protein denaturation and protein solubility. He concluded that the subject of water-protein interactions is unquestionably important but very little is known concerning the nature of "bound" water and its relation to protein solubility.

The aim of present study was to investigate whether sorption isotherms of proteins previously subjected to various heat treatments show differences that can be related to differences in solubility. Systems used in this study were flour and protein isolate from bean, *Phaseolus vulgaris*.

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EXPERIMENTAL

Materials

Crushed field beans (*Phaseolus vulgaris*, var. Alubia) obtained from a local supplier were ground to an 80 mesh size. The proximate composition of bean flour was: moisture 12.3%, protein 20.7%, starch 44.8%, lipids 2.4% and total sugar 7%.

Protein isolate was obtained by extraction with water and dilute NaOH as described previously (Pilosof et al., 1982).

The proximate composition of bean protein isolate was (dry basis): protein, 88.1%; lipids, 2.5%; total sugars, 1.1%; and starch, 0.7%.

Analytical methods

Total nitrogen was analyzed by Kjeldahl and protein was calculated by multiplying the nitrogen content by 6.25.

Sugars were extracted according to AOAC (1965) procedure and determined by a colorimetric technique proposed by Whistler and Wolfrom (1962).

Lipids were extracted with methanol-chloroform solution according to Lyons and Lippert (1966).

Starch was determined as glucose according to AOAC (1965), after acid hydrolysis of the samples subjected previously to sugar extraction. Starch was calculated by multiplying the glucose content by 0.9.

Moisture content was determined gravimetrically by placing the samples in a vacuum oven at 70°C over magnesium perchlorate during 48 hr. Soluble nitrogen was determined by dye-binding with C.I. Acid Orange 12 as described by Pilosof et al. (1982).

Water sorption isotherms

Water sorption isotherms were determined gravimetrically by placing samples over constant relative humidity solutions in desiccators and weighing periodically until equilibrium was reached.

Saturated salt solutions were used to provide the constant relative humidity atmospheres. Salts used and corresponding water activities were obtained from the literature (Iglesias, 1975). The desiccators were placed in a constant temperature cabinet at 27°C.

All measurements were made in duplicate and the average value is given.

Heat treatment

"Dry" heat. Samples of dried flour or freeze-dried isolate were sealed in metal cans, heated 48 hr at 50, 70 or 90°C, and then used for water sorption or solubility determinations.

"Moist" heat. Flours or isolates were spread in thin layers and humidified by spraying water until the desired moisture content was obtained. The moistened samples (in a sealed container) were allowed to equilibrate in a refrigerator, placed in hermetically sealed tin cans, and transferred to the oven for heat treatment, i.e. 48 hr at 50, 70 or 90°C.

RESULTS & DISCUSSION

Effect of heat treatment on sorption isotherms of bean flour and protein

Water sorption curves were determined from a water activity of about 0.65–0.97; this range was arbitrarily selected although it is to be noted that it has been also used by other workers studying protein hydration (Bull and Breese, 1968; Hagenmaier, 1972). Isotherms were determined for control and "dry" or "moist" heat treated (48

hr at 50, 70 or 90°C) flour and protein isolate samples; moistened samples of flour had 0.141g water/g dry material and moistened samples of protein isolate contained 0.111g water/g dry material.

Fig. 1 shows the effect of heat treatment at 90°C ("dry" and "moist" samples) on the water sorption isotherms of bean flour at 27°C. Fig. 2 shows similar results but for protein isolate samples. Isotherms for samples heat-treated at the other temperatures (50 and 70°C) were also determined but were not drawn for the sake of clarity of the figures.

Comparison of the isotherms of the heat-treated bean flour samples with those of the control show some differences in water sorption capacity; however, only for the samples heat-treated at 90°C (shown in Fig. 1) the change was so evident that a "visual" comparison of isotherms involves little risk of error. For this particular case (90°C) both isotherms of heat-treated samples show a reduction of the amount of water sorbed in the entire a_w range studied. For the other heat-treated samples (not shown) the comparison is not so simple, even so that the various isotherms appear to be graphically distinct.

For "dry" heated bean protein isolate samples no difference was visually observed between the isotherms of control and treated samples. For "moist" heated samples the opposite phenomenon as for bean flour was observed; heat treatment caused an apparent increase on water sorption above about 0.80 a_w. A somewhat similar phenomenon was observed by Kilara et al. (1972) who studied the effect of heat treatment on moisture sorption isotherms of sunflower meal and protein isolates. All above statements on the effect of heat treatment on water sorption isotherms should be considered only as tentative, since no statistical criterion was used to detect such change in the isotherms. Consequently, and following the method developed by Boquet et al. (1977) a statistical criterion was used for the evaluation of significant differences between sorption isotherms of the different samples. This criterion permits the comparison of the whole isotherm curve rather than "isolated" points. The details of the statistical procedure are not reported here but may be found in the original work of Boquet et al. (1977). The results of the statistical analysis were the following. Heat treatment of bean flours ("dry" or "moist" heat) resulted in all cases (treatment at 50, 70 or 90°C) in a significant change of the water sorption behavior. The results for heated protein isolate samples were different. "Dry" heating did not induce a significant change in the water sorption isotherm; with samples subjected to "moist" heat it was concluded that treatment at 90°C resulted in a significant change as compared to the control, but for samples heated at 50 or 70°C one can not practically conclude if a change occurred in the sorption behavior of the isolates.

Effect of heat treatment on nitrogen solubility of bean flour and protein isolates

Table 1 shows the effect of heat treatment at two different moisture levels on the nitrogen solubility of bean flour and protein isolates. It can be seen that for the higher moisture content, heat treatment induced a considerable decrease in nitrogen solubility in both flour and protein isolate. Heat treatment at 90°C leads in both cases to complete loss of solubility. The behavior exhibited by the samples heated in the "dry" state is somewhat different; bean flour samples show very little loss of nitrogen solubility, (maximum loss of 13.4%) while protein isolate evidenced considerable losses although always below the levels observed when the samples were heated at higher moisture content. It is concluded that protein isolate was far more sensitive to heat than bean flour and for both the greatest

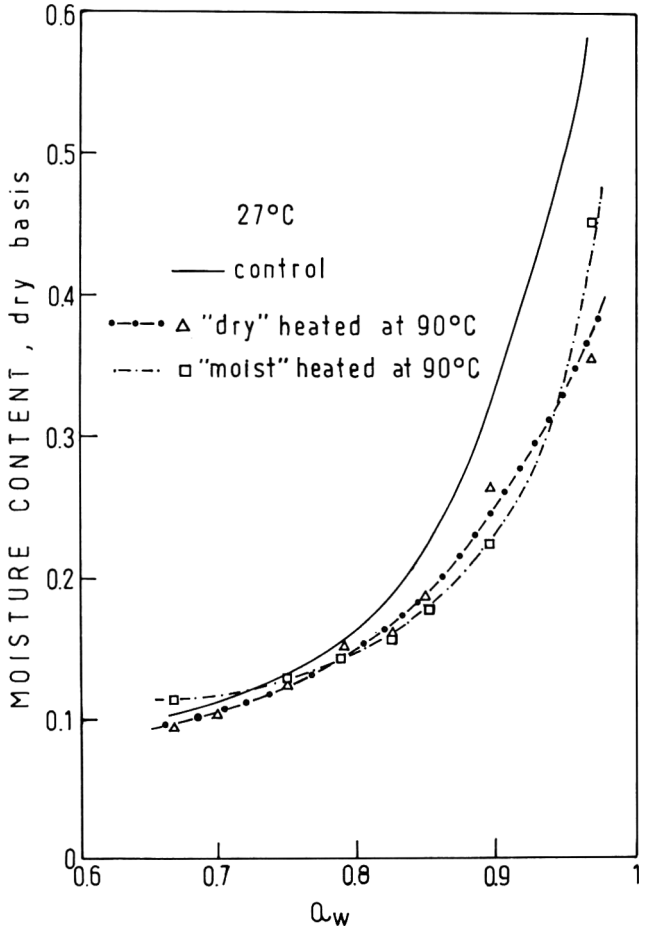

Fig. 1—Effect of "dry" and "moist" heat treatment (48 hr at 90°C) upon water sorption isotherm of bean flour at 27°C.

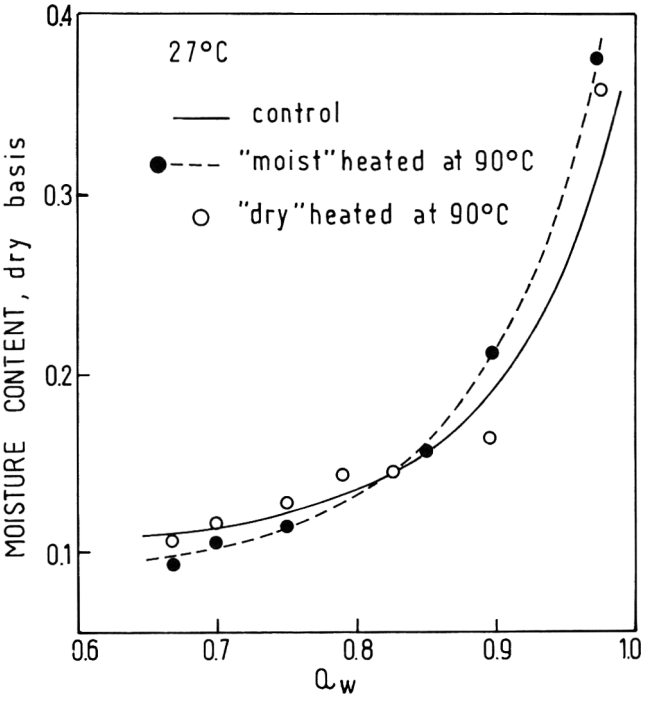

Fig. 2—Effect of "dry" and "moist" heat treatment (48 hr at 90°C) upon water sorption isotherm of bean protein isolates at 27°C.

reduction in nitrogen solubility occurred at the highest moisture content.

Correlation between changes in sorption behavior and solubility due to thermal treatment

Heat treatment ("moist" or "dry") induced a significant change (mostly a decrease) on water sorption behavior of all bean flour samples, while corresponding changes on nitrogen solubility were important only for the samples heated at 0.141g H_2O/g dry matter. The behavior of protein isolates is different from that of bean flour; that is, heating has comparatively little effect on the amount of water sorbed in the a_w range examined, and when heating does change the amount of sorbed water the change is an increase. Protein isolates also showed a decrease in nitrogen solubility upon heating, and they were far more sensitive to heat than bean flour. Bean flour and protein isolates behave in an opposite manner as regards the effect of heating upon water sorption properties. This can be explained as follows. Sorption behavior of bean flour represents the integrated sorption properties of its main components, starch (60% of the dry matter) and protein (28% of the dry matter). Thus, a change in the sorption behavior of flour is the consequence of changes experienced by sorption properties of both polymers, starch and protein. As starch comprises the major part of dry matter, its sorption properties will be dominant. Labuza (1968) noted that starch is very much affected by such pretreatments (heating) which increases the amount of crystalline water-impenetrable starch at the expense of amorphous starch, which means less sorption of water. Interactions starch-protein (of the hydrogen bond type) in the samples heated at the higher moisture content, may also be responsible for the lowered sorption (Iglesias et al., 1980). In this way the finding that heating the flour decreases, rather than increases water sorption, is not in conflict with the findings on protein isolates.

Various workers (Labuza, 1968; McLaren and Rowen, 1952) reported that heating has little effect on protein water sorption ability. The observed lack of effect of heat treatment in the "dry" state on water sorption behavior of protein isolates may be attributed to the relation between structural stability of proteins and its sorbed water under the condition of relatively low water content. Takahashi et al. (1980) have shown that the denaturation temperature of various proteins increased greatly with decreasing the content of their sorbed water; i.e. for ovalbumin, denaturation temperature at 20% moisture content (dry basis) was about 88°C while for 5% moisture content it increased to about 125°C. This explains the lack of effect of heating on water sorption by bean protein isolates, and also the relatively small effect on nitrogen solubility.

When heating is done at higher moisture content, some denaturation is likely to occur leading to an increase in water sorption above about 0.80 a_w and a decrease in nitrogen solubility. For the sake of simplicity the observed changes in nitrogen solubility were attributed to protein denaturation. It is noteworthy, however, they may result also from the effects of protein aggregation (Fennema, 1977).

As a conclusion it is important to note that the magnitude of the relative changes observed in water sorption by heated protein isolates, appear to be considerably smaller than those experienced by solubility. On this basis it may be suggested that relative water sorption changes in the water activity range studied, are not the most adequate indicators of solubility changes in "dry" or "moist" heated protein samples.

Table 1—Effect of heat treatment ("dry" and "moist") (48 hr) upon nitrogen solubility of bean flour and protein isolate samples

Moisture content gH$_2$O/g dry matter	Temp (°C)	Loss of N solubility (%)
	Bean Flour	
0.005	50	–
	70	0.32
	90	13.4
0.141	50	2.6
	70	40.5
	90	100
	Bean protein isolates	
0.016	50	3.3
	70	17.3
	90	63.5
0.111	50	7.0
	70	62.3
	90	100

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Ultrastructural Analysis of a Soybean Protein Isolate

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ABSTRACT

Various fixation techniques were employed in an effort to prepare a dry protein isolate from soybeans for analysis using transmission electron microscopy. A number of fixation schemes proved successful, and different properties of the material were brought out by the various treatments. Comparisons of electron micrographs of the isolate fixed and extracted in different ways yielded an effective means for determining the distribution of fat and protein in the sample and the effect of moisture on that distribution. Analysis of the isolate demonstrated the occluding effect of dry protein for fat located in walls of the protein shells of the isolate.

INTRODUCTION

IN THE ANALYSIS of a fat-extracted soybean protein isolate it was impossible to account for all of the fat following extraction by the usual solvents. This study was designed to determine where and why a portion of the fat remains cloistered within the protein isolate and unaffected by the extraction procedures.

A variety of fixation schemes were employed in order to reveal various ultrastructural data which could be compared to determine the true distribution of fat and protein in the isolate.

Dry materials are notoriously difficult to fix, embed, and section properly for electron microscopy. Various investigators have attempted vapor fixation of dry and imbibed materials with little success (Perner, 1965; Yatsu, 1965; Swift and O'Brien, 1972). A combination of various methods were employed in this study to achieve the desired results. The "dry-fixation" method developed by Dieckert and Dieckert (1981) was used to good advantage on the dry unimbibed isolate.

EXPERIMENTAL

A SOYBEAN PROTEIN ISOLATE was prepared by the aqueous process of Mattil et al. (1979) and supplied by The Andersons Company of Maumee, OH. All fixatives, resin components and strains were purchased from Polysciences Inc., Warrington, PA. Solvents were purchased from Fisher Chemical Co., Houston, TX.

The soybean protein isolate was fixed following six different procedures. All vapor fixation times were 3½ hr long. Post-fixation periods were 1/2 hr long. The following schemes were employed:

1. Dry isolate, acrolein vapor fixation, acetone extraction.
2. Dry isolate, osmium tetroxide vapor fixation, acetone extraction.
3. Water-moist isolate, osmium tetroxide vapor fixation, acetone extraction.
4. Moist isolate, acrolein vapor fixation, acetone extraction.
5. Dry isolate, acrolein vapor fixation, acetone extraction, osmium tetroxide (in acetone) post-fixation, acetone extraction.
6. Moist isolate, acrolein vapor fixation, acetone extraction, osmium tetroxide (in acetone) post-fixation, acetone extraction.

Stepwise hydration and acetone dehydration of fixed material preceded infiltration with Spurr's low vixcosity resin mixture (Spurr, 1969). Blocks from all series were cured at 70°C for 8 hr.

Sections were obtained with a DuPont diamond knife mounted on an LKB Ultrotome III. Strains used were magnesium uranyl acetate and lead citrate. Copper grids (100 mesh) were coated with 0.1% formvar and stabilized with carbon using an Hitachi vacuum evaporator. An Hitachi HU IIA transmission electron microscope was used to study the mounted sections.

RESULTS & DISCUSSION

THIS STUDY involved a series of fixation schemes designed to determine the distribution of fat and protein in the soybean protein isolate and the effect of moisture on that distribution. The various treatments revealed some important points.

The result of osmium tetroxide vapor fixation of dry isolate is seen in Fig. 1. The proteinaceous shells appear gray,

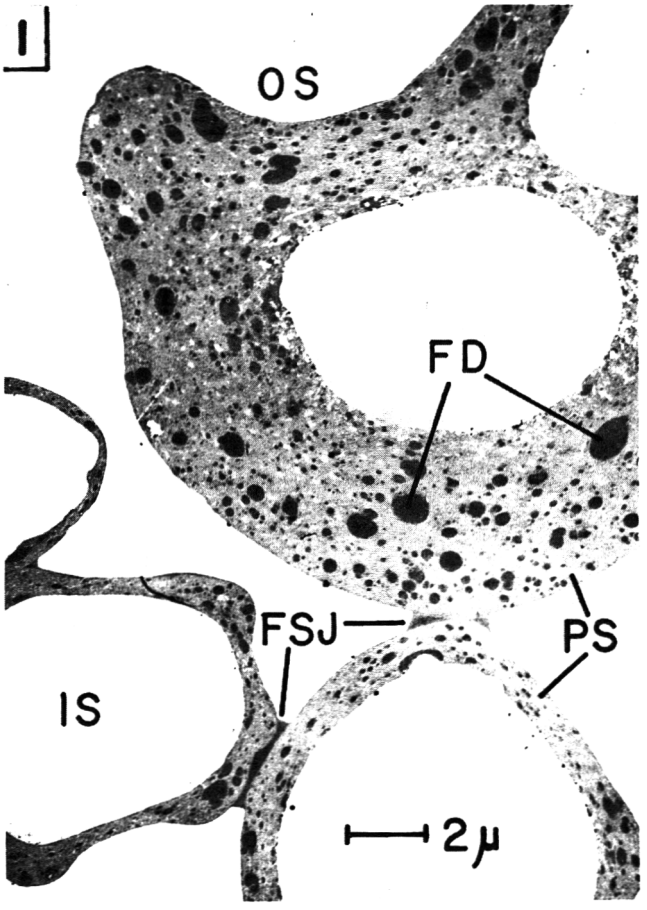

Fig. 1—Dry soybean protein isolate fixed in osmium tetroxide vapor. Fixed fat droplets (FD) are seen with protein shell (PS). Inner space (IS) is indicated within protein meal, as opposed to space outside the protein isolate (OS). Osmium tetroxide fixed fat is trapped at the junctures of the protein shells (FSJ). Magnification 8,968X.

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with embedded dark fat droplets, thus demonstrating the fixation effects of osmium tetroxide on protein and fat containing double bonds. Fat also appears at the junctures of the torus shaped figures, suggesting that the proteinaceous shells are literally "glued" together by fat. Shell interiors appear vacant.

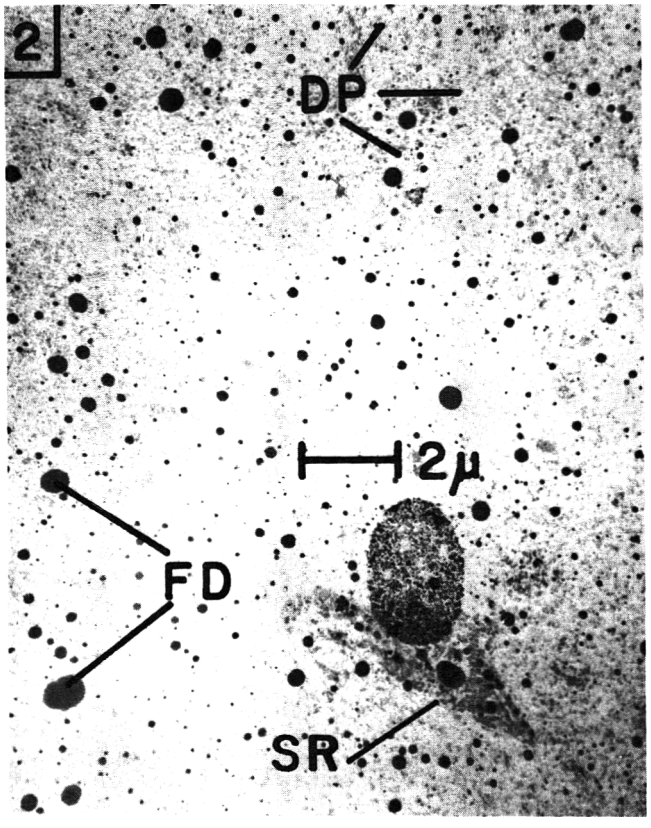

Fig. 2—Moist soybean protein isolate fixed in osmium tetroxide vapor, showing dispersed protein (DP), preserved fat droplets (FD) and remnant of protein shell (SR). Magnification 5,000X.

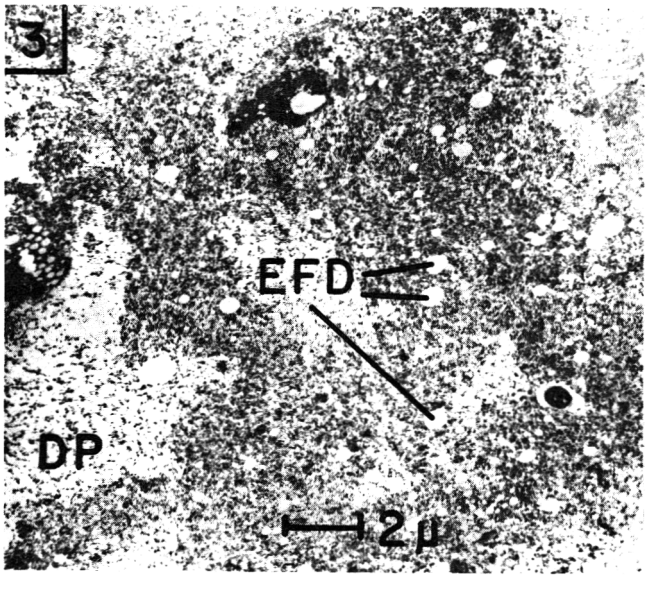

Fig. 3—Moist soybean protein isolate fixed in acrolein vapor, extracted with acetone and post-fixed with 2% osmium tetroxide dissolved in acetone. Dispersed protein (DP) and extracted fat droplets (EFD) are indicated. Magnification 5,000X.

Moisture tends to disperse the protein in the shells, as seen in Fig. 2. A remnant of the original protein shell is still discerned, but the protein appears as particles. Fat droplets are apparent in the moist isolate fixed in osmium tetroxide vapor.

The osmium tetroxide-reactive fat droplets are absent from the moist isolate fixed in acrolein vapor, extracted with acetone and post-fixed in osmium tetroxide (Fig. 3). The removal of unfixed fat by acetone extraction verifies the dark spherical inclusions, seen in the osmium treated samples, to be fat, for acrolein does not fix fat.

Fig. 4 demonstrates the effect of acrolein vapor fixation on moist protein isolate without post-fixation. The protein appears as dispersed particles, and unfixed fat droplets are extracted by the acetone. A remnant of proteinaceous shell is evident.

The dry isolate fixed with acrolein vapor, extracted with acetone and post-fixed with 2% osmium tetroxide (dissolved in acetone) is seen in Fig. 5. The "glue" between adjoining proteinaceous shells is not present, verifying that the osmium tetroxide-reactive "glue" is actually fat. This figure also demonstrates that the outside of the protein shell is accessible to organic solvents such as acetone. Much of the fat dispersed in the protein shells as droplets survives extraction with the acetone series, indicating that the protein matrix is sufficiently tight to prevent complete extraction of the fat. This property of the isolate may cause trouble in the analysis of fat by extraction procedures. The fat of the surface of the shells is expected to extract readily, while that contained in the shell is not. On the other hand, if the protein matrix is expanded by water, the problem disappears.

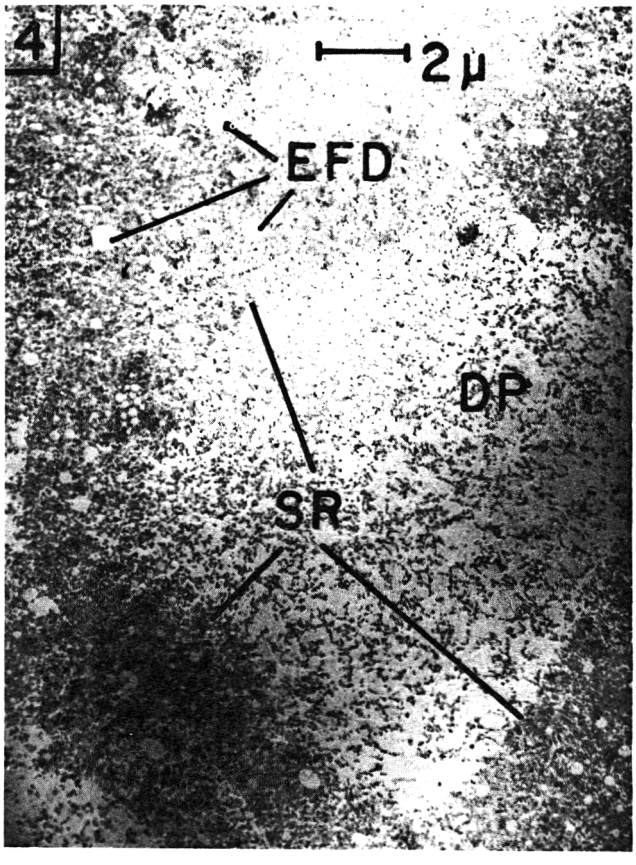

Fig. 4—Moist soybean protein isolate fixed in acrolein vapor and extracted with acetone, showing dispersed protein (DP), extracted fat droplets (EFD) and protein shell remnant (SR). Magnification 5,000X.

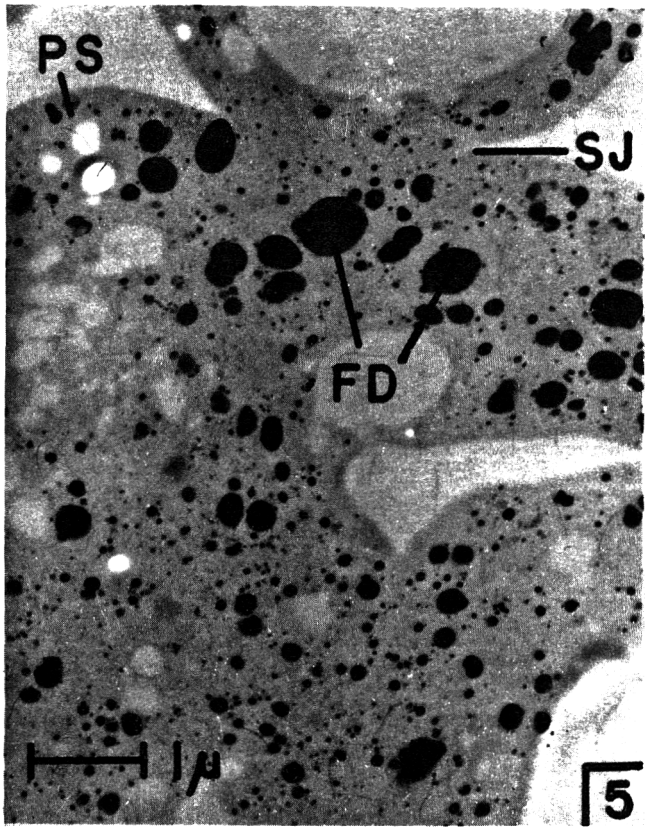

Fig. 5—Dry soybean isolate fixed in acrolein vapor, extracted with acetone and post-fixed with osmium tetroxide. Juncture (SJ) of adjacent proteinaceous shells (PS), containing fixed fat droplets (FD), is shown. Magnification 20,000X.

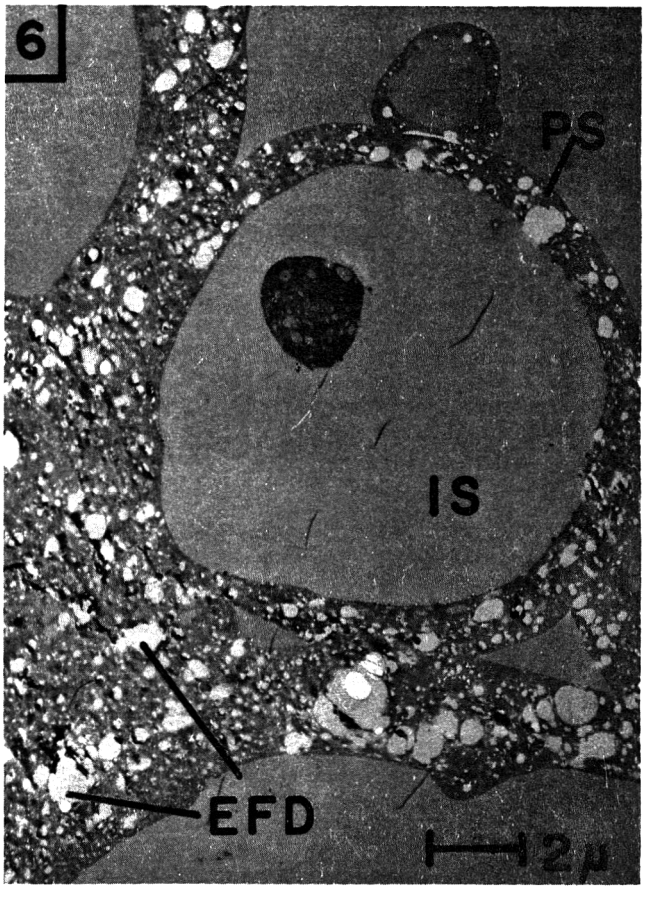

Fig. 6—Dry soybean protein isolate fixed in acrolein vapor and extracted with acetone. Protein shell (PS), containing extracted fat droplets (EFD), surrounds area of inner space (IS). Magnification 7,438X.

When the isolate is fixed dry in acrolein vapor, then processed through the acetone series and embedded, the material appears as shown in Fig. 6. The "glue" is absent between adjacent protein shells. Some of the lipid droplets appear vacant and filled with plastic while others seem to be ripped out. It appears that the fat is removed from the surface of the shells but is only partially removed from the shell walls. The space occupied by unstabilized fat is difficult to section.

These experiments are exploratory in nature. Each treatment affects the subject being examined. So, in a sense, all of the images are artifactual, and care must be exercised in making inferences about the structure of the original material. For instance, when the fat is removed before treatment with osmium tetroxide the shells seem to be fused (Fig. 5). When fat and protein are stabilized before solvent treatment, the shells seem to be separated by a thin film of fat. We believe the latter is closer to the truth. Another observation which suggests the compact structure of the protein shells was elucidated in an alternate procedure. An attempt was made to embed the fixed materials with no hydration preceding infiltration and embedment. Even with very small pieces the plastic monomer failed to penetrate the sample sufficiently to permit sectioning of the blocks. It was concluded that the shell walls of all of the samples were somewhat expanded by the aqueous hydration step preceding acetone dehydration. We took advantage of this effect to achieve "good" embedments which could easily be sectioned.

This research illustrates the useful information which may be gained using the tools of chemical electron microscopy, which can also yield quantitative data.

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Effect of *Lactobacillus fermentum, Bacillus subtilis, Bacillus cereus* and *Pseudomonas maltophilia* Singly and in Combination on the Relative Nutritive Value of Fermented Corn Meal

NANCY J. NANSON and MARION L. FIELDS

ABSTRACT

Combined *Bacillus subtilis, Bacillus cereus* and *Pseudomonas maltophilia* used to ferment corn meal over 4 days at 37°C produced a significant increase ($P < 0.02$) in % RNV (relative nutritive value) of fermented corn meal (96.3%) when compared to nonfermented corn meal (82.2% RNV). Other combinations which caused a significant increase in % RNV for fermented corn meal were: *P. maltophilia* and *B. subtilis* ($P < 0.04$, 95.0% RNV), *B. subtilis* and *B. cereus* ($P < 0.05$, 94.4% RNV). Single microorganisms used to ferment corn meal did not cause significant increases in % RNV.

INTRODUCTION

HAMAD AND FIELDS (1979) found that a natural lactic acid fermentation of corn meal increased the relative nutritive value (% RNV) significantly ($P < 0.01$) as measured by *Tetrahymena pyriformis* W.

Tongnual et al. (1981) found that % RNV increased during the first 48 hr of fermentation. After 48 hr, the pH dropped, log count of proteolytic colonies decreased and % RNV leveled off. These data suggested that either proteolysis, which increases free amino acid levels, or synthesis by proteolytic microorganisms was responsible for the increase in % RNV. Only one strain of lactic acid bacteria isolated by Hamad (1978) was slightly proteolytic, thus the proteolytic activities occurring in the fermentation were not due to the lactics, but to other members of the microflora.

In order to determine which organisms were responsible for the increase in % RNV, Tongnual et al. (1981) isolated proteolytic microorganisms from natural lactic fermentation, and identified them as pseudomonads. Proteolytic bacilli were later identified and all of the microorganisms speciated according to *Bergey's Manual* (Buchanan and Gibbons (1974). When the pseudomonads were used as pure cultures to ferment sterile corn slurries over 4 days, there was no increase in % RNV. This result indicated that proteolysis alone was not responsible for the increase in % RNV, that more than one proteolytic organism was producing an effect, or that synthesis was also involved.

The objective of this research was to mix in various combinations one pseudomonad specie, two bacillus species and one lactic acid bacterium, previously isolated from fermenting corn and to use them to ferment corn over a 4-day period. The fermented corn meal was analyzed by the RNV procedure (Stott et al., 1963) to determine which microorganism or combination thereof, was responsible, for the increase in % RNV.

MATERIALS & METHODS

Preparation of corn

Field corn, purchased locally in Columbia, MO, was passed through a sieve to remove debris and broken kernels. Approximately 2000g of the cleaned corn was ground through a 1-mm screen in a Wiley Mill and held in glass jars at 26°C until used for analysis.

Moisture and nitrogen contents

The moisture and nitrogen contents of the nonfermented and fermented samples were analyzed as described by AOAC (1975). The moisture content was measured after drying the sample at 130°C for 1 hr in a drying oven. Nitrogen was determined by the micro-Kjeldahl method.

Relative nutritive value (RNV)

RNV of the fermented and nonfermented corn was analyzed using *Tetrahymena pyriformis* W (ATCC 10542), according to Stott et al. (1963). Certified casein (Fisher Scientific Co.) was used as a reference protein.

RNV was calculated according to the formula derived by Helms and Rølle (1970) as follows: RNV = [(log ct/ml for test protein − log ct/ml for inoculum)/(log ct/ml for casein − log ct/ml for inoculum)] x 100.

pH and % RNV of corn using mixtures of microorganisms

The four microorganisms used either singly or in combination to ferment sterile corn slurries (1:16 w/v corn to water) were *Lactobacillus fermentum*, isolated by Hamad (1978), *Pseudomonas maltophilia, Bacillus subtilis* and *Bacillus cereus*, isolated by Tongnual et al. (unpublished data). The microorganisms were identified according to *Bergey's Manual* (Buchanan and Gibbons, 1974). In order to insure a constant inoculum, 18–24 hr cultures of each organism were inoculated either alone or mixed in 15 combinations into sterile distilled water and standardized at 90% T using a Bausch and Lomb Spectronic 20 spectrophotometer set at 540 nm. Ten ml of each inoculum were aseptically pipeted into a sterile corn slurry and into 100-ml sterile nutrient broth, and incubated 4 days at 37°C. Nutrient broth served as a control for growth. Uninoculated sterile corn meal was used as a nonfermented control. After fermentation, the slurries were dried 24-48 hr at 50-55°C in a Freas model 835 air-circulating oven, and reground in a Wiley Mill. Moisture and nitrogen contents of each sample were determined and the meal was then analyzed for RNV. Also, pH measurements of the meals were made with a Beckman Zeromatic pH meter prior to RNV analysis.

Statistical analysis

Individual means were analyzed by analysis of variance procedure using SAS (Barr et al., 1979). Differences among groups of means were investigated using pre-planned orthogonal comparisons (Snedecor and Cochran, 1956). The difference between means for individual slurries and between means for groups of slurries were evaluated. Differences were considered significant at $P < 0.05$.

Toxicity studies for Bacillus cereus

A chicken embryo bioassay was used to determine the toxicity of *Bacillus cereus*. Fertile eggs, obtained from the Poultry Division at UMC, Columbia, MO, were incubated at 38°C, 65% humidity in a Leland Wilson incubator, #1893918, for 5 days, and then candled to select out those with healthy embryos (Dey, 1976). The selected eggs were sterilized with iodine around the air cell area, and a hole made in it using a sterile pointed needle (AOAC, 1975). The samples were inoculated into the air cell areas using a sterile syringe and the holes sealed with sterile wax. The samples consisted of 0.1 ml of an 18–24 hr *Bacillus cereus* culture in nutrient broth, injected into each of 6 fertile eggs, and 0.1 ml of *Bacillus cereus* supernatant (obtained from centrifuging 5 min in an International Clinical Centrifuge Model 48309M-6) injected into each of six fertile eggs. Five uninoculated fertile eggs were used as controls. All of the eggs were

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incubated at 38°C for 6 days, modifying the procedure of Davis et al., 1975 (incubated eggs 4 days). The eggs were turned twice daily, and at the end of the incubation period were examined to determine whether embryos were dead (*B. cereus* toxic) or alive (*B. cereus* nontoxic). A comparison was made between uninoculated and inoculated eggs.

RESULTS & DISCUSSION

pH and % RNV of corn using mixtures of microorganisms

When fermented with different microorganisms over a 4-day period, only three corn slurries differed significantly ($P < 0.02$, $P < 0.04$ and $P < 0.05$) in % RNV (Table 1).

According to Table 1, *Pseudomonas maltophilia, Bacillus subtilis*, and *Bacillus cereus* (1) were all needed to obtain the highest % RNV (96.3%, $P<0.02$). *Pseudomonas maltophilia* combined with *Bacillus subtilis* (2), and *Bacillus subtilis* combined with *Bacillus cereus* (3) also gave a significantly higher % RNV ($P < 0.04$ and $P < 0.05$) than nonfermented corn. The three microorganisms did not cause an increase in % RNV when used by themselves in fermentation.

The combination of the highest three means (1+2+3) for % RNV was significantly ($P < 0.01$) higher than that for the nonfermented mean (16). The combination of means in which only *Pseudomonas maltophilia, Bacillus subtilis* and *Bacillus cereus* (1+2+3+6+9+12+14) were used was also significantly ($P < 0.05$) higher than the mean for nonfermented meal.

Table 1—Means[a] of pH and % RNV of corn meal fermented with mixtures of microorganisms over 4 days at 37°C

Mixture of microorganisms	pH of fermented slurry	% RNV of individual means	Significant P-value[b]
(1) *Pseudomonas maltophilia, Bacillus subtilis, Bacillus cereus*	5.0	96.3	$P < 0.02$
(2) *Pseudomonas maltophilia, Bacillus subtilis*	5.4	95.0	$P < 0.04$
(3) *Bacillus subtilis, Bacillus cereus*	5.2	94.4	$P < 0.05$
(4) *Pseudomonas maltophilia, Bacillus subtilis, Bacillus cereus, Lactobacillus fermentum*	4.3	92.0	
(5) *Psuedomonas maltophilia, Lactobacillus fermentum*	5.1	90.0	
(6) *Psuedomonas maltophilia, Bacillus cereus*	6.0	89.9	
(7) *Pseudomonas maltophilia, Bacillus cereus, Lactobacillus fermentum*	5.4	89.9	
(8) *Bacillus cereus, Lactobacillus fermentum*	5.5	89.6	
(9) *Pseudomonas maltophilia*	6.1	89.6	
(10) *Pseudomonas maltophilia, Bacillus subtilis, Lactobacillus fermentum*	4.2	88.5	
(11) *Lactobacillus fermentum*	5.7	86.2	
(12) *Bacillus cereus*	6.0	84.7	
(13) *Bacillus subtilis, Lactobacillus fermentum*	4.3	84.3	
(14) *Bacillus subtilis*	5.1	83.6	
(15) *Bacillus subtilis, Bacillus cereus, Lactobacillus fermentum*	4.2	83.0	
(16) Nonfermented corn	6.1	82.2	

[a] Means of two replications.
[b] Only % RNV values of 94.4% and above were considered significant when compared to nonfermented corn (82.2%).

The slurries which gave the lowest pH values (4.2–4.3) after 4-days fermentation are listed in Table 1. In each instance, *Lactobacillus fermentum* and *Bacillus subtilis* were present, but when fermented by themselves did not decrease the pH to such a low level.

As in the study by Tongnual et al. (1981), proteolytic microorganisms used by themselves in fermentations did not cause a significant increase in % RNV. When three of the microorganisms were used together to ferment corn meal, however, there was a significant increase in % RNV. This suggested that the enzymes of each bacterium were different and allowed either more proteolysis or synthesis during fermentation than would occur when using only one microorganism. Since nonproteolytic *Lactobacillus* Hamad, 1978) did not contribute to the largest increase in % RNV, the bacteria responsible for this increase were proteolytic.

Toxicity studies for Bacillus cereus

After incubation 6 days at 38°C only one of the control embryos was dead. Three of the six eggs inoculated with bacterial cells had dead embryos and three of the six eggs inoculated with supernatant from bacterial cell suspensions had dead embryos. Thus, the mortality rate was 33.3%, calculated as follows:

$$\frac{\#\text{ dead test embryos} - \#\text{ dead control embryos}}{\text{Total }\#\text{ test embryos for group}}$$

$$= \frac{3-1}{6} = 1/3 = 33.3\%$$

Since *B. cereus* was toxic as measured by the chicken embryo bioassay, it would not be a suitable culture to use for consumer-oriented fermentation. However, the corn meal fermented with combined *Pseudomonas maltophilia* and *Bacillus subtilis* also gave a significantly higher % RNV than nonfermented corn and should be suitable for such a fermentation.

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Interactions of Soluble Iron with Wheat Bran

A. L. CAMIRE and F. M. CLYDESDALE

ABSTRACT

Soft white and hard red wheat bran were found to contain 98.8 and 99.2% water insoluble iron, respectively. As the concentration of iron added to wheat bran was increased, less of the total added iron was bound. Ascorbic acid was found to inhibit binding of ferrous iron to wheat bran. It was found that boiling for 1 hr in a boiling water bath (BWB) had no effect on the destruction of phytic acid in wheat bran, whereas toasting for 1 hr at 178°C (350°F) and boiling for 1 hr in 1N HCl had a significant effect.

INTRODUCTION

SINCE FLOUR CONSUMED in the United States is routinely enriched with iron there is concern that addition of fiber may adversely affect its bioavailability. Bjorn-Rasmussen (1974) found that there was a decrease in iron absorption from rolls baked with added bran in amounts equal to or greater than 3.3%. The results of experiments by Simpson et al. (1981) indicate that phytate content is not the component of wheat bran solely responsible for inhibition of iron absorption. Reinhold et al. (1981) has recently investigated the binding of iron by neutral detergent fiber and acid detergent fiber prepared from wheat and maize. Iron binding by these fibers was found to be inhibited by ascorbic acid, citric acid, phytic acid, and EDTA in low concentrations. These workers found that dephytinization of wheat or maize did not affect the binding profiles of the brans. Camire and Clydesdale (1981) have investigated the effect of heat treatment and pH on the binding of metals to wheat bran and fractions of dietary fiber. Toasting had no effect on metal binding by cellulose, but had a significant effect on the binding of metals by lignin and wheat bran. Boiling for one hour had a significant effect on the binding of metals by cellulose, lignin, and wheat bran.

This research was, therefore, initiated to investigate the chemical status of iron, the effect of ascorbic acid on the binding of iron, and the effect of heat treatment on the destruction of phytic acid in wheat bran.

MATERIALS & METHODS

Wheat brans

Hard red and soft white wheat brans were obtained from the American Association of Cereal Chemists and held under refrigeration until used. The brans were used as obtained.

Stock solution of ferrous iron

A 100 ppm stock solution of ferrous iron was made up daily by dissolving 100.0 mg iron wire in 10 ml concentrated HCl and diluting to 1000 ml with distilled deionized water. No attempt was made to control or limit the dissolved oxygen content of this solution before or after dilution since results of Nojeim and Clydesdale (1981) indicate that dissolved oxygen (<1–12 μg/ml) had no significant effect on the percentage of added iron that became ionized in a potassium biphthalate buffer system.

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Determination of the chemical status of iron in wheat bran

The "iron-profile" analysis of wheat bran was done according to the method of Lee and Clydesdale (1979).

Total iron. A 2.0g sample of wheat bran was weighed into a 100 ml Kjeldahl flask to which was added 30 ml concentrated HCl and two boiling chips. The contents were heated at a boil for 10 min or until completely charred. Samples were then cooled, and filtered through Whatman #1 filter paper into 100 ml volumetric flasks.

Soluble iron. A 10.0-g sample of wheat bran was weighed into a 125 ml Erlenmeyer flask to which was added 100 ml of distilled deionized water to give a final pH of 6.8. The contents were allowed to stand for 30 min at room temperature before analysis. Approximately 40 mls were placed into 50 ml centrifuge tubes and spun at 2,500 rpm for 10 min. The iron concentration in ppm was read directly from the atomic absorption spectrophotometer on the undiluted sample.

Ferrous and ferric iron. Duplicate 10 ml aliquots of the soluble iron were placed into 60 ml separatory funnels, one containing a reducing agent, hydroxylamine hydrochloride, and the other no reducing agent. One ml of pH 4.0 buffer was added to each funnel and the total volume brought to 15 ml with water. Fifteen ml of 0.012% bathophenanthroline in 95% ethanol are added with agitation. The red colored ferrous complex was extracted with 10 mls of chloroform into 25 ml volumetric flasks which were made to volume with ethanol. The absorbance of the solutions was read within 15 min at 533 nm using a 1 cm cuvette.

Effect of ascorbic acid on ferrous iron binding by wheat bran

Ten ml aliquots of the 100 ppm stock solution of ferrous iron were pipetted into 125 ml Erlenmeyer flasks containing the wheat bran samples, along with 40 ml distilled deionized water, to give a final iron concentration of 20 μg/ml. Unfortified samples served as controls, and ascorbic acid was added at 0, 60, 120, and 180 mg per gram of wheat bran to the test samples. The pH of the samples was adjusted to 6.4 with 0.2N NaOH and the samples were placed on a shaker bath at ambient temperature for 30 minutes according to Reinhold et al. (1981) before analysis by the bathophenanthroline procedure.

Effect of heat treatment on the destruction of phytic acid in wheat bran

Soft white wheat bran was subjected to three heat treatments and the results of these treatments were compared to control samples of unheated bran. The heat treatments included: toasting for 1 hr at 187°C., heating for 1 hr in a boiling water bath (BWB), and heating for 1 hr in 1N HCl in a BWB. Phytic acid was analyzed by the method of Camire and Clydesdale (1982).

Effect of wheat bran on the binding of added ferrous iron

In this experiment, 20–60 μg iron/ml (prepared by dilutions of the 100 ppm stock solution of ferrous iron) were added to 1.00g samples of soft white or hard red wheat bran and the pH adjusted to 6.4 with 0.2N NaOH. Controls were prepared from which the fiber was omitted. The samples were placed on a shaker bath for 1 hr before centrifugation. A 1 ml aliquot was analyzed for toal ionic and ferrous iron content by the bathophenanthroline procedure.

Table 1—Iron profile of hard red and soft white wheat brans

Sample	Total iron (μg/gram)	Percent of total iron					
		Elemental	Soluble	Insoluble	Ferrous	Ferric	Complexed
Soft white wheat bran	99.83 ± 4.48*	0.0	1.17 ± .06	98.8 ± 0.0	ND	ND	ND
Hard red wheat bran	150.0 ± 4.82	0.0	1.18 ± .04	99.2 ± 0.0	ND	ND	ND

* Standard Deviation

RESULTS & DISCUSSION

RESULTS of the "iron-profile" analysis of wheat brans using the method of Lee and Clydesdale (1979) are shown in Table 1. It can be seen that there was no detectable elemental iron in either sample. Soft white wheat bran was found to contain 99.8 μg iron/gram of which 98.8% was water insoluble iron and 1.17% soluble iron. Hard red wheat bran was found to contain 150 μg iron/gram of which 99.2% was water insoluble and 1.18% soluble. There was no detectable ferrous, ferric, or complexed iron found using this method with water as the extracting solvent.

Presented in Table 2 are results of the experiment in which increasing concentrations of ferrous iron were added to 1.00g samples of soft white or hard red wheat bran. It can be seen that as the concentration of added iron in the samples was increased, less of the total added iron was bound. Results indicate that 49% of the added ferrous iron was bound when the concentration of iron added to the white wheat bran samples was 20 μg iron/ml compared to only 32% of the added ferrous iron bound when the concentration of iron added to the samples was 60 μg iron/ml. However, the total amount of iron bound increased with increasing concentration of iron even though the percentage bound decreased. Reinhold et al. (1981) reported that the total amount of iron bound per mg of wheat or maize neutral detergent fiber increased linearly with increasing iron concentration within the range of 0.0–0.7 μg iron added per ml.

Results of the experiment on the effect of ascorbic acid on the binding of ferrous iron to wheat brans are shown in Table 3. It can be seen that the addition of 60–180 mg ascorbic acid to the wheat bran samples resulted in complete inhibition of binding of ferrous iron.

The effect of heat treatment on the destruction of phytic acid in soft white wheat bran was investigated. It was found that boiling for 1 hr in a BWB had no effect on the destruction of phytic acid, while boiling for one hour in 1N HCl and toasting for 1 hr at 187°C, resulted in a loss of 36 and 19% of the phytic acid respectively. deBoland et al. (1975) reported that autoclaving for 2 hr at 115°C, resulted in a 5% loss of phytic acid in wheat. The authors suggest that the rate of phytate destruction is probably influenced by its protein and/or cation environment.

A part of this study was conducted to confirm the results of Reinhold et al. (1981). It was observed that results of this study were consistent with the results of Reinhold et al. (1981) who reported that ascorbic acid inhibits the binding of iron by fiber of wheat and maize.

This study reaffirms the complexity of the iron chemistry/bioavailability interrelationship and emphasizes the need for further chemical studies.

Table 2—Effect of wheat bran on the amount of added ferrous iron bound at pH 6.4

Sample	μg Fe Added/ml	μg/ml Ionic found	μg/ml Ferrous found	Ferrous iron as % of total added iron bound
White bran	20.0	12.35 ± 0.14	10.12 ± 0.04	49.40
	40.0	27.71 ± 0.05	26.39 ± 0.00	24.0
	60.0	41.54 ± 0.38	40.73 ± 0.19	32.1
Red bran	20.0	10.60 ± 0.23	8.40 ± 0.38	58.0
	40.0	26.56 ± 0.23	25.03 ± 0.05	37.4
	60.0	41.61 ± 0.47	41.57 ± 0.52	30.7

Table 3—Effect of ascorbate on the binding of ferrous iron to wheat bran at pH 5.4.

μg Iron Added/ml	Ascorbic acid (mg)	μg/ml Ionic found	μg/ml Ferrous found	Ferrous iron as % of total iron bound
20	0	15.05 ± 0.19	14.04 ± 0.29	30
20	60	20.73 ± 0.19	20.49 ± 0.23	0
20	120	21.26 ± 0.10	21.16 ± 0.24	0
20	180	21.20 ± 0.19	21.26 ± 0.38	0
20	0	13.09 ± 0.19	10.22 ± 0.15	49
20	60	20.72 ± 0.0	20.79 ± 0.10	0
20	120	21.50 ± 0.24	21.37 ± 0.43	0
20	180	21.84 ± 0.23	21.67 ± 0.0	0

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Unsaturated Iron-Binding Capacity of Human Milk

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ABSTRACT

The unsaturated iron-binding capacity (UIBC) and lactoferrin concentration of human milk from early stages of lactation were determined. The UIBC decreased significantly ($p < 0.01$) from 342 μg Fe/100 ml at the early transitional stage to 261 μg Fe/100 ml at the transitional stage and 215 μg Fe/100 ml at the mature stage. The lactoferrin concentration decreased significantly ($p < 0.01$) from 406 mg/100 ml at the early transitional stage to 241 mg/100 ml at the mature stage. No significant diurnal variations in UIBC or lactoferrin concentration were noted. Approximately 60% of the UIBC was retained after three different pasteurization treatments while less than 40% of the lactoferrin was retained. Seventy-five percent of the UIBC was retained after sterilization; however, lactoferrin was completely destroyed. After frozen storage, the UIBC of the initial sample increased by 37% while the lactoferrin concentration decreased by 30%. These results indicate that there is not a direct relationship between iron-binding capacity and lactoferrin concentration.

INTRODUCTION

THE PERCENTAGE of infants who are breast-fed has increased significantly during the last few years. After a long downward trend, the National Center for Health Statistics (1980) reported an increase from 25 to 35% between 1973 and 1975. Ross Laboratories reported a further increase from 33.4% in 1975 to 49.7% in 1979 (Martinez and Nalezienski, 1981). This renewed interest in breast-feeding is due in part to the increased knowledge of immunological and nutritional properties of human milk. Several studies have indicated a relationship between breast-feeding and decreased incidence of intenstinal infections (Vahlquist, 1976; Cunningham, 1977; Larsen and Homer, 1978). The American Academy of Pediatrics Committee on Nutrition (1980) considered human milk to be the best food for full-term infants during the first months of life and reported that human milk is once again preferred in intensive care nurseries. Milk banks are being established to provide milk for infants in these nurseries. With the high probability of bacterial contamination of banked milk, it appears necessary to process the milk; yet, there is concern that too severe a heat treatment may destroy the desired protective agents.

Lactoferrin, an iron-binding protein similar to transferrin in blood serum, is considered to be one of the protective constituents in human milk (Bullen et al., 1972). It contains two iron-binding sites where amino acid residues complex with ferric iron. Although the mechanism by which lactoferrin imparts protection against intestinal infection is not completely understood, one proposed theory is that lactoferrin irreversibly binds iron, making the iron unavailable for bacterial growth. Bullen et al. (1972) found that human milk containing partially unsaturated lactoferrin had a bacteriostatic effect on *Escherichia coli* 0111, a bacterial strain commonly associated with diarrhea in infants. The bacteriostatic properties were eliminated upon addition of ferric iron to equal 200% of the theoretical total iron-binding capacity of the lactoferrin in the milk. Masson and Heremans (1967) found unsaturated lactoferrin to have a similar bacteriostatic effect against *Staphylococcus albus.* Less pronounced inhibition was noted for *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Such studies suggest that unsaturated lactoferrin in human milk plays a role in providing the infant resistance to intestinal infections. While the earlier work of Bullen et al. (1972) implicated lactoferrin as the major iron-binding fraction in human milk, a recent report by Fransson and Lonnerdal (1980) indicated that lactoferrin binds only a small percentage of the naturally occurring iron. This study was undertaken to elucidate more fully the relationship between the unsaturated iron binding capacity (UIBC) of human milk and lactoferrin. Specific objectives included (1) quantitation of UIBC and lactoferrin in early transitional, transitional, and mature human milk, (2) determination of diurnal variations in UIBC and lactoferrin levels, and (3) determination of the effects of processing and storage treatments on UIBC and lactoferrin in mature human milk.

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MATERIALS & METHODS

Milk donors

Donors for the project were residents of the Athens, GA area. They were contacted initially at two local hospitals in Athens. A total of 20 women agreed to participate and signed an informed consent. All of the women were Caucasian and ranged in age from 17–35. Milk samples were collected by the donors using a Lopuco breast pump (Lopuco Ltd., Woodbine, MD). The early transitional milk was collected 4–6 days postpartum. The transitional and mature milk samples were collected 7–13 and 33–45 days postpartum, respectively. One early transitional sample was collected during the course of several early morning feedings. Two samples were collected at the transitional and mature stages, one in the late evening and one in the early morning. For these samples, donors were instructed to collect approximately equal volumes of milk from the beginning, middle, and end of the one feeding. The samples were refrigerated immediately after collection, picked up after the morning collection, and transported on ice to the laboratory. Analysis of UIBC was completed within 24 hr of collection.

Unsaturated iron binding capacity (UIBC)

The Res-O-Mat Fe 59 Diagnostic Kit, obtained from Mallinckrodt Nuclear (St. Louis, MO), was used for the quantitation of UIBC. Colorimetric methods were tried; however, they were useful only for the whey fraction of the milk. This method was highly reproducible for whole milk samples with the coefficient of variation for replicate trials less than 3%. The procedure involved adding 0.5 ml milk to a vial containing 3.0 ml of radioactively labelled ferric ammonium citrate solution. The amount of iron in each vial was provided by the manufacturer and ranged from 2.92–3.04 ug. The contents of the vial were mixed thoroughly and held for at least 10 min to allow for complete saturation of the iron-binding sites. During this holding period, a precount was taken in a Beckman LS 7500 Liquid Scintillation System (Beckman Instruments, Inc., Irvine, CA). The instrument was set to count all samples to ±2% error or 2 min. After the holding period, the unbound iron was absorbed with a resin impregnated strip supplied in the kit. The vials were

agitated for 1.5 hr, the resin strips removed, and a postcount taken. The UIBC was calculated using the following formula:

$$\frac{\mu g \text{ Fe bound}}{100 \text{ ml milk}} = \frac{\text{Net postcount}}{\text{Net precount}} \times 200^{*} \times \frac{\text{Fe content of Res-O-Mat}}{\text{Fe 59 solution vial}}$$

*Dilution factor

Lactoferrin concentration was determined using rocket electrophoresis (Laurell, 1966). Lactoferrin standards and antiserum were obtained from Calbiochem-Behring Corporation, San Diego, CA. Five μl aliquots of whole milk were assayed in duplicate. The electrophoresis was run at 7°C for 16 hr at a constant voltage of 120 mV/cm^2.

Processing and storage

The processing and storage studies were performed on three composite samples of mature human milk. Donors collected the samples during the course of several late evening and early morning feedings. The milk was refrigerated immediately after collection and processing studies were performed in the morning following collection. Each composite sample, consisting of milk from at least five donors, was mixed thoroughly and divided into five subsamples. One subsample was used as a control. The other four were subjected to one of the following heat treatments: 62.5°C, 30 min; 72°C, 15 sec; 88°C, 5 sec; and 100°C, 5 min. The low temperature long time (LTLT) pasteurization was accomplished by placing 5 ml aliquots of milk in preheated test tubes in a constant temperature water bath set at 62.5°C and holding the sample for 30 min. The two high temperature short time (HTST) pasteurizations were accomplished by pumping the milk through a stainless steel capillary heat exchanger tube in either at 72°C or 88°C constant temperature water bath. The come-up time for the HTST treatments was approximately 2.5 sec. The flow through the capillary heat exchange tubes was adjusted to give holding times of 15 sec and 5 sec for the 72°C and 88°C treatments, respectively. The cool-down time was approximately 2.5 sec. The sample to be sterilized was divided into 5 ml aliquots, placed in test tubes, and heated in the autoclave for 5 min at 100°C. The storage study involved holding an aliquot of the control and each of the four heat treated samples in frozen storage at −20°C for 30 days.

Statistical analysis was performed using the SAS (1979) package on the University of Georgia IBM 360/70 computer.

RESULTS & DISCUSSION

RESULTS OF UIBC and lactoferrin analyses for morning and evening transitional and mature milk are recorded in Table 1. Utilizing analysis of variance procedure, no significant differences ($p < 0.05$) were observed between the morning and evening samples at either lactational stage. For all additional statistical comparisons, morning and evening samples were combined.

Data for UIBC and lactoferrin levels for the three lactational stages are shown in Table 2. A large decrease in UIBC was noted during the first two weeks postpartum followed by a more gradual decline during the next three weeks. Despite the large variability noted between donors, the UIBC was significantly different at all three lactational stages. The values determined for UIBC in this study are within the range of previously reported results. Bullen et al. (1972) reported a range from 99–392 μg Fe/100 ml for skimmed human milk, while Ford et al. (1977) reported a narrower range of 310–340 μg Fe/100 ml for the whey fraction of human milk. These results are for milk from which fat or both fat and casein had been removed. Removal of the fat not only removes a large percentage of the naturally occurring iron (Fransson and Lonnerdal, 1980), but also may change the nature of the additional binding that occurs.

Although a longitudinal study of UIBC has not been reported, many of the components in human milk decrease as time postpartum increases. For example, Lonnerdal et al. (1977) reported that total protein and lactoferrin concentration decrease as lactation time increases. The most rapid decrease was observed during the first 20 days of lactation, with a more gradual decrease thereafter. The UIBC appears to follow this general trend of a large decrease during the early stages of lactation followed by a slow decline as lactation progresses.

Lactoferrin (Table 2) followed a similar but less defined trend. While the concentration decreased with each successive stage of lactation, the only significant decrease was between the early transitional and mature stages. The value of 354 mg/100 ml obtained for transitional milk is lower than

Table 1 — UIBC and lactoferrin content in morning and evening human milk[a]

Stage of lactation	Mean UIBC μg Fe/100 ml milk		Mean lactoferrin concentration mg/100 ml milk	
	A.M.	P.M.	A.M.	P.M.
Transitional[b]	255 (184 – 355)	266 (184 – 389)	333 (66 – 648)	376 (66 – 729)
Mature[c]	211 (145 – 318)	219 (152 – 370)	241 (37 – 616)	240 (26 – 616)

[a] Ranges are in parentheses below each mean. There was no significant difference betweer the A.M. and P.M. samples for UIBC and lactoferrin at either lactational stage.
[b] Number of donors = 19 for UIBC and 18 for lactoferrin.
[c] Number of donors = 16 for UIBC and 15 for lactoferrin.

Table 2 — UIBC and lactoferrin content in early transitional, transitional and mature human milk[a]

Stage of lactation	UIBC (μg Fe/100 ml milk)		Lactoferrin concentration (mg/100 ml milk)	
	Mean ± SD	Range	Mean ± SD	Range
Early transitional[b]	342 ± 74 a	236 – 497	406 ± 134 a	150 – 729
Transitional[c]	261 ± 51 b	184 – 389	354 ± 198 ab	66 – 729
Mature[d]	215 ± 41 c	145 – 370	241 ± 194 b	26 – 616

[a] Means in each column followed by the same postscript are not significantly different at $p < 0.01$ as determined by Duncan's multiple range test.
[b] Number of donors = 20 for UIBC and 18 for lactoferrin.
[c] Number of donors = 19 for UIBC and 18 for lactoferrin.
[d] Number of donors = 16 for UIBC and 15 for lactoferrin.

Table 3 – UIBC of control and heat treated samples before and after frozen storage[a]

Treatment	Before frozen storage		After frozen storage	
	UIBC μg Fe/100 ml milk (Range)	% Retention	UIBC μg Fe/100 ml milk (Range)	% Retention
Control	180 a (166 – 190)	–	248 e (168 – 296)	137
62.5°C, 30 min	99 b (91 – 105)	55	102 b (94 – 116)	57
72.0°C, 15 sec	112 c (106 – 125)	62	118 bc (116 – 119)	65
88.0°C, 5 sec	96 b (85 – 105)	53	99 b (84 – 103)	55
100.0°C, 5 min	135 d (118 – 144)	75	99 b (83 – 124)	55

[a] Means in each column or row followed by the same postscript are not significantly different at $p < 0.05$ as determined by Duncan's multiple range test. Processing and storage studies were completed on three pooled milk samples.

the 450 mg/100 ml reported by Nagasawa et al. (1972). At the mature stage the results are closer with 241 mg/100 ml in this study as compared with 210 mg/100 ml in the study by Nagasawa et al. (1972) They also determined the lactoferrin concentration for colostrum, collected 2–5 days postpartum, to be 490 mg/100 ml. The amount of lactoferrin decreased rapidly during the first two weeks postpartum followed by a more gradual decline.

Data showing effects of heat processing and frozen storage on UIBC are shown in Table 3. UIBC levels after the LTLT pasteurization and the HTST pasteurization, 88°C for 5 sec, were not significantly ($p < 0.05$) different with 55% and 53% of the UIBC retained, respectively. The other HTST pasteurization treatment, 72°C for 15 sec, gave a slightly higer retention of 62%. After sterilization, the UIBC was significantly ($p < 0.05$) less than that of the control but significantly ($p < 0.05$) greater than that of any of the pasteurized samples. The 55% retention of UIBC after LTLT pasteurization is very similar to the 56% retention reported by Eyres et al. (1978), but higher than the retention of 28% of the UIBC reported by Ford et al. (1977).

In comparison, the lactoferrin concentration declined with increasing temperature of the heat treatment (Table 4). The largest % retention, 36% was seen in the LTLT pasteurized sample followed by 27% for the 72°C sample and 15% for the 88°C sample. After sterilization, there was no detectable lactoferrin. These results are in agreement with the work of Evans et al. (1978), who reported progressively lower % retentions as the temperature of the heat treatments increased. They did report a slightly higher lactoferrin retention of 43% after LTLT pasteurization. An earlier study by Szollosy et al. (1974) indicated that heat treatment at 65°C had no effect on lactoferrin.

Frozen storage brought about several changes. The UIBC (Table 3) of the control sample increased 37%; whereas, the UIBC of the sterilized sample decreased 37%. The decrease in UIBC in the sterilized milk was large enough so that after frozen storage no significant ($p < 0.05$) differences were noted in the UIBC of any of the heat treated samples. The amount of lactoferrin remaining after frozen storage (Table 4) was determined only for the control sample. Although less lactoferrin was lost during frozen storage than during heating, freezing did significantly ($p < 0.05$) decrease the lactoferrin concentration with 70% of the original content retained.

Correlation coefficients between UIBC and lactoferrin showed low positive correlations that were significant only at the early transitional stage. The correlation coefficients of 0.66 ($p = 0.003$) for the early transitional stage, 0.42 ($p = 0.08$) for the transitional stage and 0.49 ($p = 0.07$) for the mature stage indicate that there is very little direct relationship between UIBC and lactoferrin. The results of the processing and storage study provide further evidence for this as UIBC and lactoferrin are affected differently by the various processing treatments. This supports the report of Fransson and Lonnerdal (1980) that indicated other components besides lactoferrin are responsible for the iron-binding capacity of human milk. Further, since immunological techniques are routinely used to quantitate lactoferrin, the possibility exists that modification of the antigenic properties of lactoferrin does not affect the iron-binding properties of the protein. In any event, processing required to ensure microbiologically safe human milk in a milk bank environment will modify both UIBC and lactoferrin content of the milk. Significance of the decrease in these constituents to the overall utility of the milk is not known.

Table 4 – Lactoferrin content after processing[a]

Treatment	Lactoferrin mg/100 ml	Range	% Retention
Initial	342 a	275 – 506	–
62.5°C, 30 min	122 b	44 – 197	36
72.0°C, 15 sec	91 bc	29 – 142	27
88.0°C, 5 sec	51 cd	15 – 79	15
100.0°C, 5 min	0 d	0	0
Frozen storage, –20°C, 30 days	239 e	182 – 275	70

[a] Processing and storage studies were completed on three pooled milk samples. Means in each column followed by a different postscript are significantly different at $p < 0.05$ as determined by Duncan's multiple range test.

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Antinutritive Factors in Eleven Legumes and Their Air-classified Protein and Starch Fractions

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ABSTRACT

Pin milling and air classification of starchy legumes enriched the protein content of the light fraction to 49–75%, within the range of defatted soybean flours and protein concentrates. The levels of trypsin inhibitors, hemagglutinating activity, saponin, phytic acid and fiber were high in navy bean flour and the levels in the protein fractions of navy and northern beans greatly exceeded those of soybean flour. Potential nutritional problems in the air-classified protein fractions of other legume species include trypsin inhibitor in lima bean and chickpea and saponin in lima bean, cowpea and lentil. Fababean and mung bean flours and protein fractions were comparatively lower in each antinutritive factor but fababean products contained vicine and convicine.

INTRODUCTION

LEGUME SEEDS contain a wide range of constituents which have adverse effects on enzyme activity, digestibility, nutrition and health. The inhibitors of digestive enzymes are common constituents of legumes and may reduce protein digestibility, depress growth and cause pancreatic hypertrophy (Liener, 1976). While phytohemagglutinins or lectins of soybean have limited nutritional effects on man (Turner and Liener, 1975), the hemagglutinins of *Phaseolus* species will inhibit growth and even cause the death of experimental animals when injected at low concentrations (Honavar et al., 1962). The growth depression caused by saponins may be due to reduced feed intake (Cheeke, 1976) and the formation of insoluble saponin-mineral complexes (West et al., 1978). Of course, phytic acid in its natural form as a phytate-mineral-protein complex can decrease the availability of zinc, magnesium, phosphorus, calcium and iron (Spiller and Shipley, 1977). Some workers have reported that cell wall fiber may also reduce copper, zinc and iron availability (Kies et al., 1979; Thompson and Weber, 1979). Plasma cholesterol level in man is also lowered by dietary fiber which adsorbs the bile acids and promotes their excretion (Oakenfull and Fenwick, 1978). However, the adsorption of bile acids by dietary fiber depended on the presence of saponin in the fiber source.

Many of the antinutritional factors in legumes can be eliminated or inactivated to a large degree by appropriate heating and processing during food preparation. Treatments may include dehulling, presoaking and diffusion, sterilization, steaming and cooking. Wet milling and processing techniques employed during protein concentration and isolation are known to be effective in the detoxification of seed materials.

Dry milling techniques have been used to fractionate a wide range of legumes into starch-rich and protein-rich fractions (Sosulski and Youngs, 1979). The physical, chemical and functional properties of these crude fractions have been investigated (Sosulski and Youngs, 1979; Tyler et al., 1981). The objectives of the present study were to obtain additional information on the quantitative composition of antinutritive factors in eleven legume species and biotypes, and to determine the distribution of these factors among the air-classified protein and starch fractions. Trypsin inhibitor, hemagglutinin, saponin and phytic acid were the principal components investigated in the present study. Data on oligosaccharide composition of the legume flours and their fractions are reported separately (Sosulski et al., 1982).

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MATERIALS & METHODS

THE SOURCES of seed and procedures for dehulling, pin milling and air classification of the legumes have been previously described (Sosulski et al., 1982; Tyler et al., 1981). The species and biotypes investigated were baby lima bean (*Phaseolus lunatus*), navy bean and Great Northern bean (*P. vulgaris*), chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), lentil (*Lens culinaris*), field pea (*Pisum sativum*), small fababean (*Vicia faba minor*), mung bean (*Vigna radiata*). The results obtained with these starch legumes were compared with the compositions of soybean flour (Staley F-200) and protein concentrate (Promosoy 100), and lupine flour (*Lupinus albus* var. Reuscher). Due to their low starch contents, soybean and lupine flours do not air classify into protein-rich fractions. Because high levels of lipid interfere with the air classification process, the sample of chickpea was defatted with hexane before pin milling. The soybean samples were also defatted but the lupine flour was analyzed on a full-fat (10.3%) basis.

Trypsin inhibitor activity was measured by the AACC method 71-10 and dietary fiber by AACC method 32-20 (AACC, 1980). One trypsin unit (TU) was defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of the reaction mixture. Trypsin inhibitor activity was expressed as the number of trypsin units inhibited (TUI).

A modification of the Liener (1955) procedure was used for the hemagglutinating activity test. One gram of flour, protein or starch fraction was extracted in 10 ml of 0.9% sodium chloride for 1 hr at room temperature and, after centrifugation, the extract was suitably diluted with the same solvent. The trypsin-treated red blood cells from rabbit, after 2.5 hr of reaction with the extracts, were resuspended 10 min before reading at 620 nm, as recommended by Marquardt et al. (1975). Samples were read against blanks using serial two fold dilution of the extracts. One unit of hemagglutinating activity (HU) was defined as the level of test solution which caused 50% of the standard cell suspension to sediment.

Saponins were extracted overnight in 50% ethanol using a magnetic stirrer. The mixture was centrifuged for 10 min at 2300 rpm, and the supernatant was evaporated to dryness in a rotary evaporator at 50°C. The residue was dissolved in 5 ml of isotonic buffer and saponin content assayed by the hemolysis test described by Jones and Elliott (1969) using red cells from sheep blood.

The Harland and Oberleas (1977) procedure was followed for analysis of phytic acid including determination of phosphorus by the Harris and Popot (1954) method. Convicine and vicine were determined by the Pitz and Sosulski (1979) procedure.

RESULTS & DISCUSSION

Protein content

Typical of commercial samples, the protein contents of the soybean flour and protein concentrate were 58.5 and 73.7%, respectively (Table 1). The starchy legume flours contained 19.5–31.6% of protein and the air-classification process enriched their protein levels in the fine fraction to 49.3–75.1% with an average value of 57.0%. Fababean,

mung bean and navy bean showed particularly high protein levels in the fine fractions but the lowest protein levels in the starch fractions occurred with lentil, chickpea, mung bean and field pea. Generally, the air-classification process results in nearly complete separation of starch granules, but the protein separation efficiency varied between 78–89% (Tyler et al., 1981). Several of the antinutritive factors in legumes are proteinaceous or nitrogen-containing, and their relative distributions between the fine and coarse fractions was expected to follow that of the total proteins.

Trypsin inhibitor (TI)

The TI activities in commercial soybean flour (Staley F-200) and protein concentrate (Promosoy 100) in Table 1 were within the ranges reported in the literature (Liener, 1976; Rackis et al., 1979). The TI activity in lima bean flour exceeded that of soybean while navy and northern beans, and chickpea flours contained less than one-half of the levels in soybean and lima bean flours. Fababean, lentil and field pea showed low TI values while the lupine sample appeared devoid of TI.

Early investigators (Jaffé, 1950; Liener, 1976) considered soybean, lima and kidney beans to be high in TI activity while cowpea and lentil showed intermediate levels. Recently, values of 11–16 TUI/mg were reported for navy bean and chickpea, and 2–3 TUI/mg for fababean, lentil and field pea (Bhatty, 1977; Yadav and Liener, 1977).

The data in Table 1 show that TI activity segregated with the fines during air classification, the lima bean protein fraction having a high value of 89.8 TUI/mg. The protein fractions of navy and northern beans and chickpea also showed higher TI activities than soybean flour. In general, TI separated almost completely with the protein, the starch fractions of lentil, fababean, field pea, mung bean and chickpea being almost free of TI activity. Before utilization of the protein fractions in food products, it would be necessary to evaluate the antinutritional effects of the TI and the ease of denaturation by heat during processing.

Hemagglutinating activity

The high hemagglutinating activity found in soybean flour (Table 2) was similar to values obtained by Liener (1955) but there are no comparable literature data to confirm the low HU obtained for soybean protein concentrate or lupine flour. Navy bean has been widely reported to contain a potent toxic hemagglutinin (Honavar et al., 1962) and the present value of 46.1 HU/mg was comparable to those of de Muelenaere (1965) and Yadav and Liener (1977). Northern bean contained distinctly less hemagglutinating activity, being more comparable to lentil and field bean than to navy bean. The HU values for lima bean, cowpea and mung bean were quite low, confirming the observations of Honavar et al. (1962). However, Marquardt et al. (1975) and de Muelenaere (1965) reported much lower values for hemagglutinating activity in fababean, field pea, mung bean and cowpea than was obtained in the present study. Some of the differences in values among laboratories could be due to difficulty in avoiding agitation of the sedimented red blood cells during the photometric measurement of the density of the unsedimented layer of red cells in the Leiner (1955) procedure. In addition, the soybean (Turner and Liener, 1975) and fababean (Marquardt et al., 1975; Palmer and Thompson, 1975) hemagglutinins have no apparent toxic effects on animals as compared to the growth inhibiting effects and death attributed to hemagglutinins from *Phaseolus vulgaris* (Honavar et al., 1962; Palmer and Thompson, 1975).

As for TI activity, the hemagglutinating activities concentrated with the protein fractions, giving values of from 1.3 HU/mg for lima bean to 104.0 HU/mg in navy bean (Table 2). The significance of the high levels in field pea (40.0 HU/mg), lentil (30.5 HU/mg) and fababean (20.1 HU/mg) protein fractions require further investigation to ensure the safety of these products for human consumption. The starch fractions contained from 0.3 to 3.0 HU/mg, which represented a substantial degree of detoxification as compared to the original flours.

Saponin

Navy bean and cowpea flours had the highest levels of hemolytic activity, while northern and lima beans, as well as lentil, gave intermediate values (Table 2). The other legume flours including soybean and lupine were essentially free of hemolyzing agents. Air classification was effective in concentrating the saponin into the protein fraction, and all starch fractions except those of navy bean and cowpea were almost devoid of hemolytic activity. It may be possible to adjust the cut point so that all of the saponins are excluded from the starch fractions in the case of the latter two species.

Hemolytic values of 13–16 HA/g in the protein fractions of navy bean, cowpea, northern bean, lima bean, and lentil could have adverse effects on the nutritive value of these products. Coxworth et al. (1969) reported that saponin levels of 19.5 HA/g in kochia and 30.6 HA/g in garden atriplex were sufficient to reduce feed consumption. Other than for soybean there is little published information

Table 1—Composition of protein (%) and trypsin inhibitor activity (TUI/mg sample) in eleven legume flours, protein fractions and starch fractions (dry basis)

	Crude protein content			Trypsin inhibitor activity		
	Flour	Protein	Starch	Flour	Protein	Starch
Soybean	58.5	73.7[a]	–	41.55	28.77[a]	–
Lima bean	24.0	49.5	8.2	46.81	89.78	19.70
Navy bean	26.1	61.3	7.8	18.23	53.80	4.06
Northern bean	26.5	57.5	6.4	18.08	43.58	4.05
Chickpea	23.1	49.9	4.3	18.80	50.61	2.49
Cowpea	27.2	51.0	10.4	12.20	23.88	4.94
Lentil	19.5	49.3	4.0	5.12	11.69	0.99
Field pea	21.5	56.3	5.4	7.61	23.35	1.43
Fababean	31.6	75.1	8.6	4.75	10.71	1.36
Mung bean	27.5	63.2	5.3	9.96	23.03	1.78
Lupine	36.4	–	–	0	–	–

[a] Soybean protein fractions was a commercial protein concentrate produced by a wet-processing technique.

Table 2—Hemagglutination activity (HU/mg) and hemolytic value of saponins (HA/g) in eleven legume flours, protein fractions and starch fractions (dry basis)

	Hemagglutination activity			Saponin		
	Flour	Protein	Starch	Flour	Protein	Starch
Soybean	77.43	0.44[a]	–	0	0[a]	–
Lima bean	0.89	1.32	0.30	5.9	12.9	0
Navy bean	46.12	103.99	1.94	12.6	16.1	5.9
Northern bean	16.76	28.73	0.94	7.8	16.1	0.8
Chickpea	7.89	9.84	1.46	0.5	3.8	0
Cowpea	1.99	3.94	0.34	12.8	16.2	5.8
Lentil	14.61	30.47	0.69	5.6	12.6	0.5
Field pea	15.06	39.99	1.90	0	0	0
Fababean	10.55	20.15	2.98	0	0	0
Mung bean	3.42	9.02	0.26	0.8	2.4	0
Lupine	0	–	–	0.8	–	–

[a] Soybean protein fraction was a commercial protein concentrate produced by a wet-processing technique.

on legume saponins. It would be of interest to know if there is a relationship between the present hemolytic values and the properties of foam strength, bloat production, mineral binding, reduced palatability and growth depression associated with alfalfa saponin (Cheeke, 1971; 1976). The saponins are heat stable and their removal would involve aqueous extractions with hot water or dilute alkali (Coxworth et al., 1969). The significance of the finding that saponins are bound to dietary fiber in soybean, mung bean, and chickpea, and will induce the adsorption of bile acids which ultimately reverses hypercholesterolaemia in rats (Oakenfull and Fenwick, 1978), has yet to be elucidated.

Phytic acid

Soybean, navy and northern bean flours contained 10–12 mg/g of phytic acid while lupine flour had over 8 mg/g (Table 3). Phytic acid levels ranged from 5–8 mg/g for the other legume flours except for the lower value in fababean. These results are consistent with literature values of 1.5% for soybean flour (de Rham and Jost, 1979); 7.5–15.8 mg/g in navy bean cultivars and 5.6–13.7 mg/g in northern bean cultivars (Lolas and Markakis, 1975) 6.5 mg/g in mung bean, 4.3 mg/g in cowpea and 2.8 mg/g in chickpea seeds (Kumar et al., 1978).

The proportions of phytic acid in the protein fractions were nearly three times those of the original flours (Table 3). The high levels of 18.7–26.0 mg/g, which were found in navy and northern beans, lentil and field pea, exceeded the 13.5 mg/g obtained from the soybean protein concentrate, and were within the range reported for commercial protein isolates (de Rham and Jost, 1979). The fababean protein fraction remained comparatively low in phytic acid content, and all starch fractions contained only 1.5–2.5 mg phytic acid/g sample.

The nutritional implications of phytate binding to calcium-magnesium and/or to proteins have been widely investigated (de Rhan and Jost, 1979), and the effects of these complexes on cooking properties are being studied in several legume species (Kumar et al., 1978).

Table 3–Phytic acid (mg/g) and insoluble dietary fiber (%) in eleven legume flours, protein fractions and starch fractions (dry basis)

	Phytic acid			Insoluble dietary fiber		
	Flour	Protein	Starch	Flour	Protein	Starch
Soybean	11.64	13.53[a]	–	5.94	7.91[a]	–
Lima bean	5.20	13.64	1.86	3.94	5.77	2.97
Navy bean	10.03	26.02	1.99	4.60	7.45	3.63
Northern bean	10.61	23.14	2.52	3.20	6.25	1.91
Chickpea	5.20	12.78	1.48	3.84	6.86	2.42
Cowpea	7.61	16.36	2.47	4.28	7.84	3.53
Lentil	6.39	18.74	1.49	3.74	7.51	2.51
Field pea	7.44	18.88	1.82	3.20	6.79	1.81
Fababean	3.67	8.64	1.61	2.74	5.38	2.01
Mung bean	5.12	14.37	1.58	2.75	6.19	1.10
Lupine	8.17	–	–	5.61	–	–

[a] Soybean protein fraction was a commercial protein concentrate produced by a wet-processing technique.

Table 4–Percentage of vicine and convicine in fababean flour, protein fractions and starch fractions (dry basis)

Fraction	Vicine	Convicine	Total
Flour	0.44	0.23	0.67
First pass			
Protein I	1.03	0.45	1.48
Starch I	0.24	0.15	0.39
Second pass of Starch I			
Protein II	0.89	0.40	1.29
Starch II	0.10	0.09	0.19

Dietary fiber

The soybean flour and protein concentrate contained nearly 6.0 and 8.0% of dietary fiber, resp. (Table 3). The starchy legume flours had only 2.7–4.6% of dietary fiber but the range for the protein fractions was essentially doubled to 5.4–7.8%. Much of the dietary fiber in the dehulled flours would originate from cell wall material, and this appeared to segregate with the fine fraction during air classification. Previously Tyler et al. (1981) showed that the crude fiber in legume flours segregated partially with the protein fraction. Sosulski and Youngs (1979) found that, when hulls were present in the pin milled flour, the starch fraction contained the majority of the crude fiber. In the present study, the starch fraction retained 1.8–3.6% of dietary fiber except for the lower level in mung bean starch.

There are physiological and health benefits from the consumption of high levels of dietary fiber, but the absorption of trace minerals by the gastrointestinal tract may be reduced (Kies et al., 1979; Thompson and Weber, 1979). There is also recent evidence that fiber components can be as important as oligosaccharides and trypsin inhibitors in the induction of flatulence (Fleming, 1981). For these reasons dietary fiber has been included as an antinutritive factor in the present investigation.

Vicine and convicine

The contents of the pyrimidine glycosides, vicine and convicine, were determined on the fababean flour and on the protein/starch fractions obtained by two passes through the pin mill (Tyler et al., 1981). The fababean flour contained 0.44% vicine and 0.23% convicine (Table 4). Pitz et al. (1981) analyzed the whole seeds of 242 fababean cultivars and reported a range of 0.44–0.82% vicine and 0.13–0.64% convicine.

The pyrimidine glycosides tended to concentrate with the protein fraction during the first pass but residual glycosides in Starch I were relatively high (Table 4). The next pass of Starch I through the pin mill and air classifier gave protein fraction II with a relatively glycoside content and the Starch II fraction containing only 0.19% of total glycosides. Pitz et al. (1980) obtained similar results with a high glycoside strain in which Protein I and II contained over 2.0% of total glycosides. Because of the association of vicine and convicine with favism, the high levels in the protein fractions would place a serious constraint on the use of fababean products in foods. Aqueous extraction procedures should be effective in the removal of the glycosides (Collier, 1976) but the functional properties of the vicine-free product have not been determined.

Protein fraction II

The protein fraction II's, which occurred in yields of 9.9–15.4 g/100g of air-classified flours, were not analyzed for other antinutritive factors than the pyrimidines in fababean. Presumably, their composition would be similar to those of protein fraction I, the values being somewhat lower due to the reduced protein and higher starch content of this fraction (Tyler et al., 1981).

SUMMARY

THE EFFECTIVENESS of the pin milling and air classification of starchy legumes in the separation of protein and starch fracticns has been amply demonstrated (Sosulski

and Youngs, 1979). A previous study showed that the protein fractions also contained higher levels of oligosaccharides than the original flours while the starch fractions were depleted in sugars. In the present investigation, the concentrations of trypsin inhibitor, hemagglutinating activity and phytic acid in the protein fractions were up to 300% higher than the original flour while saponins and dietary fiber also tended to segregate with the protein. The starch fractions from most of the nine legume species and biotypes were substantially detoxified by the dry milling process. The protein fractions of navy and northern beans contained particularly high levels of essentially all of the antinutritive fractions and may require extensive processing if food uses of the products are anticipated. On the other hand, mung bean and fababean products were much lower than soybean flour in most antinutritive factors although the fababean protein fraction was enriched in vicine and convicine.

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Enzymatic Dimethylamine and Formaldehyde Production in Minced American Plaice and Blackback Flounder Mixed with a Red Hake TMAO-ase Active Fraction

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ABSTRACT

A dialyzed high molecular weight (>100,000) TMAO-ase active fraction from red hake muscle produced dimethylamine (DMA) in-vitro, activated by cysteine and Fe^{++} or by methylene blue and ascorbic acid. Minced blackback flounder and American plaice were induced to produce DMA and formaldehyde (FA) when mixed with the high molecular weight fraction from red hake. A low molecular weight (<100,000) fraction did not produce DMA either in-vitro or in-vivo. Heating the high molecular weight fraction 10 min at 100°C destroyed all TMAO-ase activity. The reported results lend support to the theory that DMA and FA production in red hake is caused by an endogenous enzyme, associated with the particulate fraction in muscle tissue.

INTRODUCTION

OVER THE PAST several years, different mechanisms have been proposed to account for dimethylamine (DMA) and formaldehyde (FA) formation from trimethylamine oxide (TMAO) in gadoid fish species. Vaisey (1956) reported that in vitro, TMAO could be reduced to trimethylamine (TMA), and smaller amounts of DMA and FA, in the presence of cysteine and either iron (Fe^{++}) or hemoglobin as a catalyst. Spinelli and Koury (1979) have shown that in vitro, several reduced ionic components such as Fe^{++}, Sn^{++}, and SO_2 can induce the degradation of TMAO to DMA. They have also shown that metal chelators such as EDTA and phytic acid in the presence of Fe^{++} and Sn^{++} rapidly accelerated DMA formation. On the basis that drum-dried and freeze-dried hake produced maximal amounts of DMA at a relative humidity of 44%, and that reducing constituents may induce degradation of TMAO to DMA, they suggested that the process could be non-enzymatic. Recently, Spinelli and Koury (1981) have further elaborated this theory with findings that Fe^{++} and catabolites of cysteine (cysteine sulfinic acid, hypotaurine, and taurine) can degrade TMAO to DMA in-vitro. They suggested that because more DMA was formed in muscle homogenates which had been preheated to 60°C than in unheated samples, the cysteine breakdown products were formed enzymatically during the heating cycle. These products could then react with TMAO to yield DMA.

Several investigations have held that an endogenous enzyme directly breaks down TMAO to DMA and FA (Amano and Yamada, 1965; Tokunaga, 1965; Castell et al., 1971; Harada, 1975). The preparation of TMAO-ase active fractions have been reported from kidney tissue of cod and blue whiting (Svensson, 1979), pyloric caeca of *Theragra chalcogramma* (Tomioka et al., 1974), and liver of *Saurida tumbil* Harada, 1975). These TMAO-ase active fractions from various visceral organs do not necessarily reflect the cause of DMA and FA production in the muscle tissue, which is after all the main subject of concern. Parkin and Hultin (1981) have reported the isolation of a microsomal fraction from red hake muscle which is capable of converting TMAO to DMA and FA in the presence of iron (Fe^{++} or Fe^{+++}) and either ascorbate or cysteine. They estimated, based on yield and activity, that the microsomal fraction had the potential for producing amounts of DMA and FA found in-situ during frozen storage. Because the microsomal preparation lost activity after heating, they indicated that the rate-limiting step of the reaction was probably enzyme catalyzed.

The question that naturally arises after consideration of the above findings is how do you determine which "TMAO-ase active preparation(s)" is the one (or more) responsible for DMA and FA formation in vivo? Dingle et al. (1977) showed that the minced muscle tissue of red hake could induce the production of DMA and FA when mixed with the minced muscle tissue of two flounder species which are not normally capable of forming these two compounds. Something present in the red hake muscle was capable of catalyzing the production of DMA and FA in a 4:1 flounder:red hake mixture at nearly the same rate as in red hake alone.

We report here the isolation of a crude TMAO-ase active fraction from red hake muscle which functions both in vitro an in vivo when added to either minced blackback flounder or American plaice. The objective of this work was to demonstrate that an enzymic component present in red hake muscle associated with the particulate fraction, can be shown to induce the same reaction in two flatfish species as it does in red hake and in vitro.

MATERIALS & METHODS

AMERICAN PLAICE (*Hippoglossoides platessoides*) and blackback flounder (*Pseudopleuranectes americanus*) were obtained from a local Gloucester fish processor and filleted and skinned by hand. The fillets were then minced using a Kitchen Aid food grinder fitted with a die having 3.175 mm holes.

Red hake TMAO-ase fractions were prepared as follows: 250g of frozen red hake (*Urophycis chuss*) fillets (−63°C for 8 months) were blended at high speed for 2 min in a Waring glass Blendor jar with 500 ml of cold (4°C) distilled water. The homogenate was centrifuged at 3000 x *g* for 30 min at 4°C. The supernatant was decanted and stored at 4°C. The pellet was then reblended with 250 ml of cold distilled water for a further 2 min. This homogenate was also centrifuged, and the resulting supernatant combined with the first portion. The total volume of supernatant was 475 ml. The supernatant was then concentrated by ultrafiltration through an Amicon XM-100A membrane (exclusion limit 100,000 Daltons) to a volume of 23 ml to achieve a concentration factor of 20.5 times. The filtrate portion was collected and contained components of <100,000 Daltons. The retentate portion (>100,000 Daltons) was then diafiltered against 6–7 volumes of cold distilled water to wash out the majority of material with a size below the exclusion limit of the ultrafiltration membrane. The TMAO-ase activity of both filtrate and retentate were determined by in-vitro assay. Briefly, this consisted of incubating 0.2 ml of sample with 0.4 ml pH 5.0 phosphate-citrate buffer, 0.2 ml of 0.1M TMAO, 0.1 ml of 0.001M methylene blue, and 0.1 ml of 0.2M ascorbic acid for 30 min at 30°C. The reaction was stopped with the addition of 1.0 ml 8% (w/v) perchloric acid. The DMA content of the assay medium was then determined by gas chromatography. Corrections were made to account for TMAO breakdown from the reagents (negligable) and native DMA content in the samples.

—Continued on next page

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Five hundred gram portions of minced American plaice were mixed uniformly with either 20 ml of distilled water, 20 ml of filtrate (low molecular weight) or 20 ml of retentate (high molecular weight). Fifty gram aliquots of each mixture were placed in lacquered steel cans and sealed under vacuum after purging the cans several times with nitrogen. Anaerobic storage in nitrogen-purged vacuum-sealed cans has previously been found to accelerate DMA production in red hake (Lundstrom et al., 1981).

Five hundred gram portions of minced blackback flounder were mixed evenly with either 20 ml of distilled water or 20 ml of retentate (high molecular weight). No filtrate was mixed with the blackback flounder. Instead, another 20 ml of retentate was mixed with 500g of flesh. All samples were then packaged (in 50g aliquots) in nitrogen-purged vacuum-sealed cans as for the American plaice samples. One set of cans containing the retentate and blackback mixture was then irradiated (Co^{60} 100 Kilorads) to reduce the bacterial population in the samples. All cans were stored packed in ice in a refrigerated room (4°C). Each sample was tested daily, in duplicate, for content of TMAO-N, DMA-N, TMA-N, and free FA. DMA and TMA were determined by a modification of a gas chromatographic method described by Tokunaga et al. (1977). Modifications included substitution of a 9-ft by 2 mm i.d. glass column packed with Chromosorb 103, which allowed a baseline separation of DMA and TMA, and use of a nitrogen-phosphorous specific flame ionization detector. n-Propylamine was used as an internal standard for quantitation of the amines. TMAO was determined as TMA after reduction by titanous chloride (Yamagata et al., 1969). Free FA was determined using a modification of the Nash reagent method (Cochin and Axelrod, 1959). Using the above analytical techniques, the average coefficients of variation were found to be 6.7% for DMA, 4.0% for TMA, 4.5% for TMAO, and 25.0% for free FA. The large variability of the free FA data is thought to be due to variable binding of the FA to the proteins.

RESULTS & DISCUSSION

TMAO-ASE ACTIVITY in red hake muscle appears not to be associated with the soluble, low molecular weight (<100,000) portion of the protein. In in vitro assay at pH 5 with TMAO, methylene blue, and ascorbic acid, the filtrate fraction produced only a small amount of DMA, about 12 μg/ml sample/hr. Under the same assay conditions the retentate fraction produced 4000 μg/ml sample/hour. Clearly, the TMAO-ase activity was associated with the retentate fraction (>100,000 Daltons). This fraction was not, however, the "soluble" TMAO-ase as described by Harada (1975). Fig. 1 illustrates the sedimentation of a crude TMAO-ase preparation centrifuged at 27,000 x *g* for up to 20 hr at 4°C. After centrifuging for a specified period, duplicate samples were removed and the remainder of the samples subjected to continued centrifugation. After centrifugation, the supernatant was removed and the pellet resuspended in a volume of cold (4°C) distilled water equal to the supernatant volume. Aliquots of the supernatant and resuspended pellet were then assayed for in vitro TMAO-ase activity as described above. It is readily apparent that increased time of centrifugation caused a loss of TMAO-ase activity from the supernatant with a corresponding increase in TMAO-ase activity in the pellet. Further evidence of the particulate rather than soluble nature of red hake muscle TMAO-ase was obtained by Millipore filtration. Crude TMAO-ase was filtered by N_2 pressure through Millipore membranes with pore sizes ranging from 0.05 μ to 5.0 μ. Fig. 2 shows the relative TMAO-ase activity of the filtrates as a function of membrane pore size. Membranes with pore diameters 0.22 μ and larger allowed 90% or more of the TMAO-ase activity to pass. The 0.1 μ membrane allowed 69% of the activity through while the 0.05 mμ membrane allowed only 9.5% of the activity to pass. These data indicate a particle size of 0.1 μ and larger for the TMAO-ase activity.

Fig. 3 shows TMA–N production for minced American plaice in admixture with water (control), filtrate, or retentate during storage in nitrogen-purged, vacuum-sealed cans held at 0°C. Since TMAO is the substrate for DMA and FA as well as for TMA, we monitored TMA-N content to provide a check on our methodology. It was also necessary to know at what point TMA production began so that TMAO content could be determined. Little difference between treatments in TMA-N was seen during the seven-day storage period. There was a gradual increase in TMA-N during the first five days, after which all samples increased rapidly, causing a rapid loss of substrate (TMAO).

Fig. 4 shows the production of DMA-N in the same samples. Throughout the seven-day storage period, the minced American plaice mixed with water or with filtrate produced virtually no DMA-N. The mince mixed with the retentate, however, did produce a copious amount of DMA-N. Fig. 5 shows similar results for the production of free FA. Fig. 6 shows TMAO-N degradation. The filtrate sample shows a larger TMAO–N content than the other samples due to red hake TMAO added as part of the low molecular weight filtrate obtained during the ultrafiltration. Both the water and filtrate samples show a slight increase in TMAO-

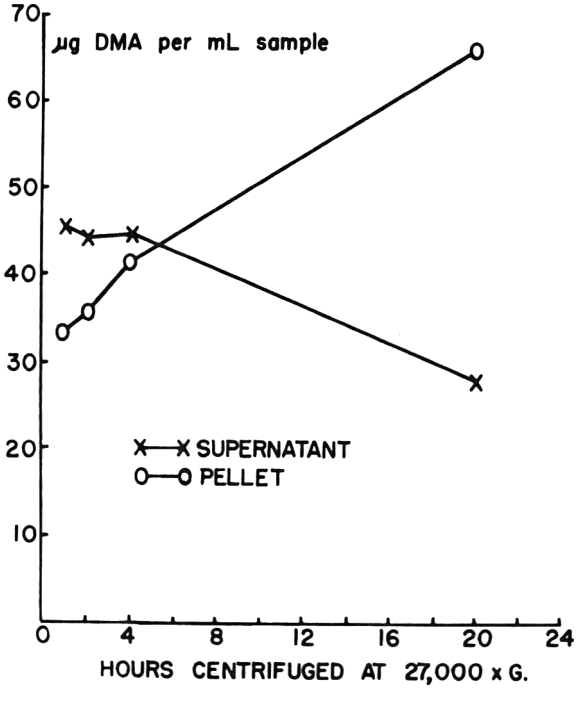

Fig. 1–Sedimentation of TMAO-ase activity.

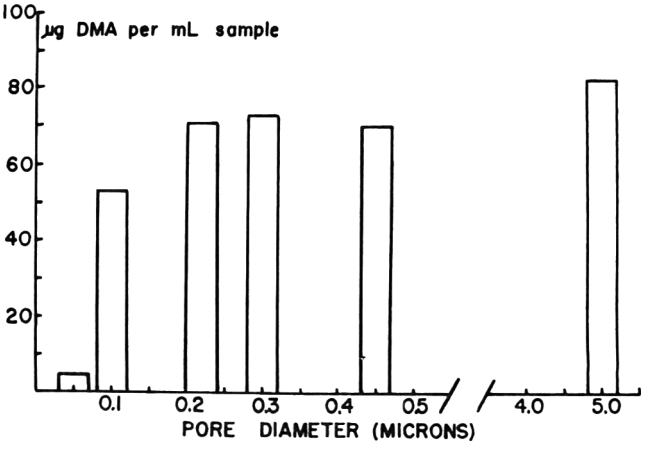

Fig. 2–Membrane filtration of suspended TMAO-ase activity.

N content during the first 5 days of storage, and a rapid decline in TMAO-N content after 5 days which corresponds to TMA-N production during the latter part of the storage period. The retentate sample shows a slight decline in TMAO-N content during the first five days which corresponds to the increase in DMA-N. After day 5 there was a rapid loss of TMAO as TMA was formed. Fig. 7 shows the net daily balance of TMAO derived nitrogen. Each day the contents of DMA-N, TMA-N, and TMAO-N were summed and plotted to follow any changes in TMAO derived nitrogen. Since DMA and TMA are presumably formed from TMAO, the sum of nitrogen contents should remain constant. No significant changes were noted for any of the samples.

The results of the previous experiment were confirmed and extended by repeating the experiment with another species, blackback flounder, known to not normally produce DMA or FA. The experimental design was modified

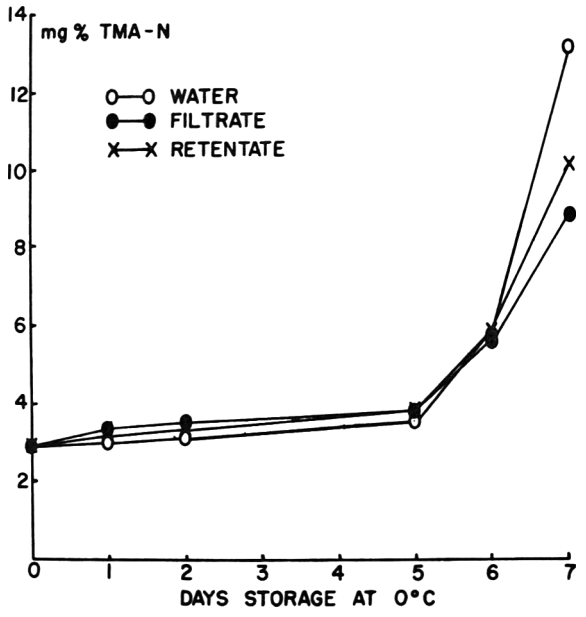

Fig. 3—Production of TMA—N in minced American plaice mixed with water (control), low molecular weight, or high molecular weight red hake muscle fractions.

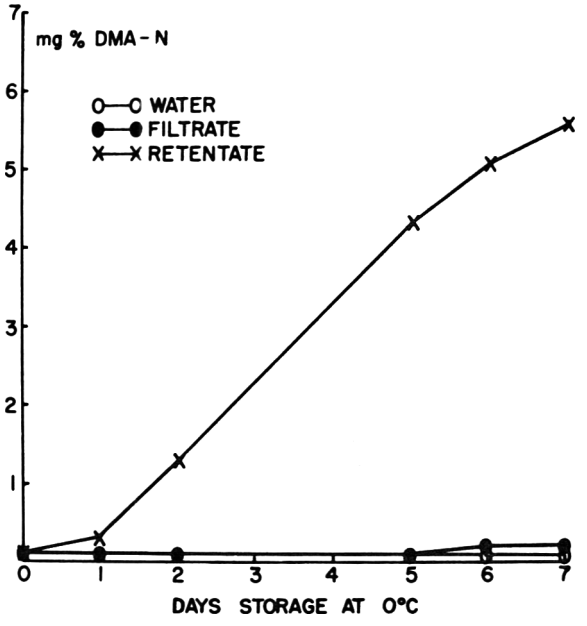

Fig. 4—Production of DMA-N in minced American plaice mixed with water (control), low molecular weight, or high molecular weight red hake muscle fractions.

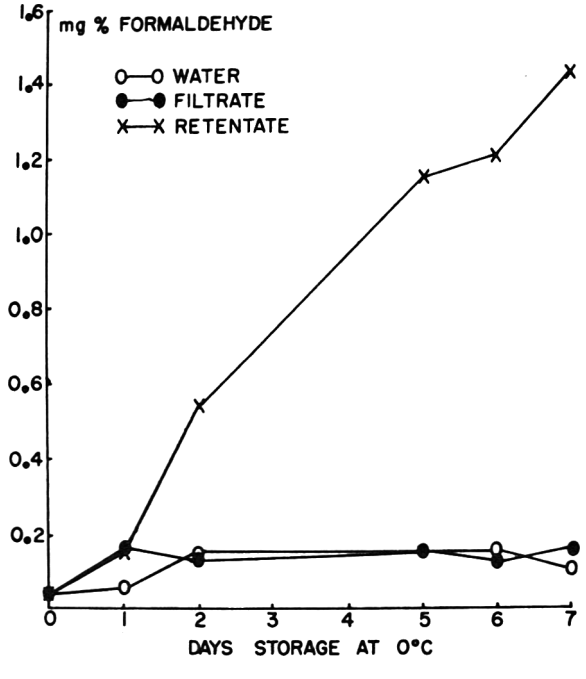

Fig. 5—Production of free FA in minced American plaice mixed with water (control), low molecular weight, or high molecular weight red hake muscle fractions.

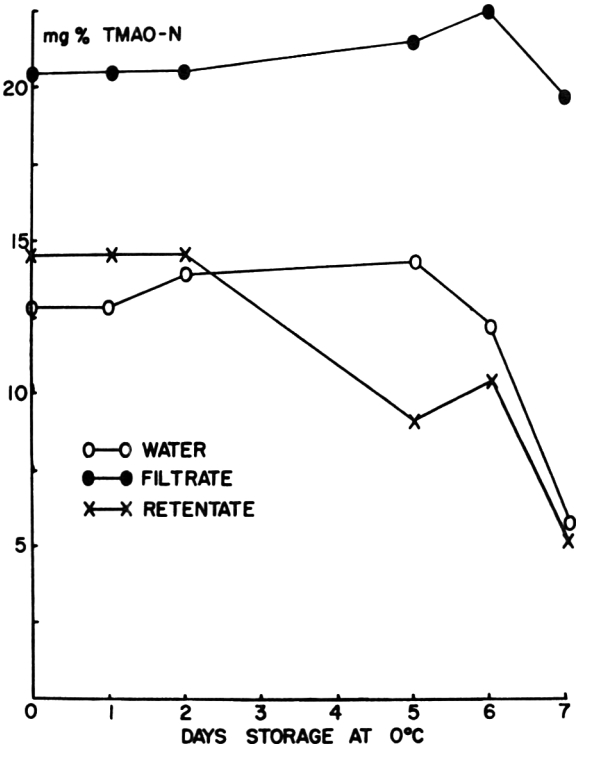

Fig. 6—Degradation of TMAO-N in minced American plaice mixed with water (control), low molecular weight, or high molecular weight red hake muscle fractions.

slightly. Since the previous experiment indicated that neither the water nor filtrate treated minced American plaice produced any DMA or FA, only the water treatment was included with the minced blackback as the control. To investigate the possibility that the observed production of DMA and FA in the mince plus retentate was due to the presence of a bacterial enzyme, an additional sample of mince plus retentate was irradiated (Co^{60} 100 Krads) to provide approximately a two-log cycle reduction in the most radio-labile bacterial population (Ronsivalli, 1967) which includes the TMA producers.

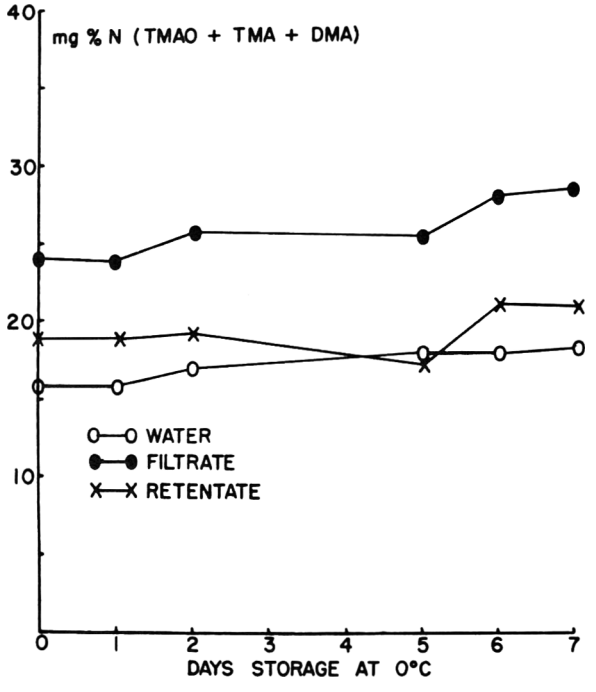

Fig. 7—Net balance of TMAO derived nitrogen in minced American plaice mixed with water (control), low molecular weight, or high molecular weight red hake muscle fractions.

Fig. 8 shows TMA—N production in minced blackback flounder mixed with water (control) or with retentate and irradiated mince mixed with retentate. The water and retentate samples were similar in TMA-N production with a rapid increase after two days. The onset of TMA-N production in the irradiated mince plus retentate was delayed until the eighth day. By the end of the two-week storage period, the irradiated sample had reached the same ultimate TMA-N content as the other two samples.

Fig. 9 shows the production of DMA-N in the same samples. The mince plus water sample produced no DMA-N, confirming that blackback flounder also lacks the enzyme necessary for degradation of TMAO to DMA and FA. The minced red hake treated with retentate, both irradiated and nonirradiated samples, produced a fairly large amount of DMA-N. The nonirradiated sample reached a level of 10.5 mg percent DMA-N by the fifth day of storage and remained at about this level for the remainder of the storage period. The irradiated sample produced slightly more DMA-N than the nonirradiated sample due to the destruction of the bacteria which would otherwise have consumed the substrate (TMAO) to produce TMA. Fig. 10 shows the content of free FA. As with the production of DMA—N (Fig. 9) there was very little free FA produced in the minced blackback plus water. The minced blackback plus retentate produced free FA through the fifth day of storage. After that point, the level of free FA dropped, due to binding with proteins and/or by bacterial degradation. In view of the fact that the irradiated mince plus retentate reached a much higher level before dropping off than the nonirradiated smple did, bacterial degradation of the FA is probably the major cause. Fig. 11 shows the content of TMAO-N. Initially the nonirradiated mince plus retentate sample lost TMAO-N at the fastest rate as both DMA and TMA were being formed. The irradiated mince plus retentate lost

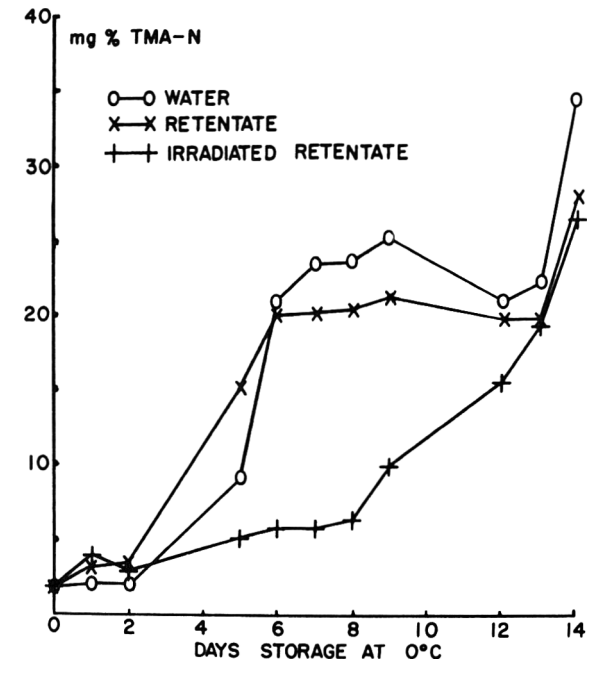

Fig. 8—Production of TMA-N in minced blackback flounder mixed with water (control) or irradiated and non-irradiated high molecular weight red hake muscle fractions.

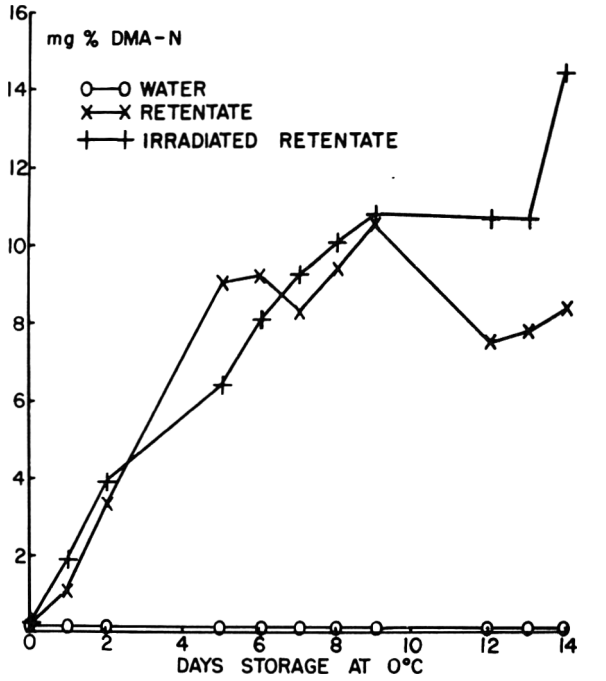

Fig. 9—Production of DMA-N in minced blackback flounder mixed with water (control) or irradiated and nonirradiated high molecular weight red hake muscle fractions.

TMAO-N at a slower rate as initially only DMA was being formed. Since the TMAO-N is converted only to DMA-N or TMA-N, the sum of "amine-nitrogen" for these three compounds should be constant at all times. Fig. 12 shows the daily sums of TMAO derived nitrogen for the three sample groups, which were constant throughout the 2-wk storage period.

It is evident from these data that published reports of no DMA production in American plaice are confirmed (Dingle et al., 1977). In addition, no DMA production was found in blackback flounder. The XM-100A filtrate isolated from red hake muscle also produced no DMA or FA in in vitro assay, or when mixed with minced American plaice. This would indicate that the red hake muscle TMAO-ase was not associated with the low molecular weight (<100,000 Daltons) soluble protein fraction. On the other hand, the high molecular weight retentate fraction (>100,000 Daltons), in in vitro assay or when mixed with minced American plaice or blackback flounder, did bring about the production of copious amounts of DMA and FA. The high molecular weight retentate fraction contained primarily molecules and cellular fragments small enough not to have been sedimented during the first centrifugation step during preparation, and large enough not to have been able to pass through the pores of the ultrafiltration membrane. Contaminating low molecular weight molecules were washed free of the concentrated retentate by passing several volumes of distilled water through the ultrafiltration cell; a type of dynamic dialysis. Thus the TMAO-ase was washed free of the majority of its substrate and other low molecular weight components. If the red hake TMAO-ase was to function in either of the flounder species, it must utilize the flounder's endogenous substrate (TMAO) and any other low molecular weight components (i.e., cofactor) needed in order to produce DMA and FA. Since the retentate did in fact produce DMA and FA, this is an indication that the XM-100A retentate did contain TMAO-ase isolated from red hake muscle, and that it was at least partially functional. Since irradiation of the minced blackback plus retentate mixture did not retard the production of DMA and FA the TMAO-ase can be assumed to have not originated from at least the most radio-labile bacteria, which includes the TMA producers. The rate of DMA and FA production is, of course, much higher in minced red hake stored under similar conditions. We have previously determined that at 32°F (0°C) minced red hake packaged in nitrogen-purged, vacuum-sealed cans can produce DMA-N at a rate of 4.5 mg percent DMA-N per 24 hr (Lundstrom et al., 1981). Since the extraction of TMAO-ase from the red hake muscle was

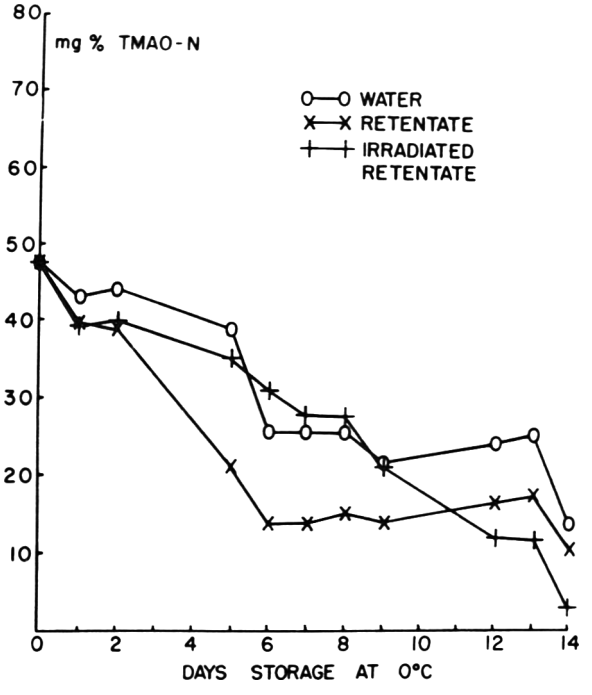

Fig. 11—Degradation of TMAO-N in minced blackback flounder mixed with water (control) or irradiated and nonirradiated high molecular weight red hake muscle fractions.

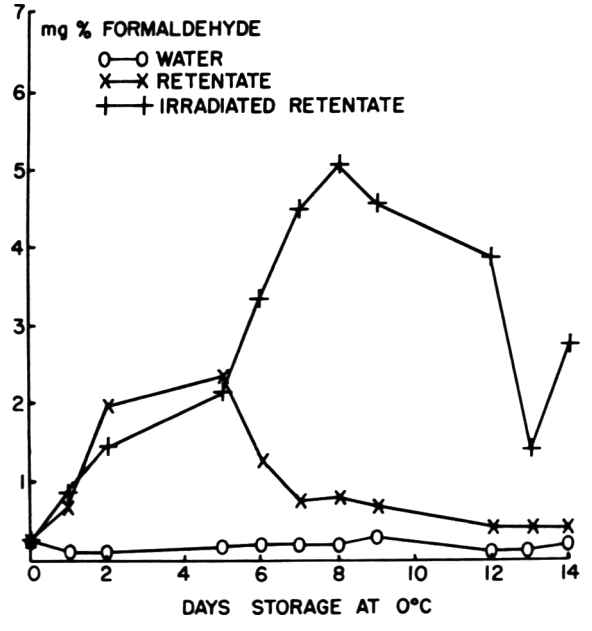

Fig. 10—Production of free FA in minced balckback flounder mixed with water (control) or irradiated and nonirradiated high molecular weight red hake muscle fractions.

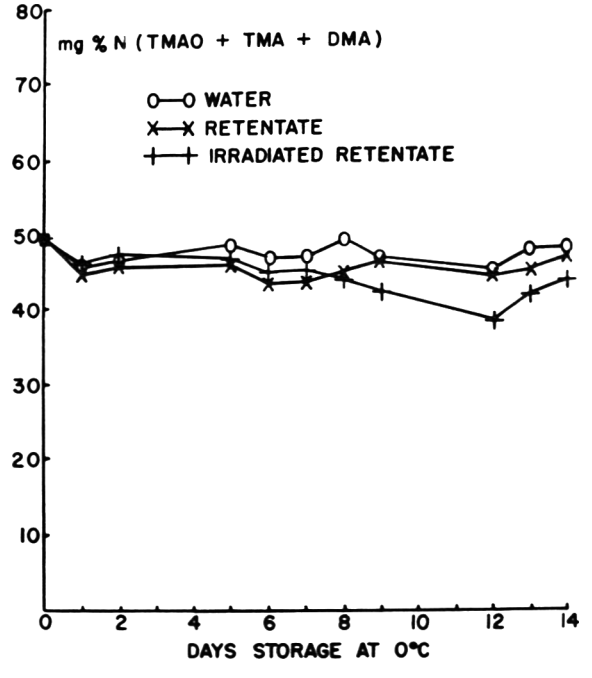

Fig. 12—Net balance of TMAO derived nitrogen in minced blackback flounder mixed with water (control) or irradiated and nonirradiated high molecular weight red hake muscle fractions.

assumed to be less than complete, and the enzyme isolated from 250g of red hake was added to 500g of flounder, it is understandable that the DMA production rate was much lower than that observed in minced red hake held under the same conditions. If we assume about half of the total TMAO-ase activity was extracted from the red hake and this was added to twice as much flounder, we would expect the rate to be a quarter. This was, in fact, what happened. Partial evidence that the TMAO-ase acted directly on TMAO to form DMA and FA was observed by the in-vitro formation of product (DMA and FA) with only TMAO, pH 6.7 histidine buffer, methylene blue, and ascorbic acid present in the assay mixture.

Table 1 shows the influence of various in-vitro assay components on the breakdown of TMAO to DMA. All of these assay mixtures contained 5 mM histidine at pH 6.7 and 10 mM TMAO. The addition of cysteine produced no DMA while adding Fe^{++} produced a small amount of DMA. Cysteine and Fe^{++} together almost doubled the rate but the amount of DMA is still relatively small. The retentate alone was capable of producing as much DMA as the cysteine plus Fe^{++}. Adding cysteine to the retentate had no effect but Fe++ with the retentate doubled the DMA production rate as compared to the retentate alone. A much greater effect was seen when both cysteine and Fe^{++} were added to the retentate. The DMA production rate was about eight times the retentate alone. Clearly, the retentate, the cysteine and the Fe^{++} are all required to produce substantial quantities of DMA. This is in agreement with Parkin and Hultin's (1981) findings with a microsomal fraction from red hake. Methylene blue and ascorbic acid with the retentate show a much greater effect than the cysteine and Fe^{++}. Without the retentate the methylene blue and ascorbic acid show almost no DMA production. If the filtrate (components <100,000 Daltons) is substituted for the retentate (>100,000 Daltons) again we can detect almost no DMA production. This clearly shows that a factor responsible for DMA production resides with the high molecular weight fraction from red hake. Cysteine and Fe^{++} must be present in order for this factor to show significant TMAO-ase activity but other compounds such as methylene blue and ascorbic acid activate the retentate, in-vitro, to a much greater degree. Retentate preparations heated at 100°C for 10 min have consistently failed to show any in-vitro TMAO-ase activity and this suggests the presence of an enzyme. Although TMAO-ase activity was detected in vitro, there is always the problem of relating what happens in the test tube with the in-situ reaction. The functionality of our retentate fraction in two flat-fish species suggests that it does play an active role in DMA and FA production in red hake, and is worthy of more detailed study.

Table 1—Production of DMA by a TMAO-ase active fraction from red hake

Assay components[a]	μg DMA/ml/hr at 30°C
A. TMAO + Cysteine	0
B. TMAO + Fe^{++}	17
C. TMAO + Cysteine + Fe^{++}	31
D. TMAO + Retentate	26
E. TMAO + Retentate + Cysteine	22
F. TMAO + Retentate + Fe^{++}	48
G. TMAO + Retentate + Fe^{++} + Cysteine	207
H. TMAO + Retentate + MB + AA	4000
I. TMAO + MB + AA	8
J. TMAO + Filtrate + MB + AA	14

[a] These assay mixtures contained components at the following concentrations:
$FeSO_4$, 1.0 mM; Cysteine, 1.0 mM; TMAO, 10.0 mM; Methylene Blue (MB), 0.1 mM; Ascorbic Acid (AA), 10.0 mM; 5 mM Histidine at pH 6.7; and 0.1 ml of either the filtrate or retentate fractions.

CONCLUSIONS

TMAO-ase, an enzyme capable of degrading TMAO to DMA and FA, was partially purified from red hake muscle as part of a high molecular weight (>100,000 Daltons) ultrafiltration fraction. This TMAO-ase active fraction appears to be associated with a particulate fraction which can be sedimented by centrifugation or filtered from suspension with a Millipore 0.1 μ filter membrane. The TMAO-ase active fraction is functional in-vitro with only substrate and buffer, and either methylene blue and ascorbic acid or cysteine and Fe^{++} present. The TMAO-ase was functional when mixed with minced muscle tissue either of two flounder species as evidenced by the production of both DMA and FA during storage under anaerobic conditions. Since irradiation of the minced flounder-TMAO-ase mixture did not retard DMA and FA production, the TMAO-ase did not originate from the most radio-labile bacterial species. We would conclude from these results that the TMAO-ase enzyme is present in significant quantities in red hake muscle and that it can be isolated in partially purified form by ultrafiltration. Additionally, the enzyme can function in the minced muscle of two different flounder species, utilizing the alien species substrate (TMAO) to produce both DMA and FA. At least in red hake, the production of DMA and FA can be attributed to the direct action of a particle bound enzyme on TMAO.

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Bacterial Histamine Production as a Function of Temperature and Time of Incubation

ALISON R. BEHLING and STEVE L. TAYLOR

ABSTRACT

Optimal temperature, lower temperature limit, extent, and rate of histamine production in a tuna fish infusion broth (TFIB) varied for the strains of *Proteus morganii, Klebsiella pneumoniae, Hafnia alvei, Citrobacter freundii,* and *Escherichia coli* studied. *P. morganii* and *K. pneumoniae* produced large quantities of histamine in a relatively short incubation period (<24 hr) at 15°C, 30°C, and 37°C; production was fastest at 37°C. *H. alvei, C. freundii,* and *E. coli* produced toxicologically significant levels of histamine (>2500 nmoles/ml) only at 30°C and 37°C on prolonged incubation (≥48 hr). At 72 hr of incubation, optimal temperature for histamine production was 37°C for *E. coli* and *C freundii*; 30°C for *P. morganii* strain 110SC2, *K. pneumoniae,* and *H. alvei*; and 15°C for *P. morganii* strain JM. The lower temperature limits for production of toxicologically significant levels of histamine in TFIB were 7°C for *K. pneumoniae*; 15°C for both *P. morganii* strains; and 30°C for *H. alvei, C. freundii,* and *E. coli.*

INTRODUCTION

FOOD POISONING EPISODES can result from the ingestion of foods containing substantial amounts of histamine (Arnold and Brown, 1978; Lerke et al., 1978; Merson et al., 1974). This type of food poisoning is often called scombroid fish poisoning because of its frequent association with scombroid-type fish such as tuna and mackerel (Arnold and Brown, 1978; Lerke et al., 1978; Merson et al., 1974) although nonscombroid fish particularly mahimahi (Anonymous, 1980; Kim, 1979) and other foods such as cheese (Doeglas et al., 1967; Taylor et al., 1982) can be involved. Histamine is formed in foods from histidine by bacteria that possess the enzyme, histidine decarboxylase. Many bacteria reportedly possess at least limited histidine decarboxylase activity (Taylor et al., 1978), but only *Proteus morganii* (Kawabata et al., 1956; Sakabe, 1973a), *Klebsiella pneumoniae* (Taylor et al., 1979), and *Hafnia alvei* (Havelka, 1967) have been implicated as causative organisms in the formation of toxicologically significant levels of histamine. However, attempts to isolate and identify histamine-producing bacteria have been made on only a few of the samples obtained from food poisoning incidents. Consequently, the possibility exists that additional species of bacteria may yet be identified as important histamine producers.

In the fishing industry, bacterial histamine production is controlled primarily by the use of low storage temperatures. The histamine-producing bacteria apparently reside in the gills and/or intestines of the fish (Frank et al., 1981; Lerke et al., 1978) and any prolonged storage at elevated temperatures results in bacterial action on tissue histidine. On a purse seiner, tuna would typically be netted in water with a temperature of approximately 29°C, and the fish might be held in the net for a matter of minutes or hours. The fish would then be transferred to a well containing refrigerated seawater at 30°F (−1°C). If the well is not immediately filled, the tuna may remain at this temperature for 2–21 days until the well is filled. The temperature in the well is then dropped to 15–20°F (−9 to −6.7°C) and the fish are frozen. The tuna are often stored frozen for several months before thawing, evisceration, and processing. The length of time that the fish are subjected to any of these temperatures is dependent on fishing conditions particularly the size of the catch. The rate of cooling or freezing is also dependent on the size of the catch. On baitboats, the tuna spend less time in the warm seawater but on-board temperature control is more haphazard and usually involves simply icing of the fish. With baitboats, the time from catching to processing is often less than 24 hr.

The general effectiveness of these temperature controls is apparent from industry experience. However, problems occasionally arise despite these control measures. Obviously, the length of time in warm seawater and the rate of cooling to −1°C would be important factors that could contribute to occasional bacterial production of histamine. The importance of other factors such as the length of time at −1°C are less certain. Although many studies have been done on the effect of storage temperature on histamine formation in fish (Baldrati et al., 1980; Catteneo and Cantoni, 1978; Durr et al., 1980; Edmunds and Eitenmiller, 1975; Frank et al., 1981; Ganowiak et al., 1979; Gheorghe et al., 1970a, b; Hardy and Smith, 1976; Kimata and Kawai, 1953; Langeland, 1978; Lerke, 1980; Park et al., 1980; Sakabe, 1973b; Smith et al., 1980), the results, particularly at low storage temperatures, have been variable. The bacterial species responsible for histamine production were generally not identified in these previous studies. Since several species of bacteria are capable of toxicologically significant histamine production, the possibility exists that these bacteria would have different capacities for histamine production at low storage temperatures. In a previous study, Arnold et al. (1980) determined that histamine production by *P. morganii* and *P. vulgaris* was delayed and diminished by incubation at 7°C as compared to 30°C or 19°C. A similar effect was noted for *H. alvei* although this species produced considerably less histamine at all incubation temperatures (Arnold et al., 1980). This study was initiated to assess the comparative abilities of several different bacterial strains to produce histamine at temperatures ranging from −3°C (26°F) to 37°C (98.6°F) in a tuna fish infusion broth in an attempt to determine if some of these strains might be capable of significant histamine production at low temperatures. Previously identified high-level histamine producers and nonproducers were included in the study. In previous studies (Taylor et al., 1978; Taylor et al., 1979), histamine-producing bacteria were distinguished from nonproducers by their ability to produce large amounts of histamine in tuna fish infusion broth within 24 hr at 32°C. It was of interest to determine if the incubation time and temperature would have any effect on this mechanism for distinction between histamine producers and nonproducers.

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MATERIALS & METHODS

Bacteria

Six strains of bacteria that had previously been studied for histamine production (Lerke et al., 1978; Taylor et al., 1978, 1979) were used in this study: *Proteus morganii*, 110SC2; *Klebsiella pneumoniae* biotype II, T2; *Hafnia alvei*, T8; *Proteus morganii*, JM; *Citrobacter freundii*, T3; *Escherichia coli*, 58. The bacteria were stored, refrigerated, on trypticase soy agar (TSA) slants until used.

Media and growth conditions

A loopful of bacteria was transferred from the TSA to 5 ml of trypticase soy broth containing 1% histidine-HCl and 0.0005% pyridoxal-HCl (TSBH) and incubated for 24 hr at 35°C (95°F); 0.2 ml of the culture was transferred to another 5 ml of TSBH and incubated 18 hr at 35°C (95°F). 2.0 ml of the overnight culture was used to inoculate 70 ml of tuna fish infusion broth (TFIB). Tuna fish infusion broth was prepared as previously described (Omura et al., 1978; Taylor et al., 1979).

The TFIB cultures were incubated without shaking at each of six temperatures: 37°C (98.6°F), 30°C (86°F), 15°C (59°F), 7°C (44.6°F), 0°C (32°F), and −3°C (26°F). Samples for aerobic plate count (APC) and histamine analysis were taken at 0, 6, 24, 48, and 72 hr for those cultures incubated at 7°C or above; at 0, 24, 48, 72, and 158 hr for those cultures incubated at 0°C and −3°C.

Histamine analysis

A modification of the AOAC method (AOAC, 1980) was used. 1.0 ml of culture fluid was added to 9.0 ml of methanol; this was heated at 60°C for 15 min and then allowed to cool. 1.0 ml of the methanol extract was pipetted onto an ion exchange column (80 x 5 mm) of Dowex 1X-8 in the hydroxide form. The column was washed with approximately 35 ml of demineralized water as previously described (AOAC, 1980). The column eluant was collected in a 50 ml volumetric flask containing 5.0 ml of 1N HCl; the volume was raised to 50 ml with deionized, distilled water.

The amount of histamine was determined using the fluorometric OPT method (Shore, 1971). 2.0 ml of the column eluant was placed into a small test tube and 0.4 ml of 1N NaOH was added and mixed; 0.1 ml of 0.1% *o*-phthalaldehyde (OPT) was added and mixed. Exactly 4 min after the OPT was added, 0.2 ml of 3N HCl was added to stop the reaction. The amount of histamine-OPT reaction product in the tube was determined fluorometrically at an excitation of 360 nm and emission of 450 nm.

Aerobic plate counts

Aerobic plate counts (APC) were conducted by using dilutions prepared in saline. Plates were poured in trypticase soy agar and incubated at 37°C for 24 hr. Duplicate plate counts were used throughout.

RESULTS

AS EXPECTED, *P. morganii* strains 110SC2 and JM and *K. pneumoniae* T2 produced the highest levels of histamine. The results obtained with *K. pneumoniae* T2 are portrayed in Fig. 1. Similar results were obtained with the two *P. morganii* strains. Histamine production by these three strains exceeded 40 μmoles/ml after 72 hr of incubation at 15°C and 30°C. *P. morganii* strains 110CS2 and JM produced 45.6 μmoles/ml and 40.8 μmoles/ml of histamine, respectively in 72 hr at 30°C compared to 40.6 μmoles / ml for *K. pneumoniae* T2. Histamine production was most rapid at 37°C. However, even with these high level histamine producers, histamine production in excess of 1.0 μmoles/ml in 6 hr of incubation was noted only at 30°C and 37°C. At 37°C with *K. pneumoniae* T2, histamine accumulation was maximal by 24 hr of incubation, declining slightly thereafter. In contrast, with the *P. morganii* strains, histamine continued to accumulate up to 72 hr of incubation but the rate of histamine formation after 24 hr was slower than at 15°C or 30°C. At incubation temperatures of 7–30°C, histamine levels continued to increase throughout the 72 hr incubation period for these three strains.

C. freundii T3, *E. coli* 58, and *H. alvei* T8 were able to produce substantial amounts of histamine (>2.5 μmoles/ml) only on prolonged incubation at the higher incubation temperatures. The results obtained with *C. freundii* T3 are shown in Fig. 2. Similar results were obtained with the *E. coli* and *H. alvei* strains. Even at 37°C, histamine production by these bacteria was substantially less than that observed with the *P. morganii* and *K. pneumoniae* strains. At 37°C and 72 hr of incubation, the accumulated levels of histamine were 10.4 μmoles/ml for *C. freundii* T3, 25.3 μmoles/ml for *E. coli* 58, and 2.63 μmoles/ml for *H. alvei* T8. With these low level or slow histamine producers, histamine levels increased throughout the 72 hr incubation period at 15°C, 30°C, and 37°C. Histamine production by these strains occurred at a much slower rate than the high level producers with a more pronounced lag period.

In these experiments, a large inoculum of approximately 10^7 organisms/ml was used. Even with this large inoculum, the production of histamine by *P. morganii* and *K. pneumoniae* corresponded with further growth of the bacteria. Under static growth conditions, no histamine production was observed with *P. morganii* and *K. pneumoniae*. The decrease in histamine concentration seen at 37°C with *K. pneumoniae* T2 was accompanied by a decrease in the number of bacterial cells. With the *P. morganii* strains at 37°C, histamine continued to accumulate beyond 24 hr despite a decrease in the aerobic plate count. Similarly, the increased histamine production observed at 37°C with *C. freundii* T3 and *E. coli* 58 was accompanied by a decrease in the aerobic plate count. These organisms apparently have the ability to produce or release histamine during the stationary or death phases of growth. The temperature resulting in the highest histamine production varied with the different bacterial strains and the incubation time. At 72 hr of incubation, the temperature yielding the maximal

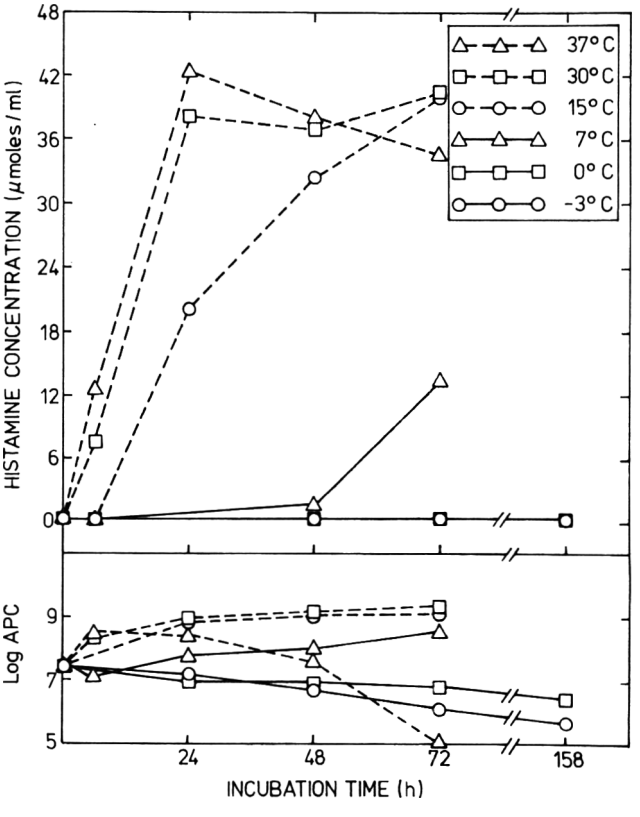

Fig. 1—Growth (aerobic plate count, APC) and histamine production by Klebsiella pneumoniae T2 as a function of incubation time and temperature.

histamine accumulation was 37°C for *E. coli* 58 and *C. freundii* T3; 30°C for *P. morganii* 110SC2, *K. pneumoniae* T2, and *H. alvei* T8; and 15°C for *P. morganii* JM. Because of the decrease in histamine level observed at 37°C beyond 24 hr of incubation and the faster rate of histamine production at 37°C, the greatest histamine accumulation after 24 hr of incubation was obtained at 37°C for the *P. morganii* and *K. pneumoniae* strains.

The lower temperature limit for substantial histamine production (in excess of 2.5 μmoles/ml) was 7°C for *K. pneumoniae* T2, 15°C for *P. morganii* strains 110SC2 and JM, and 30°C for *H. alvei* T8, *C. freundii* T3, and *E. coli* 58. The rate of histamine production for the high level histamine producers slowed appreciably when the incubation temperature was dropped from 15°C to 7°C. At −3°C and 0°C, both bacterial growth and histamine production virtually ceased. Slow production of histamine by *K. pneumoniae* T2 occurred at 0°C but the maximal level of histamine reached only 715 nmoles/ml at 158 hr of incubation.

These incubations were performed without shaking. To determine the effect of shaking at 100 rpm on bacterial histamine production, a comparative study was done with and without shaking in TFIB at 32°C with the same six bacterial strains. Essentially no differences in bacterial histamine production were observed between static and shaking incubation.

DISCUSSION

THE OPTIMAL TEMPERATURE, the lower temperature limit, the extent, and the rate of bacterial histamine production are functions of the bacterial strain being studied (Fig. 1 and 2). The variability in the amount of histamine produced and the rate of its production among different bacterial strains had been determined previously in comparisons of large numbers of bacterial strains (Taylor et al., 1978, 1979). These earlier studies on the rate and extent of bacterial histamine production indicated that while many bacterial strains possessed the capability for histamine production in TSBH medium, only *P. morganii, Enterobacter aerogenes,* and selected strains of *K. pneumoniae* were capable of rapid production of large quantities of histamine in TSBH and TFIB (Taylor et al., 1978, 1979). Incubation periods in these earlier studies were limited to 24 hr or less at 32°C. The results presented here indicate that the selected *P. morganii* and *K. pneumoniae* strains were capable of producing several-fold more histamine in TFIB if the incubation periods were extended to 48 or 72 hr at 15°C, 30°C, and 37°C. The *K. pneumoniae* and *P. morganii* strains produced toxicologically significant levels of histamine in TFIB within 24 hr at 15°C. *K. pneumoniae* T2 produced a toxicologically significant level of histamine in 72 hr at 7°C. Even the *H. alvei, C. freundii,* and *E. coli* strains previously identified as nonproducers of histamine (Lerke et al., 1978; Taylor et al., 1978) were able to produce toxicologically significant levels of histamine at 30° and 37°C on prolonged incubation.

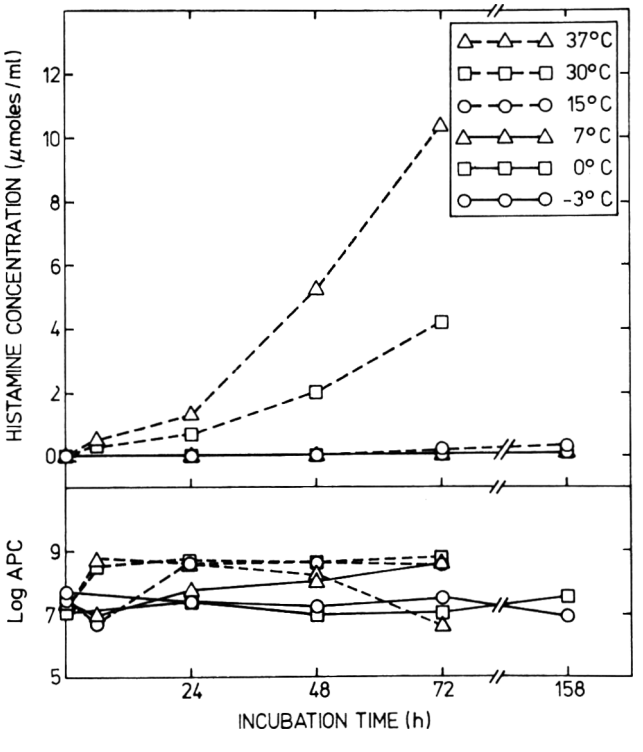

Fig. 2—Growth (aerobic plate count, APC) and histamine production by Citrobacter freundii T3 as a function of incubation time and temperature.

A toxicologically significant level of histamine would be a level equivalent to the FDA hazard action level for histamine in tuna fish of 50 mg/100g. Based on the wet weight of the tuna used to prepare the TFIB, a histamine level of 2.5 μmoles/ml in TFIB would correspond to approximately 50 mg/100g. Histamine production in TFIB should be a reasonable indicator of an organism's ability to generate histamine in tuna fish, since TFIB is prepared from raw tuna. Obviously, differences exist between TFIB and tuna fish including the liquid nature of TFIB, the aerobic incubation conditions, the addition of glucose, and the lack of competing organisms. The histamine levels produced in these experiments were often so far in excess of 2.5 μmoles/ml that these differences would assume less importance.

This study was performed with inoculation of rather large numbers of bacteria into TFIB. An inoculum of approximately 10^7 organisms/ml is several orders of magnitude higher than the level of contamination expected to occur on scombroid fish. If a smaller inoculum had been used, the rate of histamine accumulation would certainly have been slower. The large inoculum was used in an attempt to determine which bacteria would be capable of histamine production at the lower incubation temperatures. Additional studies would be necessary to determine if histamine can accumulate to a similar extent with smaller inocula.

Earlier, Arnold et al. (1980) showed that histamine production by *P. morganii, P. vulgaris,* and *H. alvei* in TFIB was highest at 30°C and was diminished by incubation at 19°C and 7°C. They used a large inoculum also. In their study, histamine production was delayed considerably by incubation at 7°C. The lag period for histamine production at 7°C was 4 days for *P. morganii* and *P. vulgaris* and 14 days for *H. alvei.* Our results indicate that the lag time for *K. pneumoniae* T2 may be even shorter. Apparently, the potential for histamine production at low temperatures is much greater for some bacteria such as *K. pneumoniae* than for others.

No differences were observed in bacterial histamine production in comparisons of shaking versus static incubation conditions. Arnold et al. (1980) noted appreciable differences in bacterial histamine production between aerobic, microaerophilic, and anaerobic incubation conditions. In both the static and shaking incubation conditions used in our experiments, aerobic growth conditions would prevail. Although histamine formation and spoilage are both the result of bacterial action, histamine is not necessarily a good index of spoilage (Arnold and Brown, 1978). A spoiled fish with high histamine content will likely not be consumed whereas a fish with good appearance and a high

histamine content may be consumed. Consequently, the bacteria such as *P. morganii* and *K. pneumoniae* that are capable of producing large amounts of histamine in a short period of time are of the greatest concern. Histamine production by these bacteria must be controlled during fish handling, storage, processing, and preparation. The histamine production by *H. alvei, C. freundii,* and *E. coli* at 30° and 37°C may have little practical significance due to the rapid spoilage of fish at these temperatures. The abilities of *P. morganii* and *K. pneumoniae* to produce significant levels of histamine at incubation temperatures of 15°C and even 7°C may be important also due to the slower rate of spoilage at these temperatures. The rate of cooling of a large tuna catch on the boat may be a critical factor in the generation of histamine.

The variability in the optimal temperature and the lower temperature limit for bacterial histamine production among different bacterial strains may explain the differences observed in previous studies on histamine formation in fish as a function of storage temperature. The earliest studies of histamine formation in scombroid fish indicated that maximal histamine production in mackerel occurred on storage at 17°C (Kimata and Kawai, 1953). In that study, 354 mg/100 g histamine was found after 75 h at 17°C in fillets that organoleptically appeared to be acceptable. Likewise, Ganowiak et al. (1979) noted a rapid rise in histamine levels in sardines and tuna within 24 hr at 18°C; Durr et al. (1980) found 66–99 mg/100g histamine in sardines and mackerel after storage for 14–16 days at 15–20°C; and Baldrati et al. (1980) observed a histamine level of 67 mg/100g in mackerel stored at 18°C for 3 days. Kimata and Kawai (1953) found negligible levels of histamine in mackerel on storage at 35°C. Again, this finding agrees with the observations of Baldrati et al. (1980) in mackerel at 30°C. In contrast, Frank et al. (1981) indicated that 37.8°C was the optimal temperature for histamine formation in skipjack tuna. Other investigations pinpointed temperatures in the range 20–25°C as optimal for histamine formation in fish (Durr et al., 1980; Edmunds and Eitenmiller, 1975; Hardy and Smith, 1976; Langeland, 1978; Park et al., 1980; Smith et al., 1980). Variability exists even among these six studies with maximal histamine production ranging from a high of 300 mg/100g in sardines after 48 hr at 25°C (Park et al., 1980) to a low of 33 mg/100g in mackerel after 48 hr at 24°C (Edmunds and Eitenmiller, 1975) and 42–52 mg/100g in mackerel after 96 hr at 23°C (Smith et al., 1980). In contrast to the other studies, Sakabe (1973b) found little difference in histamine production in tuna extracts in the temperature range 10–37°C. Particularly disturbing is the lack of uniformity in data reflecting on the effectiveness of low temperature storage as a means for control of bacterial histamine formation. Several studies have indicated that little histamine is formed in fish at temperatures in the range 2–10°C (Edmunds and Eitenmiller, 1975; Gheorghe et al., 1970a, b; Hardy and Smith, 1976; Smith et al., 1980). However, in other investigations, substantial histamine formation has been noted at such temperatures. Dabrowski et al. (1968) showed that histamine could be produced in low levels in herring at 0–2°C. Sakabe (1973b) found substantial histamine production by *P. morganii* in a tuna extract at 10°C. Cattaneo and Cantoni (1978) found histamine levels of 118–274 mg/100g in mackerel and 88–278 mg/100g in tuna after 4 days of storage at 6°C. Baldrati et al. (1980) observed 24 mg/100g histamine in mackerel after 5 days at 4°C, while Park et al. (1980) detected histamine levels of 130 mg/100 g in mackerel after 4 days at 10°C and 250 mg/100g in sardines after 5 days at 10°C. All studies seem to agree that storage at 0°C or below limits histamine formation to negligible levels (Baldrati et al., 1980; Cattaneo and Cantoni, 1978; Gheorghe, 1970a, b; Hardy and Smith, 1976; Smith et al., 1980).

The differences noted in the above cited studies on the effects of temperature on the formation of histamine in fish may be due to differences in the type and level of microbial flora of the fish used in the various studies. As noted, individual bacterial strains vary considerably in their optimal temperatures and lower temperature limits for histamine production. Fish storage studies would be affected also by the presence of histamine-producing bacteria on the fish. There is no reason to believe that all of the fish in these studies possessed histamine-producing bacteria or that histamine producers were among the predominant microflora in all cases. The incidence of histamine-producing bacteria in scombroid fish is unknown. The identity of the histamine-producing bacteria was established in only two of the fish storage studies. Sakabe (1973b) inoculated tuna extracts with *P. morganii* and Ganowiak et al. (1979) contaminated sardines and tuna with *P. morganii* prior to storage. Certainly, the level of histamine-producing bacteria in the fish would affect the rate of formation of histamine in a storage study. In our studies and those of Arnold et al. (1980), large inocula resulted in the accumulation of high histamine levels at 7°C. However, such high numbers of histamine-producing bacteria would not be expected in most fish storage studies, and this might have resulted in a lower level of histamine formation at low temperatures in those experiments.

Current fishing practice for tuna involves either immediate icing (baitboats) or storage in refrigerated seawater at approximately −1°C before freezing (purse seiners). Histamine formation by the bacteria employed in this study would be controlled by such storage temperatures. However, the production of relatively large amounts of histamine at 4–6°C in the fish storage studies of Baldrati et al. (1980) and Cataneo and Cantoni (1978) may suggest the existence of bacteria capable of histamine production at such temperatures. However, another explanation is possible. Several investigators have shown that fish subjected to storage at 20°C for a short period of time (1 day) will yield high levels of histamine following subsequent storage at refrigeration temperatures (Sakabe, 1973b; Smith et al., 1980). Consequently, prior storage of fish at high temperatures may influence the findings of subsequent cold storage studies. Alternatively, Lerke (1980) has shown that histamine is produced slowly in fish stored at −8°C prior to subsequent storage at 29°C. Presumably, freezing injures the histamine-producing bacteria and limits their capacities for histamine production. Low temperature storage of tuna on fishing boats has been a successful practice. However, the safety factor may not be large particularly with certain bacterial strains. Therefore, precautions should be taken such as careful control of storage temperatures, rapid cooling in refrigerated seawater, and limitations on the length of storage in refrigerated seawater before freezing.

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Correlation of Ethanol Concentration with Sensory Classification of Decomposition in Canned Salmon

THOMAS A. HOLLINGWORTH, JR. and HAROLD R. THROM

ABSTRACT

Canned salmon samples of four species (sockeye, pink, coho, and chum) were analyzed for ethanol content. A highly significant correlation ($r = 0.71$, $p < 0.001$, Pearson; $r = 0.80$, $p = 0.001$, Spearman) was found between the ethanol content and the sensory classification of decomposition in canned salmon. This relationship has potential use as a means of objectively confirming the initial sensory classification of canned salmon.

INTRODUCTION

THE ACCEPTED and established method for categorizing the quality of seafood products is by sensory analysis. Since the technique is subjective in nature it has long been recognized that confirmation by an objectively based independent method would be highly desirable.

To this end a number of workers (Holaday, 1939; Hillig, 1958; Lerke and Huck, 1977; Khayat, 1979; Human and Khayat, 1981) have investigated the potential use of ethanol as a chemical index for decomposition in fish. Since ethanol can be derived from carbohydrates via anaerobic fermentation (glycolysis) and/or the deamination and decarboxylation of amino acids such as alanine, it is a common metabolite of a variety of bacteria. Thus one might reasonably expect increasing ethanol concentration to be associated with increasing decomposition (Lerke and Huck, 1977). Recently several investigators, Miller and Throm (1977) and Crosgrove (1978), have suggested that ethanol alone might be a useful index of decomposition in canned salmon. The purpose of the present investigation was to extend these studies to determine if a relationship between ethanol content, sensory analysis and the degree of decomposition in canned salmon exists and, if so, to determine if it could be utilized in a practical way to objectively confirm an initial sensory classification of the product.

EXPERIMENTAL

Analysis of canned salmon sample for ethanol

Canned salmon samples of four species of Pacific salmon, sockeye (*Oncorhynchus nerka*), pink (*Oncorhynchus gorbuscha*), coho (*Oncorhynchus kisutch*) and chum (*Oncohynchus keta*) were collected from a variety of sources which included commercially packed cans from various processors and locations, salmon that contained naturally occurring decomposition which was canned and retorted under FDA supervision, and from salmon that was allowed to decompose in Alaskan canneries under as near normal conditions as possible.

The can of salmon was opened and the liquid phase was decanted into a 250 ml beaker, the salmon remaining was retained for sensory analysis (see below). The liquid phase was transferred to a 250 ml separator and the oil and aqueous phases allowed to separate. The aqueous phase was then drained into a 50 ml glass stoppered graduated cylinder. Five ml of the aqueous phase was filtered through Whatman No. 41 paper; the first 1 ml of filtrate was discarded and the next 1 to 2 ml were collected in a small screw cap glass vial. Recoveries of added ethanol to the salmon aqueous phase were of the order of 95% indicating minimal ethanol loss during handling. The vial was sealed and stored in the freezer until analysis.

A Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector and a 6 ft x 4 mm i.d. glass column packed with Porapak QS (100–120 mesh) operated at 166–170°C with a carrier gas (nitrogen) flow rate of approximately 50 ml/min was used for analysis. Injector and detector temperatures were 200°C and 250°C, respectively. Flow rates of detector gases were 43–46 ml/min hydrogen and 421-430 ml/min air. Approximately 5 μl of sample was injected for analysis. The ethanol peak area (R.T. $\approx$ 2.6 min) was quantitated by the external standard method. The calibration curve was constructed from standard solutions of ethanol in distilled water which encompassed the range of ethanol concentrations in the samples. The method of analysis was similar to that reported by Crosgrove (1978). To reduce accumulated contamination, the gas chromatographic column was routinely purged and cleaned with multiple injections of the ethanol standard solutions at the beginning and end of each day. An analysis turn around time of greater than 45 min was required due to late eluting peaks. Work is in progress to refine the method to further minimize the above problems.

GC/MS confirmation of ethanol

The presence of ethanol in canned salmon was confirmed in one sample of each of the four species by chemical ionization (methane) gas chromatography/mass spectometry on a VG 7070 double focusing mass spectrometer. A 30m DB-5 widebore capillary column at 150°C was used for analysis. The injector and source temperatures were 200°C and the methane pressure was 10^{-4} torr.

Sensory classification of sample

Immediately after the can of salmon was opened and the liquid phase was removed, the salmon remaining in the can was examined by sensory analysis and assigned to one of the three sensory classes of decomposition that are utilized by the Food & Drug Administration (Table 1).

RESULTS & DISCUSSION

THE RESULTS of the analysis of the canned salmon samples are shown in Table 2. A total of 154 samples were analyzed which included 46 samples of sockeye, and 36 samples each of pink, coho, and chum.

Table 1–Sensory classifications of decomposition

Class 1	Passable
This category includes fishery products that range from very fresh to those that contain fishy odors or other odors characteristic of the commercia product, not definitely identifiable as decomposition.	
Class 2	Decomposed (slight but definite)
The first stage of definitely identifiable decomposition. An odor Is present that, while not really intense, is persistent and readily perceptible to the experienced examiner as that of decomposition.	
Class 3	Decomposed (advanced)
The product possesses a strong odor of decomposition which is persistent, distinct and unmistakable.	

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To determine the degree of statistical significance and correlation between ethanol concentration and decomposition, both the Pearson's correlation and the nonparametric Spearman's correlation tests were run. Pearson's correlation gave $r = 0.7102$, $p < 0.001$ and Spearman's correlation gave $r = 0.7983$, $p = 0.001$ indicating a highly significant level of correlation between ethanol concentration and the degree of decomposition.

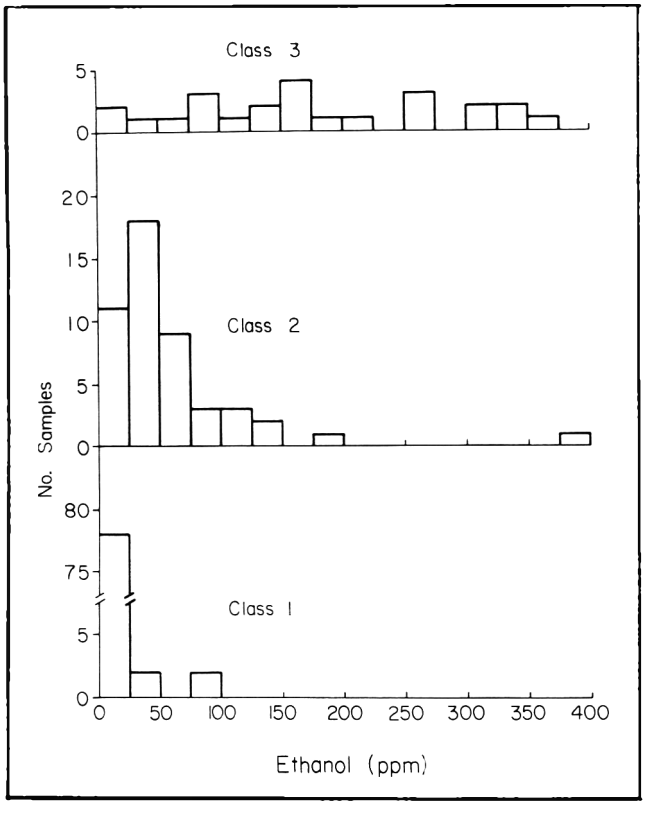

Fig. 1—Ethanol concentration vs sensory classification.

In order to graphically represent the distribution of ethanol concentration as a function of sensory class, the data from Table 2 were combined into the data presented in Fig. 1. For each sensory class, the number of samples found to contain ethanol within 25 ppm intervals are shown. Fig. 1 demonstrates that in general the ethanol content increases with increasing decomposition. The few cases of overlap observed between classes is not unexpected since seafood decomposition is a complex process affected by many factors such as type of bacterial contamination, the manner in which the fish was handled, and storage conditions, to name a few.

If the relationship between ethanol content and sensory classification of decompsition observed in this study is to be utilized in a practical way, guidelines must be set. Thus, if a sample is found to contain ethanol within a specified range, it will be considered as good evidence that the sample falls within the sensory classification determined by a sensory specialist.

The following tentative ranges were selected: sensory class 1, 0–24 ppm ethanol; sensory class 2, 25–74 ppm ethanol; and sensory class 3, 75 ppm ethanol and above. It is recognized that as more data relating the ethanol content in canned salmon to its sensory classification becomes available, these tentative ranges might have to be slightly modified. Applying these criteria to the data in Table 2 and Fig. 1, the following results are obtained. Of the 82 class 1 samples, 2 samples (2.4%) fall in the class 2 range of ethanol content and 2 samples (2.4%) fall in the class 3 range.

Table 2—Relationship between ethanol concentration (ppm) and sensory classification in various species of canned salmon

Sockeye[a]			Pink[b]			Coho[c]			Chum[d]		
Class 1	Class 2	Class 3	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
TR[e]	11	41	2	3	205	1	4	19	TR	22	90
TR	12	59	3	9	261	1	12	24	TR	31	115
TR	12	82	4	23	261	1	20	362	TR	32	126
TR	29	92	4	25	265	1	24		TR	39	161
TR	30	149	4	31	303	2	25		1	42	173
TR	32	151	5	32	324	2	27		1	58	184
TR	41	170	5	48	346	3	29		2	65	341
TR	48		7	54		4	36		2	92	
TR	55		9	57		4	45		3	113	
1	65		9	65		4	65		3	117	
1	72		10	92		4	377		3	123	
1	96		14	127		4			4	138	
1			21	196		6			6		
2			30			7			19		
2			32			10			23		
3			88			11			24		
3						11			86		
5						12					
6						12					
6						13					
6						15					
12						21					
12											
13											
15											
15											
22											

[a] Total number of canned sockeye salmon samples = 46
[b] Total number of canned pink salmon samples = 36
[c] Total number of canned coho salmon samples = 36
[d] Total number of canned chum salmon samples = 36
[e] TR = Trace (less than 1 ppm ethanol)

In the same manner, of 48 class 2 samples, 11 samples (22.9%) fall in the class 1 range and 10 samples (20.8%) fall in the class 3 range. Finally, of the 24 class 3 samples, 2 samples (8.3%) fall in the class 1 range and 2 samples (8.3%) fall in the class 2 range.

The fact that a particular chemical indicator of decomposition such as ethanol is not present in a sample in the amount that confirms an initial classification by an experienced sensory analyst does not necessarily mean that the initial classification is incorrect, since, as stated previously, seafood decomposition is a complex process. Thus, if the amount of ethanol found in a sample is less than the amount needed for confirmation of an initial classification, this classification could be confirmed by a second sensory analyst. Using the data and criteria here, this situation occurred with 11 of the 48 class 2 samples (22.9%) and with 4 of the 24 class 3 samples (16.7%).

The other possibility is that the amount of ethanol found in a sample is greater than the amount needed for confirmation of an initial classification. Again, using the data and criteria presented here, this occurred with 4 of the 82 class 1 samples (4.9%) and with 10 of the 48 class 2 samples (20.8%).

The fact that only 4.9% of the class 1 samples contained levels of ethanol above the selected criterion is important since this indicates a high level of reliability for this criterion and it is possible that these samples were misclassified. Class 2 samples containing amounts of ethanol greater than the 25–74 ppm range would be interpreted as confirmation that these samples exhibit class 2 decomposition at a minimum.

In conclusion, there appears to be a useful relationship between the ethanol content of the aqueous phase of canned salmon and the extent of decomposition. Furthermore, it would appear that this relationship can be used in a practical way to confirm the initial sensory classification.

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Sorption Isotherms and Drying Rates for Mullet Fillet and Roe

K. V. CHAU, J. J. HEINIS, and M. PEREZ

ABSTRACT

Desorption isotherms at 25°C and 43°C and adsorption isotherms at 25°C were determined for salted and unsalted female roe of mullet (*Mugil cephalus*). Isotherms for ground white muscle of mullet were also determined for desorption at 25°C, 43°C, and 54.5°C, and for adsorption at 25°C. Adsorption-desorption hysteresis was evident for the sigmoid shaped isotherms. At 45°C and 15% RH, and also at 45°C and 25% RH, mullet fillets dry almost four times faster than mullet roe. An air temperature lower than 45°C should be used to obtain an acceptable color for the roe.

INTRODUCTION

FISHERY PRODUCTS are a good source of protein and high quality foods. However, many fish species such as mullet are underexploited. Striped mullet (*Mugil cephalus* Linnaeus) is an abundant fish that can be found in coastal waters and estuaries throughout the tropical and subtropical areas (Furch, 1976). In the United States, the State of Florida is by far the largest producer of mullet, with an annual catch of approximately 15,000 tons (Cato et al., 1976).

Roe from mullet is a product which is widely consumed in the Orient as a delicacy commanding a high price. At present, the roe is either shipped within whole sexually mature fish or separated from the fish and packed in a polyfilm bag and shipped separately to the processing nation of Taiwan or Japan (Dougherty, 1979). Once in the processing nation, the roes are removed, salted, desalted and presspiled to give a uniform surface. After sun drying for up to 10 days on boards which are shielded from direct sunlight by bamboo blinds, the process yields a product called karasumi (Asano, 1980; Tanikawa, 1971). Generally the roe is sliced thin, warmed and eaten as an appetizer.

The University of Florida is conducting research on the utilization of solar energy to dry mullet fillets and mullet roe. However, while information on the drying characteristics of codfish and other products are available (Cooper and Noel, 1966; Legendre, 1955; 1961) there is very little information on mullet and especially mullet roe. The purpose of this investigation is to study the drying characteristics of mullet fillets and roe, specifically the rates of drying and the equilibrium moisture contents at different air temperatures and relative humidities, to support the solar drying research work now underway.

MATERIALS & METHODS

Equilibrium moisture content determination

Adsorption and desorption isotherms for mullet roe (salted and unsalted) and white fish muscle were found by placing the samples in wire baskets within glass jars holding 750 ml of sulfuric acid solutions of appropriate concentrations to provide the desired relative humidities in the jar. Constant temperatures were maintained by placing the jars within an insulated box heated with three strip heaters controlled at the set temperature by a proportional controller. A small fan circulated the air in the insulated box to assure a uniform temperature within the box. Samples were weighed daily until there was no weight loss or gain which took from two to four weeks. At the completion of the test, samples were removed for moisture determinations and the sulfuric acid solutions titrated against a standard base to determine final relative humidities of the air inside the jars.

Solution preparation. Sulfuric acid solutions were selected for these tests since they offer several advantages over saturated salts. They are less sensitive to slight changes in temperature, their vapor pressures are known for a wide range of temperatures, they can be easily titrated against a standard base to determine their concentration and hence final relative humidity, they are simple to prepare and are low in cost. Titrations of the sulfuric acid solutions were made against a standard sodium hydroxide solution using phenophthalein as an end-point indicator. Once the acid concentration was known, the vapor pressure inside the jar was readily obtained from a table presented by Perry (1963) from the data of Greenwalt (1925). The relative humidity was then calculated from the vapor pressure and drybulb temperature using standard psychrometric equations.

The final equilibrium moisture contents presented in this paper are the average values of three replications.

Sample preparation. Mullet roe were prepared according to the method of Hsu (1977) which is similar to the traditional Japanese method. Roe was removed from the freezer (−35°C) thawed under cold running water and dry salted. Salting was on a 15% weight to weight basis and the roe cured for 8.5 hr and desalted for 6 hr in cold water. Roe samples were 15g cross-cut slices.

White fish muscle was prepared by thawing the whole mullet under cold running water, filleting the fish and separating the dark muscle. The white muscle was then ground and 15g samples placed in wire baskets for the equilibrium moisture determinations.

Moisture contents were determined by the vacuum oven method (24 hr at 70°C, 20 mm Hg).

Drying tests

The facility used for the drying experiments, shown in Fig. 1, can control the air temperature, relative humidity and air flow rate accurately. The spray chamber saturates the air to the desired dewpoint temperature, and the electric heater heats the air to the desired dry-bulb temperature. Inside the spray chamber, an arrangement of horizontally stretched plastic filaments was used to produce a good contact between the air and the spray water. A temperature controller regulates the cold water pump to maintain the desired water temperature in the mixing tank, and another controller regulates the electric heater. A gate valve controls the air flow through the system.

Mullet fillets and roe were placed on a 25 cm by 25 cm tray inside the drying chamber. The fillet samples used were slabs of mostly white muscle with skin but no scales, 7.5 cm long, 5 cm wide, and 1.25 cm thick. The roe samples were complete roe (2 lobes). 36-gauge copper constantan thermocouples were inserted under the skin and at the center of the roe and fillets to record the change in temperature in the samples. The samples were weighed periodically to determine their moisture loss. All the air, water, and samples temperatures were recorded by a data acquisition system. At the end of each test, the moisture content of the samples were determined by the vacuum oven method, at 70°C and 20 mm Hg, 24 hr.

RESULTS & DISCUSSION

Equilibrium moisture contents

In order to check the accuracy of the values of the rela-

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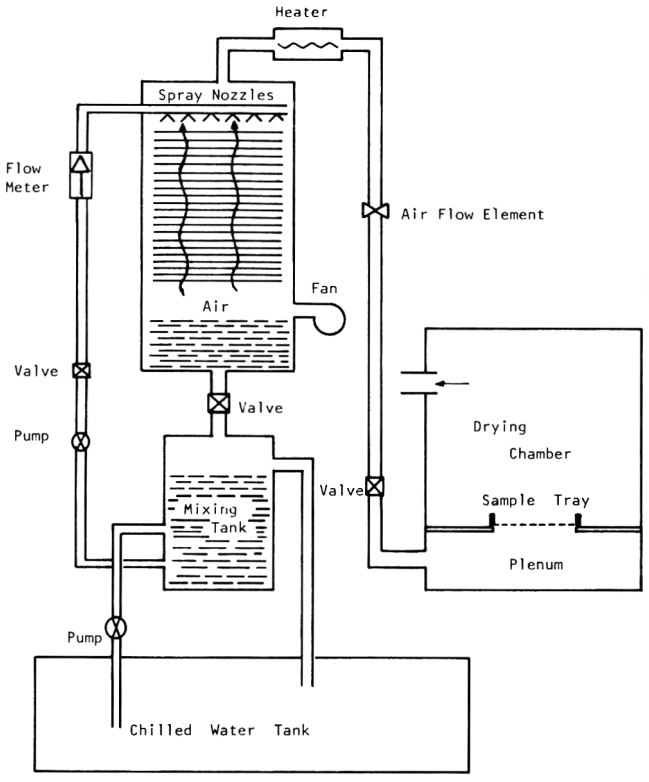

Fig. 1—Schematic diagram of the drying facility.

tive humidity of the jars as given by calculations based on the acid concentration and temperature, a General Eastern dew point hygrometer (mirror condensation type) was used to determine the dew points of the air inside three jars containing acid solutions at different concentrations. Values of dew point temperatures calculated from the acid concentrations were within 0.5°C of the values given by the General Eastern hygrometer.

Salted roe. Desorption isotherms were obtained for salted roe at 25 and 43°C. Higher temperatures were not used because roe are not expected to be dried at temperatures higher than 43°C due to excessive browning of the roe. Only one temperature (25°C) was used for the adsorption isotherm. In the desorption test at 25°C, molding occurred at relative humidities greater than 30% and at 43°C molding occurred only at relative humidities greater than 90%. In the adsorption test at 25°C, molding occurred at 94% relative humidity. The results of this test are presented in Fig. 2. Fig. 2 shows that at the same relative humidity, the equilibrium moisture content is lower at higher temperatures. It also shows that at the same temperature and relative humidity, the desorption equilibrium moisture content is higher than the adsorption moisture content (hysteresis effect). These characteristics are typical of food products.

Unsalted roe. Desorption isotherms at 25 and 43°C and adsorption isotherms at 25°C were also obtained for unsalted roe. In the adsorption test at 25°C, molding occurred at 95% RH. The results are presented in Fig. 3. Again, at the same relative humidity, the equilibrium moisture content is lower at higher temperatures, and the adsorption moisture content is lower than the equilibrium desorption moisture content at the same temperature and relative humidity. However, the differences are not as pronounced as in the case of salted roe.

When isotherms for unsalted roe are compared against those for salted roe, it can be seen that under similar con-

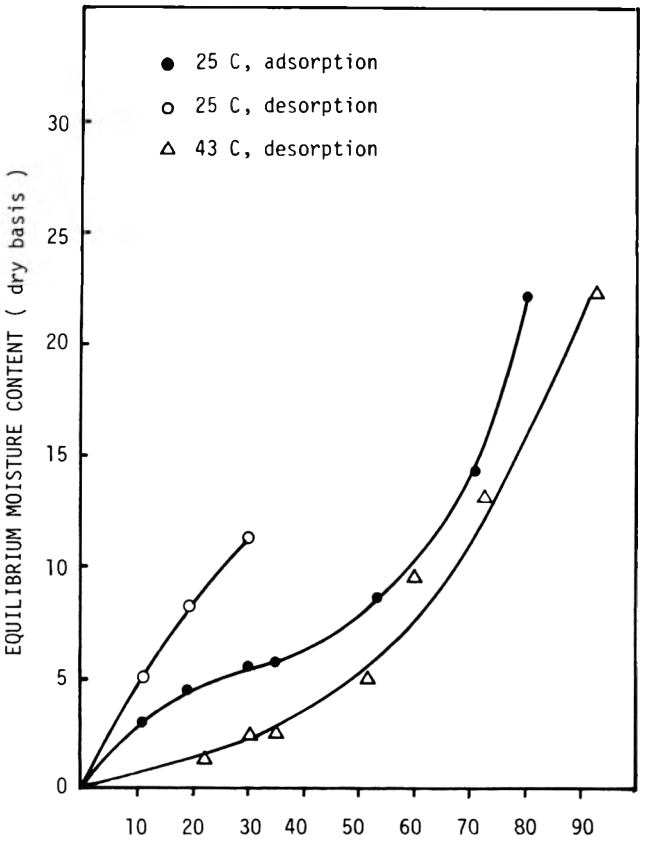

Fig. 2—Isotherms for salted mullet roe.

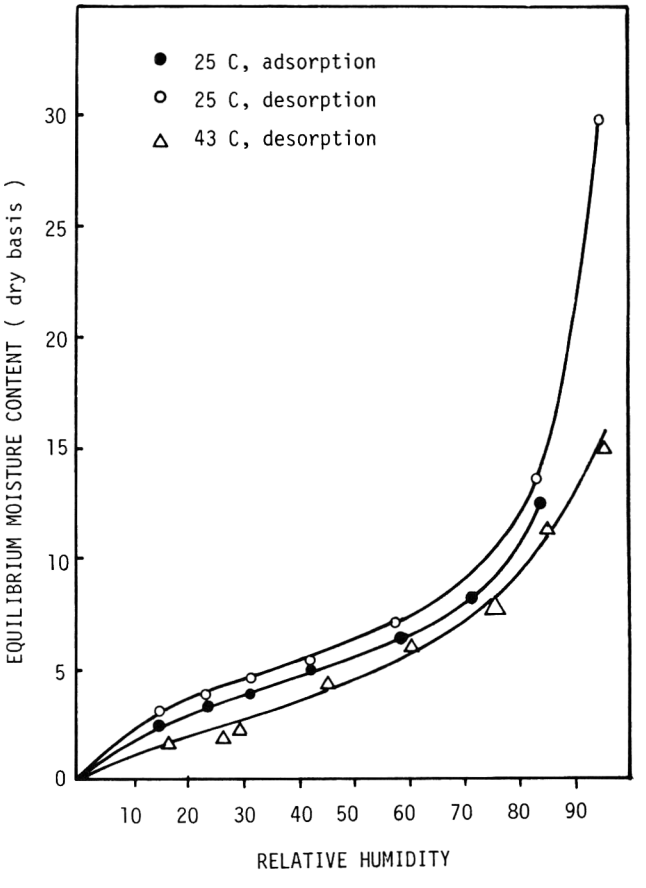

Fig. 3—Isotherms for unsalted mullet roe.

ditions of temperature and relative humidity, the unsalted roe always has a lower equilibrium moisture content than the salted roe. Gal and Bankay (1971) found a similar effect of NaCl on the equilibrium moisture contents of casein. They found that for any given relative humidity, the equilibrium moisture content of casein increases steadily with increasing amount of bound NaCl in the casein.

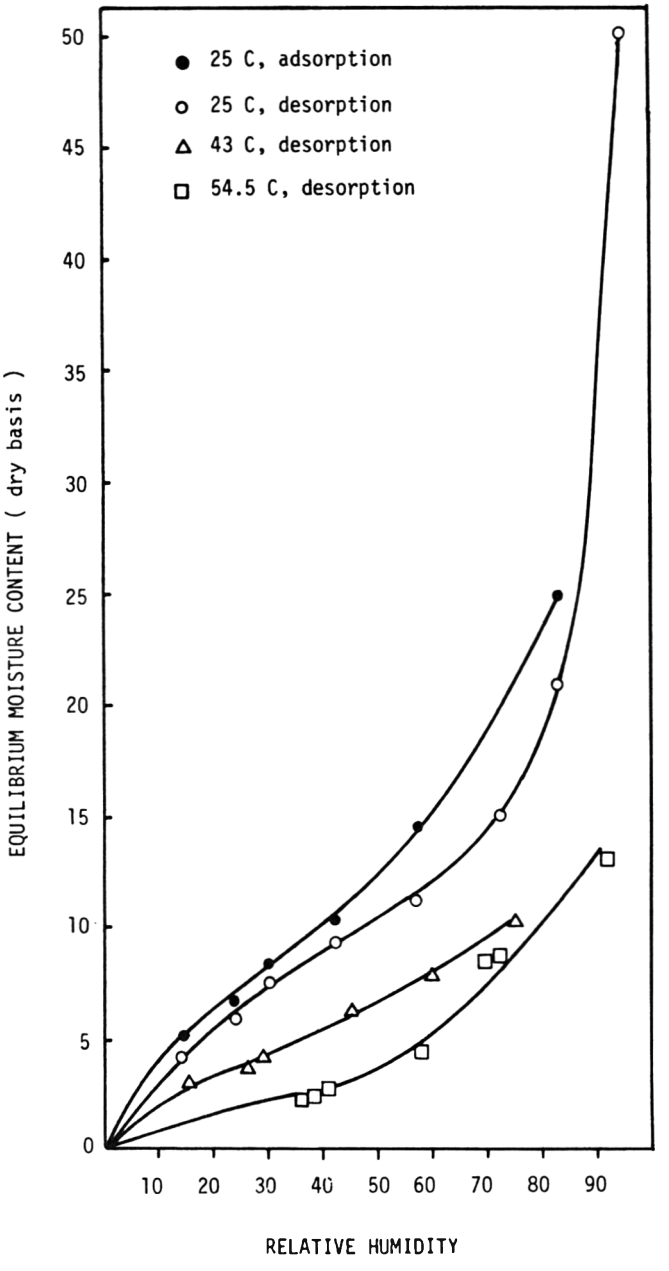

Fig. 4—Isotherms for ground white muscle of mullet.

Ground fish white muscle. Desorption isotherm experiments were conducted at 25, 43, and 54.5°C. The adsorption isotherm experiment was conducted at 25°C. These results are presented in Fig. 4. As expected, the isotherms are sigmoid shaped, and show the usual effect of temperature and the adsorption-desorption hysteresis.

Mold prevention. An attemp was made to prevent the growth of mold on the samples by spraying with 0.025% potassium sorbate in water as recommended by Hsu (1977). Although this level was successful in preventing molding of whole roe when dried in a mechanical drier, it led to total disintegration of the structure for the roe slices with the individual eggs separating from the ovarian membrane. Therefore, this method of sample preparation was not used in the isotherm determinations.

Drying tests

The fillets and roe were dried at 45°C, 15% RH and 45°C, 25% RH. The temperature of 45°C was chosen because it is thought to be the maximum temperature that the products can withstand without serious injury. The relative humidities used were those we can normally expect to have when ambient air is heated to 45°C, except on very hot, muggy days. Table 1 shows a summary of the test conditions. The roe sample 3 was kept on a separate tray placed in a corner inside the drying chamber, away from the heated air stream. This was done to see if the air flow rate has much effect on the drying rate of roe.

Fig. 5, 6, 7, 8, and 9 show the variation of the moisture ratio, (M-Mo)/(Mo-Me), with drying time for roe and fillets. M is the moisture content of the sample at any given time, Mo is the original moisture content, and Me is the equilibrium moisture content. All the moisture contents are on a dry basis. These five figures show that during the first several hours, the drying rates were rather fast, then followed by a falling-rate period when the drying rate was governed by the rate of moisture migration within the product. In the falling-rate period, the drying process may be represented by:

$$MR = \frac{M - Me}{Mo - Me} = Ae^{-kt}$$

where t is the drying time, and k is sometimes referred to as the drying constant; k is the slope of the straight line portion of the ln(MR) versus time curve, and A is the y-intercept. The average values of k and A for the roe and fillets tested are given in Table 1.

For mullet roe, the two replications for each test behaved very similarly as indicated by the closeness of the two sets of data points in Fig. 5 and 6. The drying rates at the two relative humidity levels were approximately the same

Table 1—Summary of drying tests

Air conditions	Air conditions: 45°C, 15% RH				Air conditions: 45°C, 25% RH				
	Roe		Fillet		Roe			Fillet	
Sample No.	1	2	1	2	1	2	3	1	2
Sample wt (g)	140.08	155.72	64.95	63.19	198.0	171.63	165.80	62.27	63.08
Initial MC (d.b.)	113.9	143.0	201.8	224.9	166.0	122.5	126.2	190.8	240.00
Final MC (d.b.)	20.4	18.4	4.2	4.3	20.8	21.1	18.0	4.8	3.9
Air flow (m^3/sec)	88.0	88.0	88.0	88.0	88.0	88.0	0.0	88.0	88.0
Drying time (hr)	217.2	217.2	217.2	217.2	166.1	166.1	166.1	166.1	166.1
Average k	0.0208		0.0357		0.0226		0.0234	0.0365	
Average A	0.80		0.78		0.80		0.73	0.61	

as indicated by the values of A and k in Table 1. The drying rate at 25% RH was slightly faster; the moisture ratio reached a value of 0.1 (about 30% moisture content, dry basis) in 96 hr as compared to 100 hr in the case of 15% RH air. The difference is certainly small and can very well be due to factors other than the relative humidity.

The roe sample that was away from the air stream (sample 3) dried even faster than the two samples that were directly exposed to the air stream. The moisture ratio reached a value of 0.1 in only 85 hr (Fig. 7). This could

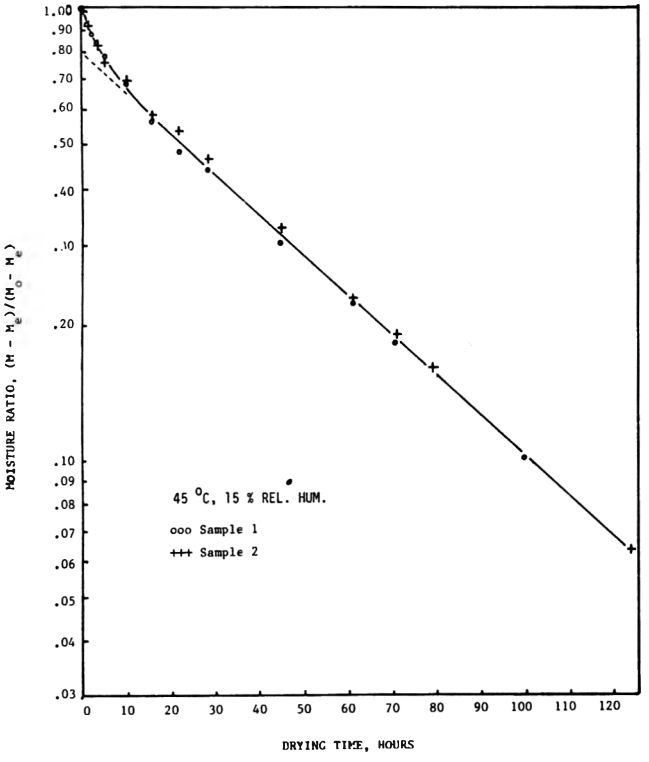

Fig. 5—Drying curve for mullet roe (45°C, 15% RH).

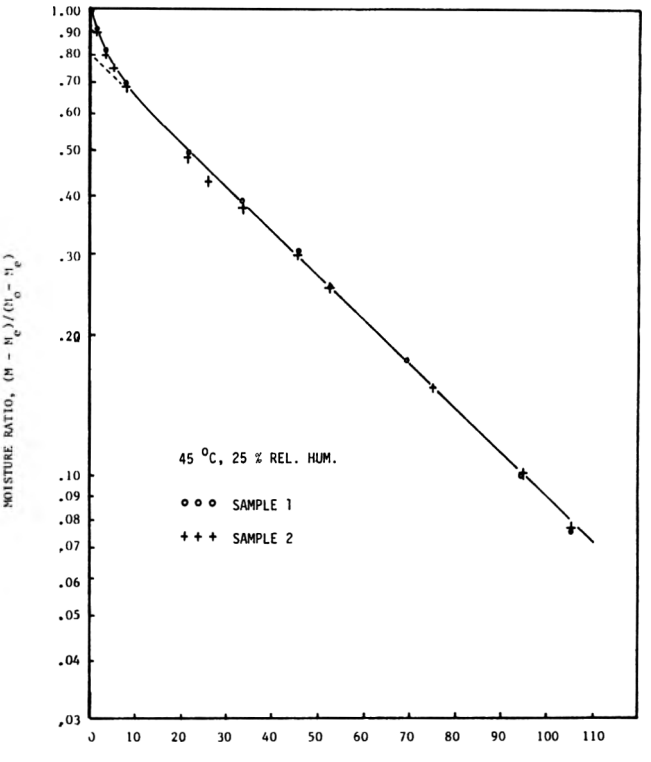

Fig. 6—Drying curve for mullet roe (45°C, 25% RH).

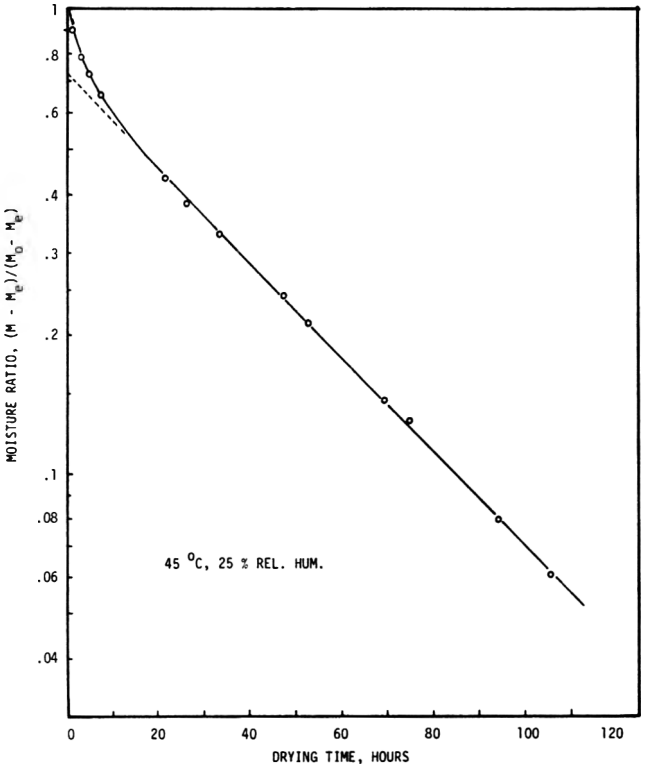

Fig. 7—Drying curve for roe sample #3 (still air).

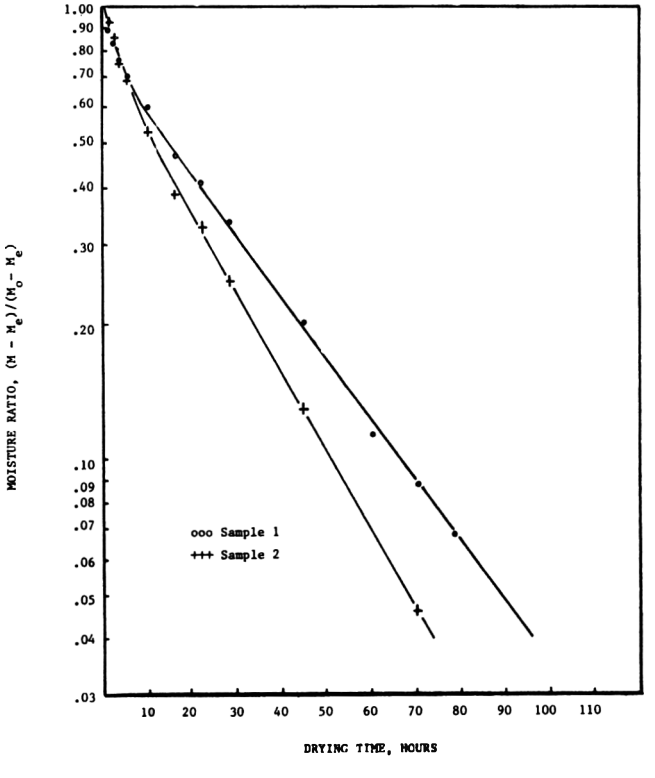

Fig. 8—Drying curve for mullet fillet (45°C, 15% RH).

be due to the difference in the roe themselves, especially in their fat content and salt concentration; it is also possible that the overian membranes of the roe that were exposed to the air stream hardened quickly and hindered the passage of the moisture through the membranes.

Fillets dried much faster than roe as indicated by their high k values. In the test with 45°C, 15% relative humidity (Fig. 8), the two samples dried at different rates. Since the two samples did not come from the same fish, the difference was most likely due to the difference in fat content or the relative freshness of the two samples. Fat contents have been shown to affect the water diffusion in fish muscle (Futch, 1976). The values for A and k listed in Table 1 were the average values for the 2 samples.

When compaing the drying rates between the two air conditions, the k values were similar (0.0357 vs 0.0365), but the A values were quite different (0.78 vs 0.61). indicating a marked difference in drying rate during the beginning drying phase. The moisture ratio reached a value of 0.1 (about 25% moisture content, dry basis) in 58 hr at 15% RH but only in 50 hr at 25% RH.

When comparing roe with fillets, fillets dry about 4 times faster than roe. Fillets also have much higher initial moisture contents, over 200% dry basis compared to about 125% for roe.

Fig. 10 shows the temperatures inside the fillet and roe. The temperature under the ovarian membrane of the roe approached the air temperature very quickly, within a few hours, whereas the surface temperature of the fillet remained several degrees lower. This was due to the higher evaporative cooling of the fillet which dried at a much faster rate, especially at the beginning of the drying phase. The temperatures at the center and under the ovarian membrane of the roe become equal after 20 hr.

One important quality control parameter of interest in roe drying is the final color. Premium prices are paid for roe with a bright, yellow-brown color. Color development is due to both Maillard browning (between the reducing sugars and free amino groups of lysine) and to lipid oxidation. Free radicals derived from peroxide degradation can then interact to form brown polymeric compounds. Both reactions are highly dependent on the water content and product temperature.

In the first 22 hr of drying at 15% RH, the roe developed a shiny, glossy pellicle. Browning began on the tips and the browning continued toward the center.

Similarly, color development at 25% RH followed the same pattern. Browning was slightly less extensive and continued evenly over the entire surface. The color of the roe for the conditions tested was marginal at best. Lower air temperature and possibly higher relatively humidity should be used to obtain a product similar to sun-dried roe currently sold in the Orient.

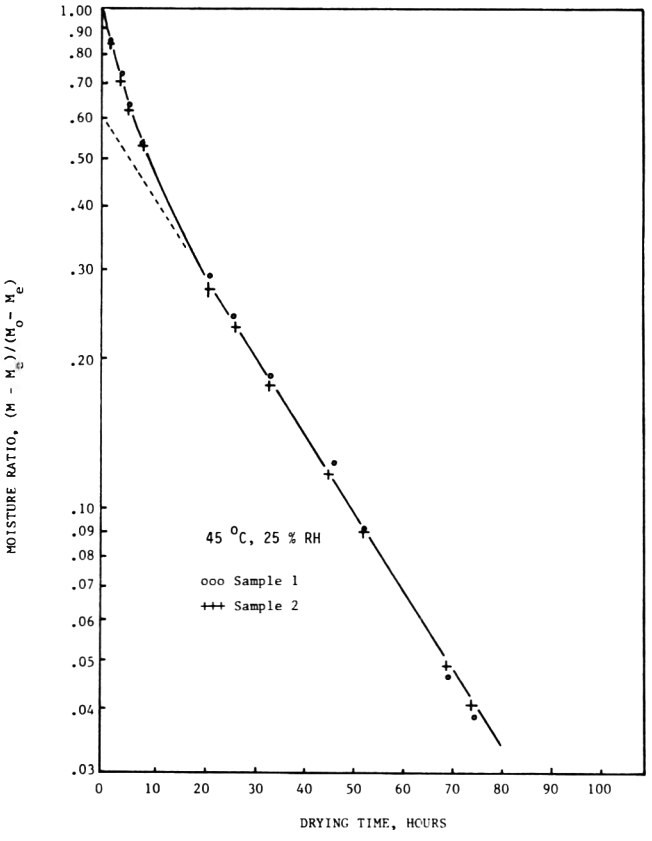

Fig. 9—Drying curve for mullet fillet (45°C, 25% RH).

SUMMARY

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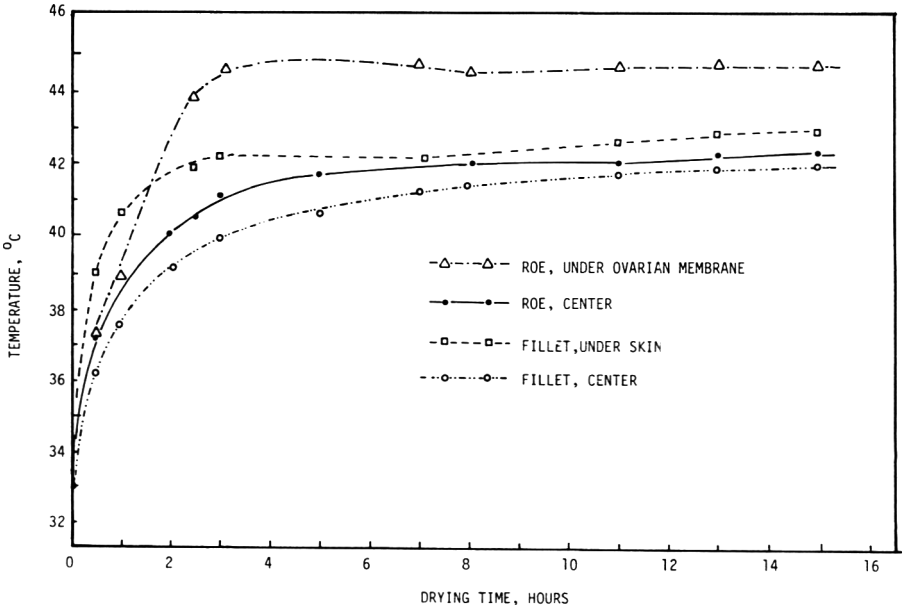

Fig. 10—Temperatures inside fillet and roe.

Functional Properties of Guar Seed (Cyamopsis tetragonoloba) Meal Detoxified by Different Methods

RAZIA TASNEEM, S. RAMAMANI, and N. SUBRAMANIAN

ABSTRACT

Functional properties of guar meal detoxified by autoclaving, extraction with aqueous ethanol, methanol, isopropanol or dilute hydrochloric acid were compared with those of defatted soy meal. Acid washed and the autoclaved meals gave low nitrogen solubility (NS) at pH 7.0. Detoxified guar meals had greater water absorption capacity (WAC) than the raw meal, while defatted soy meal was far superior to defatted guar meal. Fat emulsification capacity (EC) showed a similar trend as NS and fat absorption capacity (FAC) inversely correlated with the bulk density of samples. Autoclaved guar meal had higher EC and FAC than the alcohol or acid extracted meals. The foam capacity (FC) and foam stability (FS) of the 80% isopropanol extracted guar meal were the highest among the samples.

INTRODUCTION

DATA on the properties of the total guar seed proteins, isolation and characterisation of the major globulin fraction, and the functional properties of defatted guar meal and protein isolate have been reported earlier from this laboratory (Nath et al., 1978, 1980; Nath and Narasinga Rao, 1981).

Guar meal, the by-product of the guar gum industry, is rich in protein (~55%) but has some toxic and anti-growth principles. Heat treatment of the meal, or selective extraction with aqueous alcohols or dilute HCl has been reported to improve the nutritional value of the meal. However, the protein isolated from the meal by conventional alkali-extraction and isoelectric precipitation was reported to be more toxic than the meal (Subramanian and Parpia, 1975; Khopkar, 1976). Since the detoxification procedures have an effect on the overall composition as well as on proteins of the meal (Nath et al., 1981) it was of interest to study the functional properties of the meal detoxified by different methods. Data on nitrogen solubility, water and fat absorption capacity, foaming and emulsification properties of detoxified guar meals are presented in this paper.

MATERIALS & METHODS

Commercial grade (Durgapur Safed) guar seeds were procured from a guar gum industry.

Raw guar meal and defatted guar meal

Eighty kg of the seeds were split in a plate mill and the protein rich germ fraction was separated from gum splits and fines by sieving, winnowing, air classification and finally hand picking. The germ fraction constituted 45% of the seed and accounted for 90% of the guar seed protein. The germ was equilibrated to 20% moisture for 2 hr, flaked to 0.2 mm thickness by passing through twin flaking rolls and then dried in a current of air (25°C). The flakes were extracted with food grade hexane (five successive extractions by steeping) and dried in a current of air (25°C). The defatted flakes were further processed to obtain the detoxified meals as detailed in the following.

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(a) Defatted guar flakes were extracted by steeping over night in different aqueous solvents such as 80% ethanol, 70% methanol or 80% isopropanol separately (five successive extractions by steeping), and dried in a current of air (25°C) overnight.

(b) Extraction of the defatted flakes with 1N HCl was carried out by steeping the flakes in the acid for 30 min (the flakes to solvent ratio being 1:5). The acid extract was decanted, the residue washed free of acid using tap water and testing the pH of the washings. The washed meal was dried in a current of air (25°C). 0.25N HCl extraction of defatted flakes was carried out at 60°C for 4 hr and the residue washed free of acid and dried as above.

(c) The defatted flakes were autoclaved at 1 kg/cm^2 steam pressure (120°C) for 20 min and dried in a current of air (25°C).

Defatted soybean meal, used for comparison, was obtained from Bragg variety of soybean, after dehulling, flaking and defatting with hexane.

All the treated guar flakes and defatted soybean flakes were powdered to pass through 36 mesh (BSS). The powdered flakes were analyzed for moisture, total nitrogen, crude fat and total ash by the standard AOAC procedures (AOAC, 1975). The yield data and the chemical composition (on dry basis) are presented in Tables 1 and 2.

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Table 1—Yield data of processed guar meals and solids and nitrogen extracted by aqueous acid and alcohols

Detoxification method	% yield	Solids extracted g/100 g flakes	Nitrogen extracted g/100 g flakes	Nitrogen as % of extracted solids
1N HCl extraction	31.7	68.3	4.09	5.99
0.25N HCl extraction	45.0	53.7	3.40	6.33
80% ethanol extraction	79.5	22.7	0.41	1.80
70% methanol extraction	72.8	24.6	0.46	1.87
80% isopropanol extraction	82.2	18.1	0.38	2.10
Autoclaving	100.0	–	–	–

Table 2—Proximate composition of raw and processed guar meals

Sample	Protein (Nx6.25)	% Fat	% Ash	% carbohydrate (by diff.)
Raw guar meal	60.0	9.6	5.7	24.7
Defatted guar meal	68.9	0.8	6.0	24.3
1.00N HCl extracted guar meal	79.8	1.0	1.7	17.5
0.25N HCl extracted guar meal	82.4	1.1	2.2	14.3
80% Ethanol extracted guar meal	81.4	0.2	5.8	12.6
70% Methanol extracted guar meal	84.3	0.3	4.4	11.0
80% Isopropanol extracted guar meal	77.2	0.2	5.6	17.0
Autoclaved guar meal	67.8	0.7	5.9	25.6

Nitrogen solubility (NS)

To 2g of meal 20 ml of distilled water were added and the pH of the suspension adjusted to the desired value by adding 1N HCl or 1N NaOH. The suspension was then shaken for 1 hr at room temperature (25°C), centrifuged at 4000 rpm for 20 min and the pH of the supernatant noted. Aliquots of 10 ml were taken for nitrogen estimation by the Kjeldahl method. Similarly NS in 1M NaCl was also measured in the pH range 1–11.

Bulk density

The method of Wang and Kinsella (1976) was used to measure the bulk density of the powdered samples.

Water absorption capacity (WAC)

The WAC was determined by the method of Sosulski (1962) and is expressed as the amount of water (g) retained by 100g of the residue or 100g of protein in the residue.

Fat absorption capacity (FAC)

This was determined by the method of Sosulski et al., (1976) and expressed as the amount of oil (g) bound by 100g of the material or 100g protein.

Emulsification capacity (EC)

The method of Beuchat et al., (1975) was used and EC expressed as ml of oil emulsified by one g of protein. The amount of protein used was kept constant for these measurements. EC was also determined as a function of pH and NaCl concentration.

Foam capacity (FC) and foam stability (FS)

A known amount of the material equivalent to 2.77g protein was taken along with 100 ml of distilled water in a Braun electric blendor. The suspension was whipped at 1600 rpm for 5 min, the mixture poured into a 250 ml measuring cylinder and the volume was recorded after 30 sec. FC was expressed as percent increase in volume (Lawhon et al., 1972) and was determined as a function of pH and NaCl concentration.

$$FC = \frac{\text{Vol. after whipping} - \text{Vol. before whipping}}{\text{Vol. before whipping}} \times 100$$

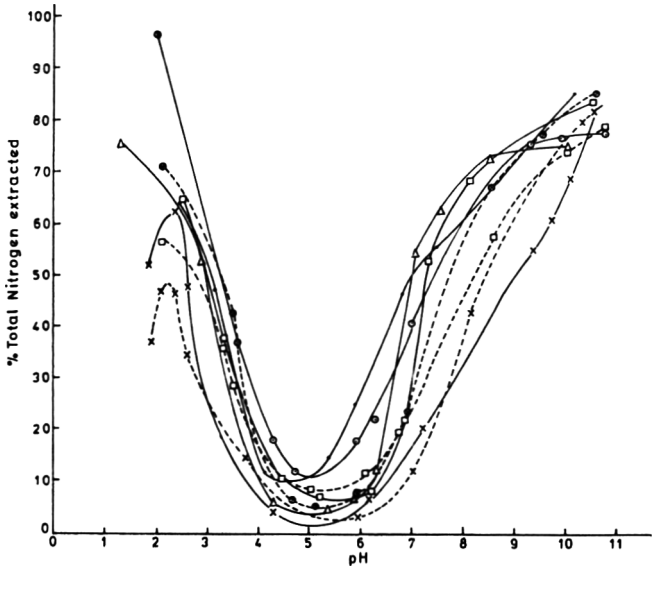

Fig. 1—Nitrogen solubility of detoxified guar meals in water: ● — ● Defatted soymeal; ○ - - - ○ Raw guar meal; ⊖ — ⊖ Defatted guar meal; □ - - - □ Autoclaved guar meal; x — x 1.00N HCl extracted guar meal; x - - - x 0.25N HCl extracted guar meal; □ — □ 80% ethanol extracted guar meal; Δ — Δ 70% methanol extracted guar meal; ⊕ - - - ⊕ 80% isopropanol extracted guar meal.

The foam volume was recorded 2 hr after whipping to determine FS (Ahmed and Schmidt, 1979).

$$FS = \frac{\text{Foam vol. after 2 hr}}{\text{Initial foam vol.}} \times 100$$

All measurements were made in duplicate and the average of the two values was taken.

RESULTS & DISCUSSION

YIELD DATA of the variously processed guar meals and the solids extracted by dilute acids or alcohols are given in Table 1. It is seen that the yields of acid extracted meals are very low being 31.7 and 45.0%, respectively, for 1N and 0.25N HCl treatments; also, the total nitrogen extraction is very high. The extraction with aqueous alcohols gives good yields (73–82%) of the detoxified meal. The composition of the detoxified meals is given in Table 2. All values have been expressed on a dry weight basis. The crude protein content (N x 6.25) of the processed meals varied from 67.8% to 84.3% whereas for the raw meal it was 60%. The higher concentration of protein in the extracted meals is due to the leaching out of soluble sugars, saponins and free amino acids. The acid extracted meals had a higher fat content but lower ash content than the alcohol-extracted meals.

Nitrogen solubility

The effect of pH on NS of the variously processed guar meals is presented in Fig. 1. All the samples gave a U-shaped curve in the pH range 1–11 with minimum solubility around pH 5.0, which is similar to many oil seed and legume flours (Lawhon et al., 1972). The solubility profile of guar proteins in water and NaCl was reported to be similar to those of soy and groundnut proteins (Nath et al., 1978). Defatted guar meal gave a value of 41% at pH 7 which is lower compared to 50% for the defatted soybean meal. The autoclaved guar meal gave a value of 24% at pH 7 which is lower than that of defatted guar meal. This observation is in agreement with the report on heat treatment of soy meal (Wu and Inglett, 1974). Unlike in other cases the acid extracted meals gave a peak in the pH range 2.0–2.5. These meals gave lower NS at all pH values compared to other samples, the values at pH 7 being 12% and 17%, respectively, for 0.25N and 1N HCl extracted meals. Among the

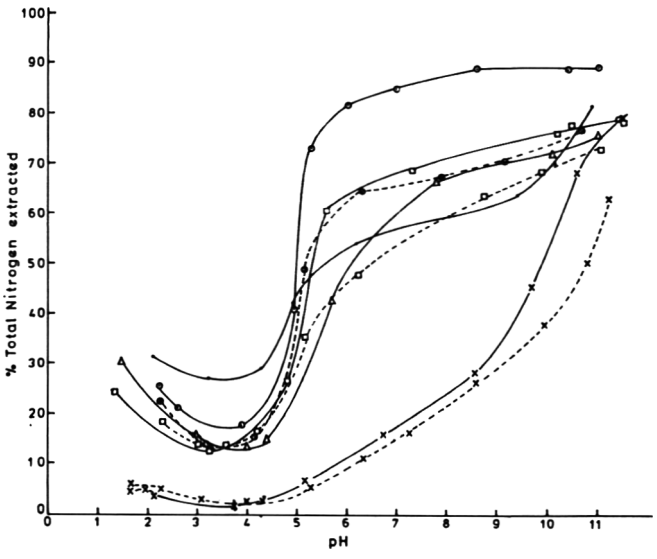

Fig. 2—Nitrogen solubility in 1M Nacl. Symbols same as Fig. 1.

alcohol extracted samples, the methanol extracted meal gave the highest solubility of 50% compared to 30% for ethanol extracted meal and 26% for isopropanol extracted meal at pH 7. It has been reported that soymeal proteins are denatured by aqueous alcohol extractions and the 70% methanol extracted meal has a much lower NS than the 80% ethanol or isopropanol extracted meals (Smith et al., 1951). Also acid extracted soy protein concentrates have been reported to have higher NS than alcohol extracted concentrates (Meyer, 1969). The results of this study on guar meal are, however, not in agreement with the above observations. Thus the mode of denaturation of proteins by aqueous acid or alcoholic solvents in relation to NS appears to differ among seed proteins.

Effect of 1M NaCl on nitrogen solubility

In the presence of 1M NaCl all samples showed lower NS below the isoelectric pH while the values were much higher in the neutral and alkaline range (Fig. 2). The lower solubility in the acid range may be due to the salting out effect of NaCl. Defatted guar meal had higher NS than soybean meal at pH 5. The NS values at pH 7 of the detoxified samples except acid extracted meals were improved in 1M NaCl; the defatted meal gave the highest value of 85%. Aqueous alcohol extracted samples gave values ranging from 60–68% and the autoclaved meal 53%. Acid extracted meals gave very low NS of about 15%. The isoelectric point of the proteins was shifted to the pH range 3.5–4.0 and such shift has been reported earlier (Nath et al., 1978). The acid extracted guar meals had lower NS than the raw or alcohol extracted meals.

Water absorption capacity

The WAC values are presented in Table 3. Since a considerable amount of soluble solids including protein was extracted in the procedure, the solids and protein leached out in the extracts were determined and corrections applied. The WAC values thus represent the moisture absorbed by the residual material and the protein present in it. 1N and 0.25N HCl extracted meals gave higher values of 325 and 295 per 100g, respectively, while all other guar samples gave lower values ranging from 147–268g/100g. This could be due to factors such as: (a) the acid extracted samples represent only a minor portion of the whole meal due to considerable loss of solids and protein, and (b) the proteins in the residue are also denatured as evident from the NS pattern.

Autoclaving of the defatted guar meal did not alter the WAC as the values are comparable. Thus none of the detoxification treatments decreased WAC; if anything they only increased the value. Defatted soy meal gave a value of 312/100g residue. When the WAC values were calculated on protein basis, defatted soy meal gave the highest value of 654/100g. Among the guar meals, 1N HCl extracted meal gave a value of 449, while the methanol extracted sample and 0.25N HCl extracted meal gave comparable values of 383 and 376, respectively. The value for defatted guar meal was 297; this differed from the earlier results of Nath and Narasinga Rao (1981) who reported a value of 552. This could be due to (a) corrections made for the soluble solids and protein in calculating WAC values in the present study, and (b) complete removal of gum from the meal. Guar gum which is an impurity in the meal is intensely hydrophillic and binds more water (Goldstein et al., 1973). The varietal differences in guar seeds used in the two studies might also contribute to the variations. From the table it is seen that WAC values do not follow the same order as the protein content. This suggests that apart from protein, the other constituents of the meal also bind water.

Fat absorption capacity

FAC has been attributed to physical entrapment of oil (Kinsella, 1976). The FAC data on the variously detoxified guar meals along with the bulk density of the samples are presented in Table 4. Defatted soy meal gave the highest value of 132 g/100g sample compared to 109 for raw guar meal and 101 for defatted guar meal. Among the detoxified samples, 70% methanol extracted meal had a better FAC of 111 compared to others (87–97). FAC expressed on protein basis gave a value of 179 for raw guar meal which was higher than that of defatted meal (134) as well as the autoclaved meal (125). The values for other samples were in the range 97–119. FAC was found to have an inverse correlation with the bulk density of the samples. Thus, the defatted soy meal having the lowest bulk density of 0.476 g/ml had the highest FAC of 262 g/100g protein. Defatted guar meal and the autoclaved meal had higher bulk density and lower FAC compared to the raw guar meal. Among the alcohol extracted samples the methanol extracted meal had the highest FAC (119) and the lowest bulk density (0.645). Both the acid extracted samples gave comparable values for FAC and bulk density. Dench et al. (1981) have shown a similar relationship between FAC and bulk density for sesame and soy meals and protein isolates. It has been reported that alfalfa leaf protein after extraction with ace-

Table 3—Water absorption capacity of soy and guar meals

Sample	Protein content of the residue %	Water absorption g/100g Residue	Water absorption g/100g Protein
Defatted soybean meal	47.7	312	654
Raw guar meal	63.2	147	232
Defatted guar meal	73.3	218	297
1.00N HCl extracted guar meal	72.5	325	449
0.25N HCl extracted guar meal	78.4	295	376
80% ethanol extracted guar meal	77.3	259	335
70% methanol extracted guar meal	69.9	268	383
80% isopropanol extracted guar meal	74.8	244	326
Autoclaved guar meal	72.4	216	299

Table 4—Fat absorption capacity of soy and guar meals and the bulk density of samples

Sample	Fat absorption g/100g sample	Protein content %	Fat absorption g/100 g protein	Bulk density g/ml
Defatted soybean meal	132	50.5	262	0.476
Raw guar meal	109	60.0	179	0.540
Defatted guar meal	101	68.9	134	0.656
1.00N HCl extracted guar meal	97	79.8	109	0.667
0.25N HCl extracted guar meal	97	82.4	104	0.702
80% ethanol extracted guar meal	87	81.4	97	0.690
70% methanol extracted guar meal	111	84.3	119	0.645
80% isopropanol extracted guar meal	94	77.2	111	0.714
Autoclaved guar meal	90	67.8	125	0.714

tone showed an increase in bulk density (2–3 fold) and a decrease in FAC by 50% compared to the controls (Wang and Kinsella, 1976). In the present investigation the FAC value of 179/100g protein for raw guar meal decreased after extraction with hexane, alcohols or acids and also after autoclaving.

Emulsification capacity

EC of the guar meals as affected by pH (2–11), is presented in Fig. 3. The pH-EC profiles have a similar trend to that of pH-NS profiles (Fig. 1). The EC values (ml oil/g protein) were minimum at the pH of minimum solubility for the different samples. It is interesting to note that the defatted guar meal had the highest EC among the samples at the pH of minimum NS. The acid extracted meals gave low EC values at all pH values. At pH 7, the EC value for methanol extracted meal was much lower (54), compared to that of isopropanol or ethanol-extracted meal (75 and 82, respectively); the raw and defatted guar meals as well as the autoclaved meal gave comparable EC values in the range 87–90. Even though autoclaving reduced NS, it did not affect EC. McWalters and Holmes (1979) have also observed that higher levels of NS were not necessarily associated with maximum EC in the case of autoclaved soy and peanut flours.

Effect of NaCl on EC

Fig. 4 presents the effect of NaCl on EC. In all cases the samples were taken in distilled water containing the required levels of NaCl and no adjustment of pH was made. The pH of the solutions was in the range 6–6.5. For soy meal the highest EC was at 0.4M NaCl which was higher than that for defatted guar meal. Nath and Narasinga Rao (1981) had also found that the EC of the defatted guar meal differed from that of soy meal. It is seen that the pattern obtained for soy meal was somewhat different from that for guar meals. With increasing concentration of NaCl up to 0.4M, soy meal showed increased EC while, in the case of guar meal EC was maximum at 0.8M NaCl. The highest value of 97 was given by defatted guar meal in the presence of 0.8M NaCl, while soy meal gave a value of 93 at 0.4M NaCl. EC is known to increase with moderate salt concentration because of salting in of proteins. At higher salt concentration, the EC does not increase as there is likely to be salting out of proteins. The results are in conformity with the findings on other proteins (Hegarty et al., 1963; Swift and Sulzbacher, 1963; Carpenter and Saffle, 1964; Wang and Kinsella, 1976; Ramanatham et al., 1978).

Foam capacity

The FC-pH profile of the samples is presented in Fig. 5. In general the FC-pH pattern was similar to NS-pH profiles, the values being low in the pH range 4.5–6.5. Defatted guar meal was far superior to defatted soy meal as well as raw guar meal. The present findings differ from the earlier report of Nath and Narasinga Rao (1981) who found that guar meal had lower FC than defatted soy meal in the pH range 4.5–6.5. Autoclaved guar meal had a slightly increased FC in the region of minimum solubility as compared to defatted guar meal. Acid extracted samples gave the lowest FC among the detoxified samples. Among the alcohol washed meals, isopropanol extracted sample showed the highest FC at all pH values and the methanol extracted sample had lower FC than ethanol extracted sample. A striking feature of the results is that the isopropanol extracted sample gave the highest FC of 95 at pH 4.7 compared to 20–80 for other samples. Alcohol treatment of soy meals or soy proteins is known to alter their functional properties (Eldridge et al., 1963).

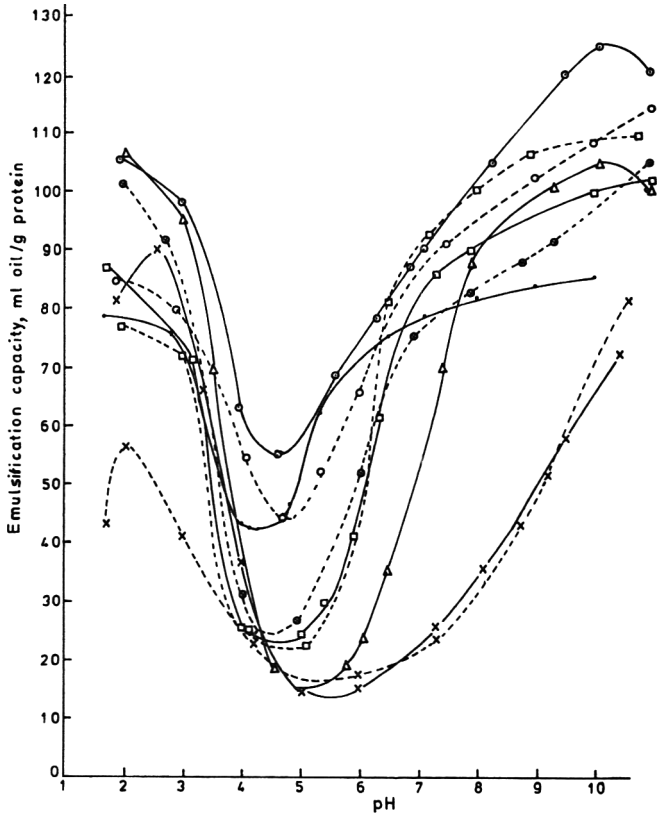

Fig. 3—Effect of pH on emulsification capacity. Symbols same as Fig. 1.

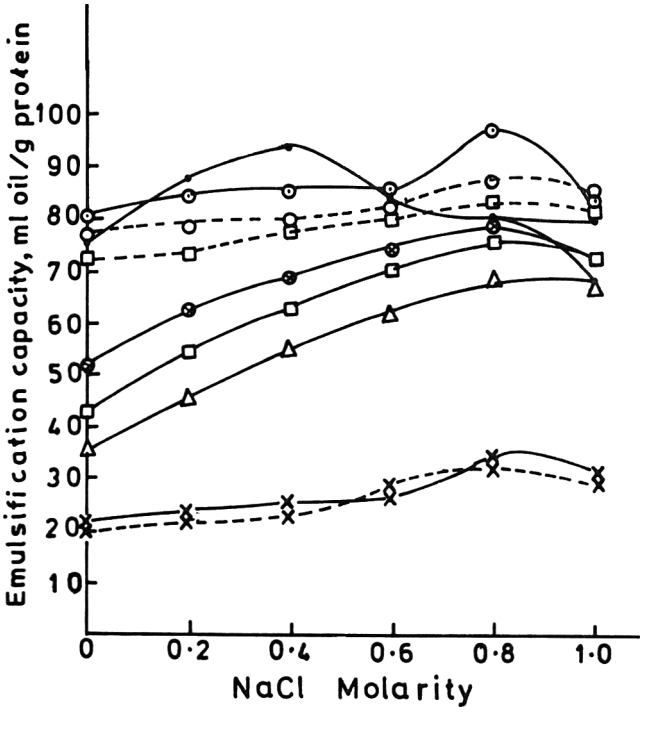

Fig. 4—Effect of NaCl concentration on emulsification capacity. Symbols same as Fig. 1.

Effect of NaCl on foam capacity

The effect of NaCl on FC (Fig. 6) indicates that there is a beneficial effect up to a point. In general, increase in FC was observed in the range 0.2–0.6M NaCl concentration. The defatted guar mal gave appreciably higher values than the defatted soybean meal, but raw guar meal was inferior to soy meal. Autoclaved guar meal had a lower FC at all molarities compared to defatted meal. Among the alcohol extracted meals, the isopropanol extracted sample gave better response than the ethanol or methanol extracted sample. In the range 0–0.8M NaCl the FC of ethanol extracted meal gradually increased with a value of 124 at 0.8M; while for methanol extracted and isopropanol extracted meals the highest values were 152 and 95, respectively, at 0.6M NaCl. The values obtained for 0.25N HCl and 1N HCl treated guar meals were much lower at all molarities compared to the rest of the samples even though the FC increased with NaCl concentration up to 0.6M NaCl, the values being 60 and 66 ml, respectively.

Foam stability

The FS of the guar meals is presented in Fig. 7. The FS of defatted guar meal (44%) was better as compared to raw guar meal (33%), autoclaved meal (24%), and also soy meal (30%). Considerable differences were observed in the case of alcohol extracted samples. The isopropanol extracted sample had the highest foam stability (51%) and the methanol extracted sample gave a value of only 18%. The FS of 0.25N and 1N HCl extracted samples were appreciably lower, the values being only 3 and 6.5%, respectively.

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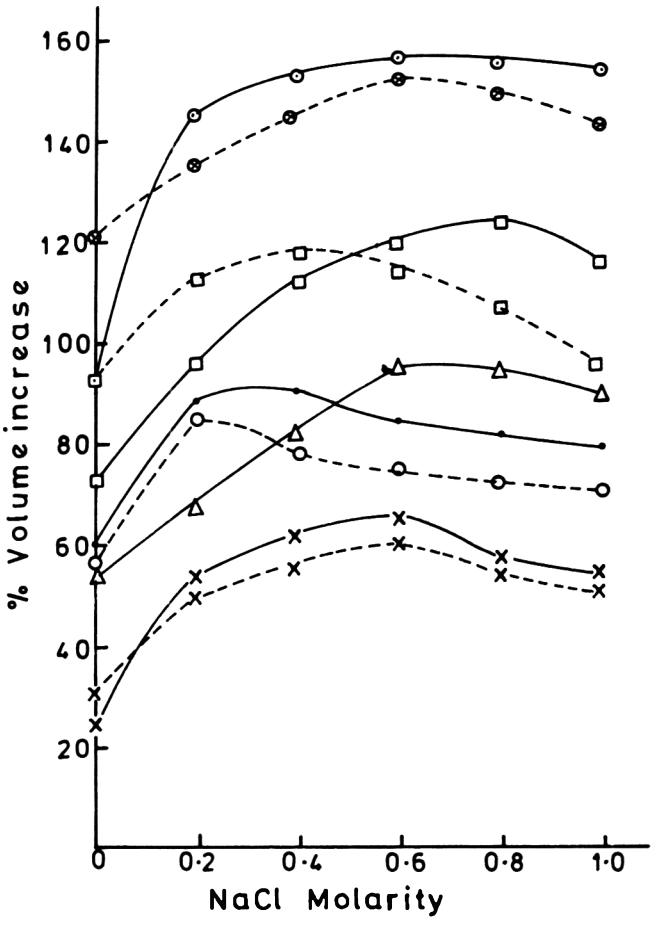

Fig. 6–Effect of NaCl concentration on foam capacity. Symbols same as Fig. 1.

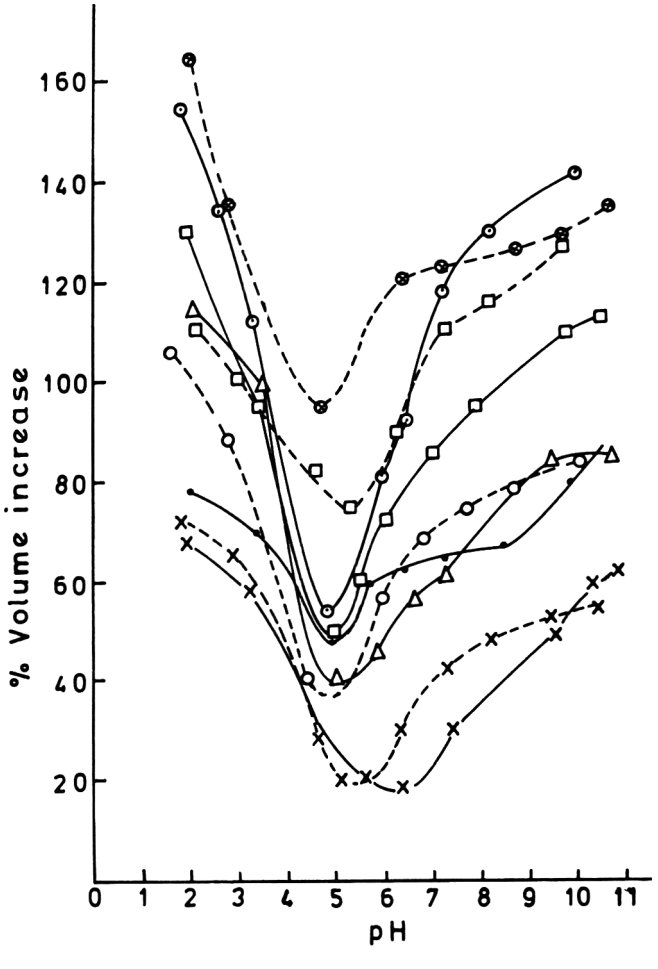

Fig. 5–Effect of pH on foam capacity. Symbols same as Fig. 1.

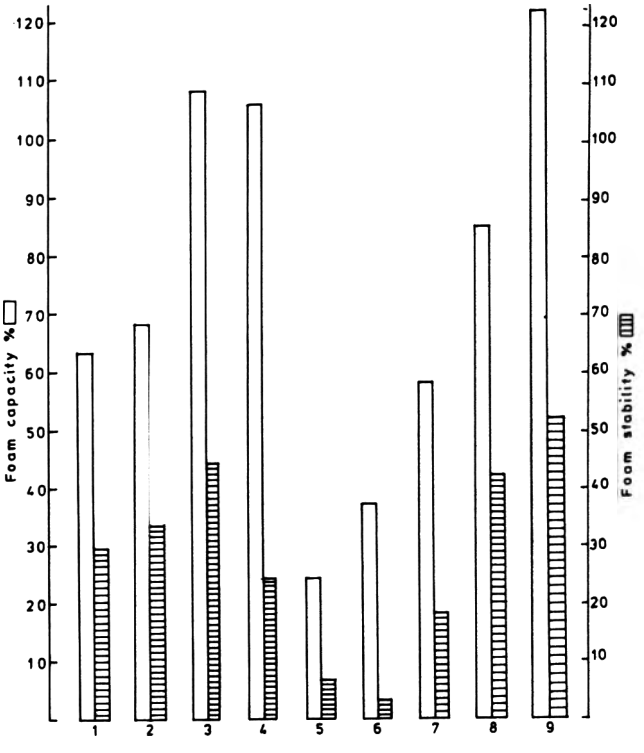

Fig. 7–Foam stability: 1. Defatted soybean meal; 2. Raw guar meal; 3. Defatted guar meal; 4. Autoclaved guar meal; 5. 1.00N HCl extracted guar meal; 6. 0.25N HCl extracted guar meal; 7. 70% methanol extracted guar meal; 8. 80% ethanol extracted guar meal; 9. 80% isopropanol extracted guar meal.

From the data reported on the various functional properties of guar meal, it appears that the method of processing greatly influences the quality of the samples. It is however, seen that acid extraction of guar meal as a method of detoxification adversely affects most of the functional properties as compared to other types of detoxification such as autoclaving or alcohol extraction.

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ISOTHERMS AND DRYING RATES FOR MULLET . . . From page 1322

temperatures were determined for salted and unsalted female roe of mullet, and ground white muscle of mullet. All isotherms are sigmoid shaped, typical of isotherms for food products. Adsorption-desorption hysteresis was also evident for salted and unsalted roe, and for ground white muscle. At any given temperature and relative humidity, the equilibrium moisture content for unsalted roe was always lower than that for salted roe.

Under the conditions tested, mullet fillets dried almost four times faster than roe. Roe placed in an air stream did not dry faster than roe in still air at the same temperature and relative humidity. This indicated that the restriction to moisture removal was in the moisture migration within the roe rather than at the roe surface. Air temperature should be lower than 45°C to prevent excessive browning of the row. Browning began on the tips and continued toward the center of the roe.

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Effect of Antioxidants on Malonaldehyde Production and Fatty Acid Composition in Pieces of Bovine Muscle and Adipose Tissue Stored Fresh and Frozen

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ABSTRACT

TBA-reactive material was produced in pieces of bovine semitendinosus muscle and adipose tissue during storage at 2 ± 2°C and −10 ± 2°C. The process was faster in muscle than in adipose tissue and the total content, higher at 2°C than at −10°C. The effect of sprying butylated hydroxytoluene and a citric acid-EDTA-ascorbic acid mixture on the production of malonaldehyde was studied. Declines in both saturated and unsaturated fatty acid proportions of the polar lipids without increases in the content of free fatty acids suggests that enzymes involved in lipid catabolism remain active at low temperatures. Whereas lipid breakdown was unaffected, malonaldehyde production was inhibited by sprying antioxidants in early stages of the slaughtering process.

INTRODUCTION

TIME-RELATED ORGANOLEPTIC CHANGES taking place in stored meat products, such as off-odors and off-flavors, are associated frequently with lipid peroxidation changes. These in turn result in the accumulation of malonaldehyde and other oxidative reaction products potentially harmful to human health (Shamberger et al., 1974, 1977; Mukai and Goldstein, 1976). Many factors contribute to this process, and it is difficult to inhibit it completely. Phenolic antioxidants and the combination of ascorbic acid with chelating agents often give the best protection against lipid oxidation. Different antioxidants have been shown to delay lipid and meat pigment oxidation (Greene et al., 1971; Ragnarsson et al., 1977) and chelators by sequestering metallic ions (Liu, 1970a, b) are able to act synergistically with antioxidants.

Most previous studies have been carried out with ground meats where the additive can be intimately mixed with the sample (Rhee, 1978; Wilson et al., 1976; Govindarajan et al., 1977). However, in whole meat cuts antioxidants are put on the external surface either by dipping or spraying, and hence lipid-related alterations can proceed in the interior of the cut. In this paper we examine the effect of spraying butylated hydroxytoluene (BHT) and a citric acid-EDTA-ascorbic acid mixture (CEA) on both malonaldehyde (MA) production and the fatty acid composition of major lipid fractions in relatively large pieces of semitendinosus muscle and adipose tissue during storage at low temperature. It is well known that MA production and lipid breakdown proceed even at temperatures below zero. However, whereas the former can be retarded by antioxidants, the latter is not affected, pointing to two different aspects of lipid involvement in meat food damage during storage.

MATERIALS & METHODS

BOVINE semitendinosus muscle (ST) and subcutaneous adipose tissue (AT) from three young animals (300–400 kg) were obtained immediately after slaughter, wrapped in polyethylene film and transferred to the laboratory packed in flaked ice. Cubes of about 2 cm per side, (2–3g) were cut within 6 hr after slaughter. Several groups of cubes, each consisting of samples from one of the three animals, were prepared. Using nitrogen as a propellant, samples were sprayed either with solution (a) 0.01% aqueous citric acid-EDTA-ascorbic acid; of (b) 0.2% ethanolic butylated hydroxytoluene (BHT). In controls no antioxidants were treated. Additives were were applied at the rate of 0.1 ml of solution per gram of tissue and the samples were stored either at (1) 2 ± 2°C or (2) −10 ± 2°C. Antioxidant levels were used according to maximum concentrations permitted by the Argentinian Alimentary Codex.

Meat samples

At appropriate times, samples at each treatment, from each of the three animals, were homogenized with distilled cold water (4°C) in a Potter Elvejhem homogenizer to form a 15% solution (w/v). Five ml of 10% trichloroacetic acid (TCA) (cooled at 4°C) were added to 5 ml of the homogenate and the precipitate separated by centrifugation. Malonaldehyde was assayed on 4 ml of supernatant after adding 1 ml of 1% (w/v) thiobarbituric acid (TBA) and boiling for 15 min (Shamberger et al., 1977). Proteins were estimated from the precipitate, after treating it with 0.5N NaOH, according to Layne (1957).

Lipids were extracted from the remainder of the homogenates with chloroform-methanol (1:2) (Bligh and Dyer, 1959) and the extracts were dried under nitrogen. Neutral lipids, polar lipids, and free fatty acids (FFA) were separated by a modification of the gradient-thickness TLC technique developed by Bazán and Joel (1970) which consisted of spotting the samples on the thinnest edge of the plate and developing them towards the thickest edge. Two mixtures of hexane:ether:acetic acid were used: (a) 60:40:1.2; (b) 40:60:1.2. The plates (20 x 20 cm) were run in system (a), dried under nitrogen and then rerun in system (b) about 7 cm from bottom (1/3 height).

Total lipids were estimated as fatty acid methyl esters, which were prepared from the lipid fractions according to Morrison and Smith (1964) and analyzed by GLC using a stainless steel column packed with DEGS 6% on Diatoport S, at 190°C. Methyl-nonadecanoate was used as an internal standard.

Adipose tissue samples

Subcutaneous adipose tissue samples were homogenized with chloroform-methanol (2:1) (Folch et al., 1957) and the homogenates were filtered. A portion of the extracts was spotted on TLC plates, and the lipid fractions were resolved as previously indicated. Aliquots of the chloroform solution were put into tubes and the solvents were evaporated under nitrogen at 40°C. Four ml of 5% TCA and 1 ml of TBA solution were added and the samples were processed as mentioned above for meat.

In this paper the TBA number is expressed as the absorbance read at 535 nm per gram of lipid in the samples. Pure methyl linoleate (Sigma Chemical Co.) was peroxidized by heating at 45°C in an air stream until a constant value of absorbance (usually 48 hr) was obtained. This preparation resulted in a TBA test with a linear curve from 10–1000 μg peroxidized methyl-linoleate (Fig. 1). Aliquots of the lipid extracts were chosen so that their absorbances fell between these values. Methyl-linoleate was used because it is the major source of conjugated dienes present in meat and fat samples (See Fig. 4). Moreover, linoleic acid hydroperoxides react with TBA to yield a red pigment identical to those formed from polyunsaturated fatty acid hydroperoxides (Ohkawa et al. 1978) and produce an absorption spectrum similar to that obtained from 1,1,3,3-tetramethoxypropane, a malonaldehyde producing agent used as a standard in many laboratories.

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RESULTS & DISCUSSION

THE TOTAL CONTENT of polar lipid fatty acids in semitendinosus muscle diminished significantly as a function of storage time and its composition was gradually modified (Table 1). After 5 days of storage at 2°C polyunsaturated fatty acids represented 12% of the total acyl groups compared to the initial 20%, while at 10 days this percentage was again 20%. Surprisingly enough, a similar decrease affected phospholipids at temperatures below 0°C. Thus, analogous values were observed after 10 days of storage at 2°C and after 14 days at −10°C (Table 1). The fatty acid content continued to decrease up to 70 days in samples stored at −10°C, the polyenoic fatty acids being the most affected.

The total content of free fatty acids in meat stored at a low temperature was extremely low (Table 2). This fraction showed no significant changes as a function of time, except after long periods of storage, when unsaturated free fatty acids were produced in a greater proportion than saturated ones. As the increases in FFA did not reflect the decreases observed in polar lipids (Tables 1 and 2), this suggests the possibility that phospholipase C-type activities were affecting the latter, rather than A-type. Diacyglycerols were not measured but were included in the neutral lipid fraction together with triacylglycerols. The latter represented 90% of the muscle lipids and may explain the observation that only limited changes were detected under the experimental conditions surveyed. Spraying with the two antioxidants employed in this work did not affect any of the changes described in phospholipids, free fatty acids or neutral lipids.

Malonaldehyde production estimated by the TBA test increased markedly as a function of time (Fig. 2). The oxidation process was more rapid at 2°C than at −10°C and spraying with BHT inhibited the production of malonaldehyde at both temperatures compared to the control at both final storage periods. The CEA mixture proved effective only in the case of samples stored at 2°C, but it was not adequate for samples maintained at −10°C. This is possibly the result of a longer period of contact between ascorbic acid and oxygen rather than an influence of storage temperature. It has been reported that ascorbic acid stability is dependent on the oxygen level present (Ford, 1967; Khan and Martell, 1967). Furthermore, a pro-oxidant action of ascorbic acid has been proposed, both in meat preserved at −1°C for 10 days (Benedict et al., 1975) and in model systems (Dennison and Kirk, 1978). However, these effects depend on ascorbic acid concentration (Watts

—Text continued on page 1332

Table 1—Changes in phospholipid fatty acid content in semitendinosus muscle[a] during storage

	Storage temperature					
	2 ± 2°C			−10 ± 2°C		
Fatty acid	0 day	5 days	10 days	7 days	14 days	70 days
	μg/100 mg of protein					
16:0	195 ± 7	141 ± 40	109 ± 40	111 ± 5	77 ± 4	83 ± 9
16:1	25 ± 3	14 ± 3	14 ± 3	24 ± 6	16 ± 1	14 ± 1
16:2	29 ± 7	4 ± 0.2	4 ± 1	30 ± 10	10 ± 4	11 ± 4
18:0	122 ± 8	124 ± 15	52 ± 19	56 ± 9	45 ± 2	56 ± 7
18:1	286 ± 51	261 ± 40	140 ± 50	159 ± 17	132 ± 2	140 ± 23
18:2	112 ± 24	95 ± 21	66 ± 23	61 ± 10	50 ± 4	41 ± 4
18:3	40 ± 4	25 ± 7	20 ± 7	19 ± 3	16 ± 2	11 ± 2
20:4	87 ± 3	42 ± 9	48 ± 20	49 ± 9	40 ± 7	20 ± 2
20:5	42 ± 7	18 ± 1	26 ± 9	36 ± 3	23 ± 6	6 ± 2
22:5	53 ± 13	25 ± 4	30 ± 11	34 ± 3	26 ± 5	11 ± 1
22:6	2.5 ± 0.1	2.4 ± 0.8	2.0 ± 0.1	3.3 ± 1.0	2.3 ± 0.4	1.5 ± 0.3
Total	1,145 ± 21	894 ± 198	577 ± 200	647 ± 88	515 ± 12	435 ± 26

[a] Figures represent mean values ± S.D. from three samples obtained from different animals.

Table 2—Changes in free fatty acids in semitendinosus muscle during storage[a]

	Storage temperature					
	2 ± 2°C			−10 ± 2°C		
Fatty acid	0 day	5 days	10 days	7 days	14 days	70 days
	μg/100g mg of protein					
16:0	7.3 ± 2.4	5.9 ± 1.1	7.1 ± 2.4	6.0 ± 1.5	5.9 ± 1.8	9.3 ± 1.0
16:1	1.6 ± 0.6	1.1 ± 0.2	1.5 ± 0.3	1.5 ± 0.7	1.4 ± 0.2	2.7 ± 0.01
16:2	0.2 ± 0.08	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.09	0.7 ± 0.1
18:0	4.9 ± 1.0	4.4 ± 0.4	4.9 ± 1.8	5.3 ± 1.0	5.3 ± 1.3	5.8 ± 0.6
18:1	10.6 ± 4.5	10.2 ± 4.0	11.7 ± 3.1	12.9 ± 5.8	14.7 ± 0.9	19.3 ± 3.7
18:2	1.5 ± 0.4	1.4 ± 0.2	1.7 ± 0.6	1.6 ± 0.1	1.9 ± 0.5	4.8 ± 0.4
18:3	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	1.3 ± 0.2
20:4	0.8 ± 0.3	0.8 ± 0.1	0.9 ± 0.3	1.0 ± 0.2	1.2 ± 0.3	2.3 ± 0.6
20:5	0.3 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	1.2 ± 0.3
22:5	0.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.3 ± 0.4	1.9 ± 0.5
22:6	0.2 ± 0.03	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.03	0.2 ± 0.04	0.3 ± 0.04
Total	30.7 ± 9.4	27.1 ± 5.6	32.0 ± 9.3	32.7 ± 9.1	34.7 ± 6.4	55.2 ± 9.1

[a] Results are presented as in Table I.

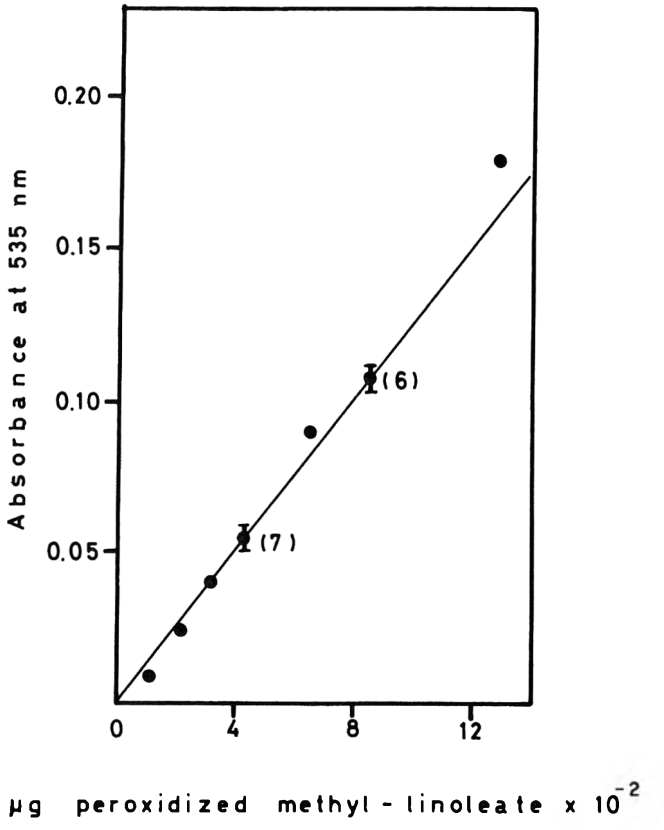

Fig. 1—Absorbances obtained for the TBA reaction from peroxidized methyl-linoleate. Methyl 18:2 was peroxidized under air and resuspended in chloroform. Aliquots were taken and subjected to the TBA test. Figures in brackets represent the number of determinations, the rest are duplicates. Results from four different methyl 18:2 peroxidations are included.

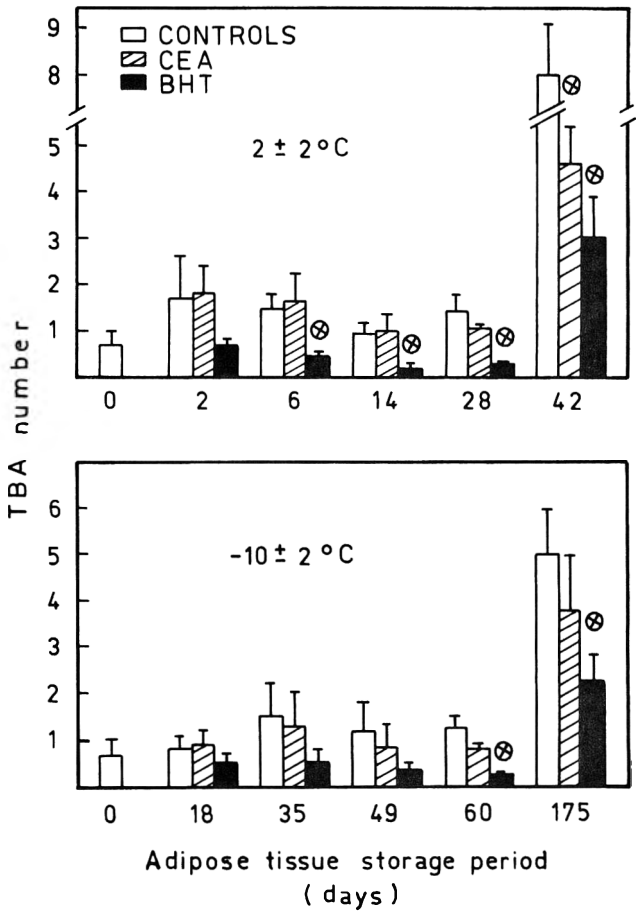

Fig. 3—Effects of antioxidants on TBA number in adipose tissue stored at low temperatures. Results are presented as in Fig. 2. ⊕P < 0.005.

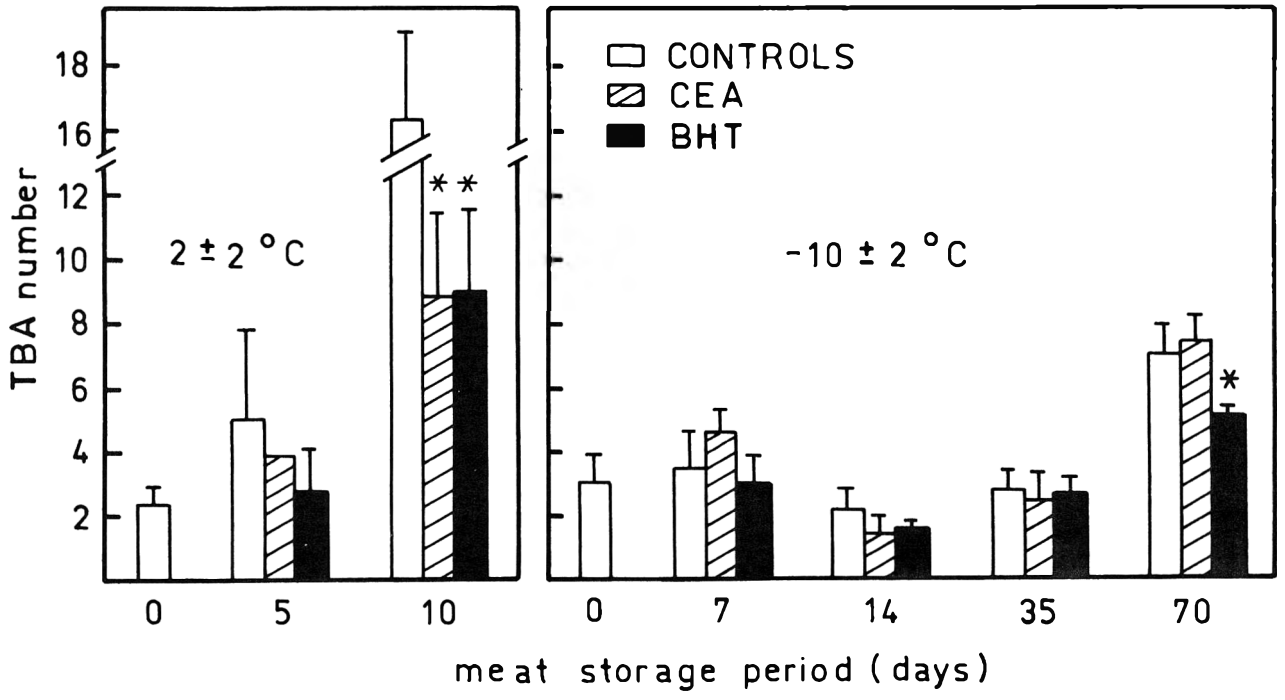

*Fig. 2—Effect of antioxidants on TBA number in semitendinosus muscle stored at low temperatures. Bars represent mean values ± SD from three samples in each case. Results significantly different from controls, *P < 0.05. CEA = Citric acid-EDTA-Ascorbic; BHT = Butylated Hydroxytoluene.*

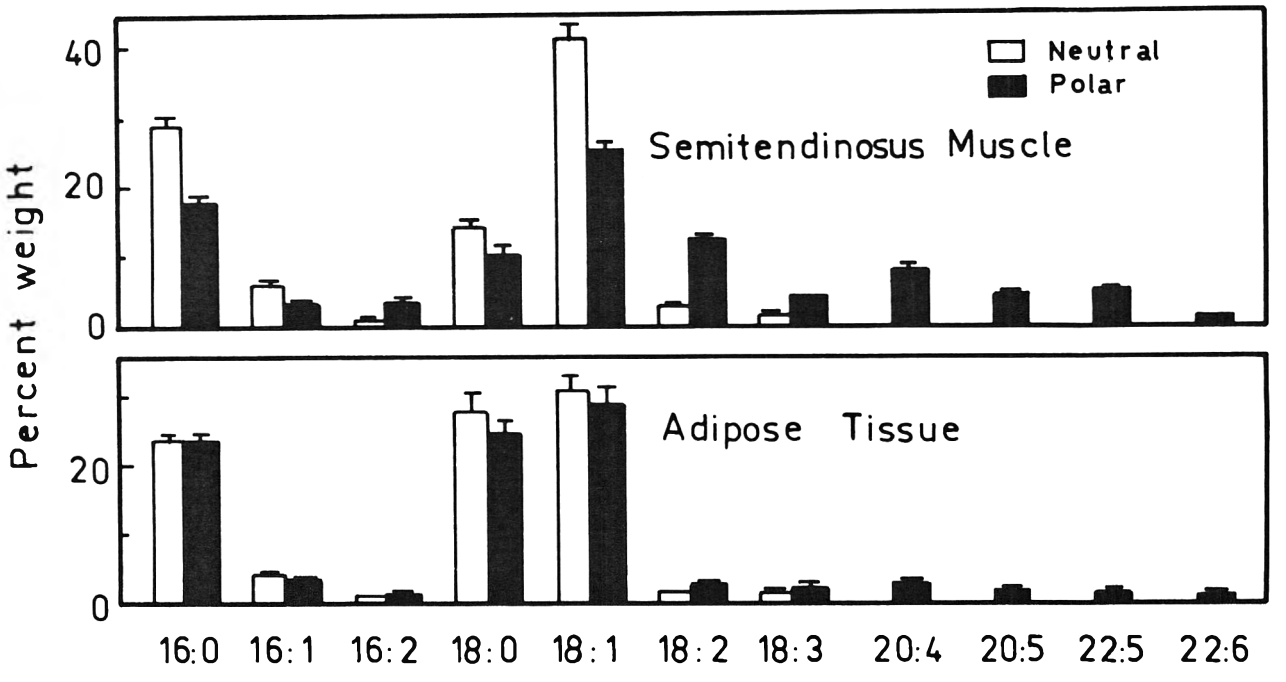

Fig. 4—Fatty acid composition of neutral and polar lipids from semitendinosus muscle and adipose tissue. Lipid extracts were prepared from nonstored samples (time 0) and subjected to TLC. Polar lipids represent the fraction at the origin of the plates, and the neutral fraction includes di- and triglycerides. Methyl esters were prepared and analyzed by GLC. Results are mean values ± S.D. from three samples obtained from different animals.

and Wong, 1951; Love and Pearson, 1974) and may disappear in the presence of EDTA salts (Liu, 1970).

The TBA numbers obtained for stored subcutaneous adipose tissue are shown in Fig. 3. The degree of peroxidation was slower in fat than in muscle, requiring storage three times as long to reach similar levels. A decline in TBA numbers was noted after the initial rise during storage at low temperature for both semitendinosus and adipose tissue (Fig. 2 and 3). This could result from the formation of less stable and/or volatile compounds reactive with TBA. However, some metabolites may be susceptible to oxidation yielding products unreactive with TBA (Dugan, 1961; Moledina et al., 1977; Gokalp et al., 1978).

The slower peroxidation rate in fat than in muscular tissue should be expected since triacyglycerols, which conform the bulk of adipose tissue mass, are less susceptible to peroxidation than phospholipids (Chipault and Hawkins, 1971; El Gharbawi and Dugan, 1965; Younathan and Watts, 1959, 1960; Keller and Kinsella, 1973). A relationship between the susceptibility to peroxidation and the proportion of unsaturated fatty acids has been reported (Wilson et al., 1976; Moerk and Ball, 1974), suggesting that phospholipids may play a preponderant role in the development of unpleasant flavor and odor in foods (Wilson et al., 1976). The percentage of polyenoic fatty acids in the fraction containing the phospholipid was higher than the amount present in neutral lipids in muscle as well as in subcutaneous fat (Fig. 4). Although in both tissues polar lipids represent a minor fraction, the higher level of polyenoic fatty acids in muscle may play an important role in the triggering of peroxidation.

Other factors affecting the differences in the rate of damage observed between muscle and adipose tissue lipids may be the presence of metallic ions (Barber, 1966) and of hemoproteins which catalyze lipid oxidation (Tappel, 1955; Froning and Johnson, 1973).

The results presented here suggest that two kinds of phenomena are occurring simultaneously in stored meat. The decreases observed in phospholipids may represent metabolic activities in the tissue mainly unrelated to autoxidation, since they affect both saturated and unsaturated fatty acids and cannot be prevented by antioxidants. Though peroxidation occurs even at low temperatures, its velocity can be significantly reduced by applications of antioxidants to the surface at early stages of the slaughtering process.

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Composition of Serum from Cooked-Frozen-Thawed-Reheated Scrambled Eggs at Various pH Levels

G. E. FEISER and O. J. COTTERILL

ABSTRACT

The volume of serum pressed from the Cooked-Frozen-Thawed-Reheated (CFTR) scrambled egg decreased as pH was increased from 6.0 to 7.0. Solids, protein and lipid levels were maximal at pH 7.0. Ash and phosphorus contents decreased with increased pH. Sodium and potassium remained constant. Chloride concentration increased from pH 6.2 to 6.8. Iron had maximal levels at pH 6.2 and to 6.8 to 7.2. The lipid and phosphorus levels in the serum were very low when compared to those in the original liquid whole egg. Ovomacroglobulin, lipovitellin and some of the globulins and livetins were absent in the electrophoretograms of the serum. Also, a band appeared in the ovomucoid area which was not normally present in uncooked egg.

INTRODUCTION

IN RECENT YEARS several egg products have been developed as Cooked-Frozen-Thawed-Reheated (CFTR) items, such as scrambled eggs, quiches, souffles, meringues, omelets and egg rolls. Palmer et al. (1974) published a report that contained many formulated products for this purpose. Huber and Engebretsen (1978) developed an egg sandwich loaf. Sauter and Petersen (1980) worked with omelets from frozen whole egg and fresh, freeze-dried or spray-dried egg white.

With the market for these products expanding, problems such as syneresis, off flavor development, color deterioration and rubberiness must be resolved. One approach was by altering process conditions. Davis et al. (1952) concluded that ice crystal formation caused these changes and the damage could be reduced by supercooling or by the addition of calcium carbonate. Hasiak (1978) worked with different methods of freezing to reduce syneresis. Immersion in liquid Freon, as compared to liquid nitrogen or carbon dioxide, was the better process. Shamwell and Stadelman (1976) varied rate of freezing and temperature to reduce weepage from souffles. Jokay and Meyer (1961) developed a freeze-dried precooked scrambled egg product by regulating cooking temperature and rate of freezing.

The other approach was the addition of various ingredients. Rivoche (1963) added methylcellulose and Cimino et al. (1967) used flour and methylcellulose to improve frozen souffles. Low methoxyl pectin and soy protein were ingredients used by Katz (1968) in a CFTR scrambled egg mix. Latham and Seeley (1973) developed omelets with many additives. Starch was used by Hawley (1970), which reduced rubberiness and syneresis in CFTR egg white. Chin and Redfern (1968) improved color in scrambled eggs by adding monosodium phosphate.

Presumably the problem of syneresis in CFTR egg products is caused by the cooked egg white fraction. Cotterill (1973) stated that cooked egg white is easily damaged by freezing.

This research is based on the premise that the textural problems mentioned above are common to most CFTR egg products. Therefore, information on one product should help resolve the problems in others. The objective of this research was to determine the influence of pH on egg components involved in or altered by the CFTR process of scrambled whole egg by studying CFTR scrambled egg serum. The main focus is the effect of initial and final pH on composition of the serum expressed from the freeze damaged product.

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MATERIALS & METHODS

Whole egg

The liquid whole egg (WE) was prepared from fresh unwashed shell eggs held at 4°C for less than 1 wk. The yolks and whites were blended in a Waring Blendor at reduced speed with an Erylenmyer flask immersed below the surface of the liquid to prevent foaming. The pH of this milled WE was adjusted with 2M citric acid and 1M sodium hydroxide with constant mixing on a magnetic stirrer. Triplicate samples were made and analyzed.

Cooking, freezing, thawing, and reheating

The WE was scrambled in 1.4L glass double boilers. The inside of the upper unit was sprayed with PAM to prevent sticking. The lower unit contained 340 ml of water maintained at 100°C on a gas burner. One hundred grams of WE were cooked for 6 min while being stirred constantly with a rubber spatula. After preparation, the cooked WE was placed in a 10.8 cm X 10.8 cm X 3.2 cm dish suitable for use in a microwave oven with the contents spread around the perimeter, leaving a hollowed center. The dishes were partially covered with aluminum foil and allowed to cool to room temperature. After closing, the cooked WE was frozen in still air at −23°C and held for 1 wk. The samples were then thawed in a refrigerator at 4°C. A Sharp Carousel R-6770 microwave oven was used to reheat the samples for 5 min. This product is termed: Cooked-Frozen-Thawed-Reheated (CFTR) scrambeld egg.

Expressing and collecting serum

The serum was expressed with a test cylinder on a Carver Press. To prevent leakage, the test cylinder was modified as shown in Fig. 1. It was necessary to place an O-ring between the base plate and cylinder and a clamp to hold the two units together. A gasket was used between the piston and the sample. The CFTR scrambled egg (90g) was wrapped in four layers of cheesecloth and placed in the cylinder. A 136 kg force was applied to the piston and held for 1 min. The serum was collected in 50 ml graduated conical centrifuge tubes under a vacuum of 60 mm Hg. After centrifugation at 1000 RCF to remove insoluble matter, the serum volume was noted. The coagulated scrambled egg was discarded and the centrifuged serum was held for further analyses.

Serum solids-protein-ash

Total solids were determined by placing five gram samples of serum in an aluminum weighing dish and then drying in a forced draft air oven at 105°C for 24 hr. After drying, samples were cooled in a desiccator for 20 min and weighed.

Total protein was determined by micro-Kjeldahl procedure (AOAC 1980) using the factor of 6.25 for converting total N to protein.

Ash was performed as follows: One gram of serum was placed in a Coors porcelain dish, size number 1, then placed in a muffle furnace at 500°C for 3 hr. After these samples were ashed, they were cooled in a desiccator for 20 min and weighed.

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Serum lipids

Total lipids in the CFTR serum were determined by an ethyl alcohol, ethyl ether, petroleum ether extraction in an ammoniacal system (Cotterill, 1961). After solvent evaporation and drying, the amount of lipids was determined gravimetrically.

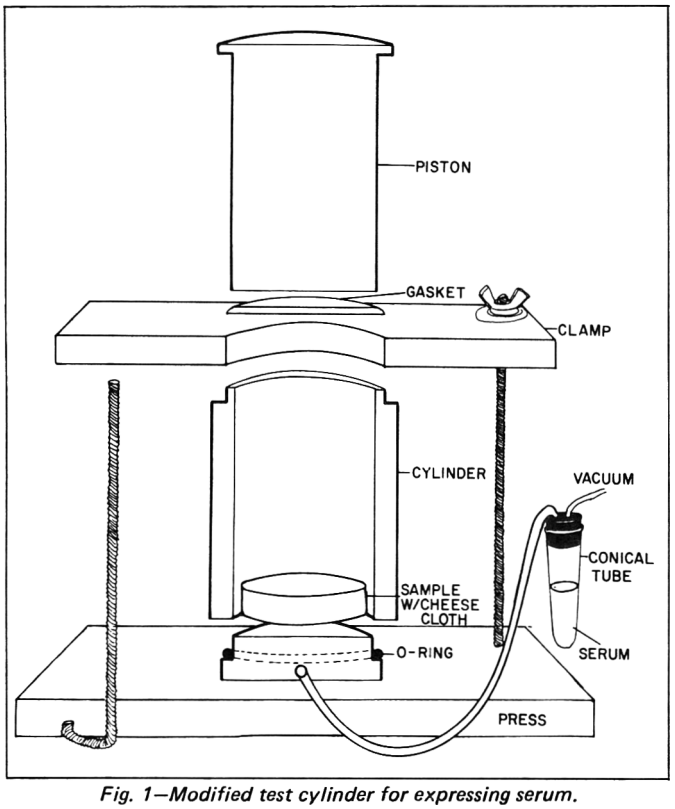

Fig. 1—Modified test cylinder for expressing serum.

Iron, phosphorus, sodium, potassium, chlorine analysis

Samples of iron, phosphorus, sodium and potassium were prepared by a wet ash procedure using nitric and perchloric acids according to AOAC (1980). Iron was analyzed using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer at 248.3 mm according to the directions given in their manual (1980). Phosphorus was determined colorimetrically as phosphomolybdic acid after treatment with 1,2,2,-aminonaphtholsulfonic acid (Fiske-SubbaRow) according to Bartlett (1959) with minor modifications. Two ml aliquots of the wet ash solution were placed in test tubes containing nine ml of water, then one-half ml each of 2.5% ammonium molybdate and the Fiske-SubbaRow reagent were added. The spectrophotometer readings were made at 640 mm. Sodium and potassium were determined by an automated flame photometric procedure according to Neuner et al. (1975). Soluble chlorine was determined by a titration method according to AOAC (1980). A known quantity of silver nitrate was added to sample aliquots to precipitate chlorine as silver chloride. Excess silver nitrate was then back titrated with potassium thiocyanate.

Serum electrophoresis

Separation of proteins in CFTR serum was performed on a Canalco Model 1200 electrophoretic unit (Miles Laboratories, Inc., Elkhart, IN) utilizing a discontinuous 3 gel polyacrylamide system consisting of: (1) sample gel (2.5% polyacrylamide in pH 6.6–6.8 Tris HCl buffer), (2) stacking gel (2.5% polyacrylamide in pH 6.6–6.8 Tris HCl buffer), and (3) separating gel (7.0% polyacrylamide in pH 8.8–9.0 Tris-HCl buffer). Sample size ranged from 250–350 μg of protein in 5 μl of serum. Current was carried by a pH 8.3 Tris glycine buffer. A constant current of 4 milliamps per gel was applied for 30–45 min. Separation was monitored with a Bromophenol Blue tracking dye. After completion of separation, gels were placed in 12% trichloracetic acid for 30 min, rinsed with distilled water, then stained with 0.025% Coomassie Blue in 7% acetic acid for 1.5 hr. The gels were destained in 40% methanol-7% acetic acid solution overnight. Gels were then stored in 7% acetic acid.

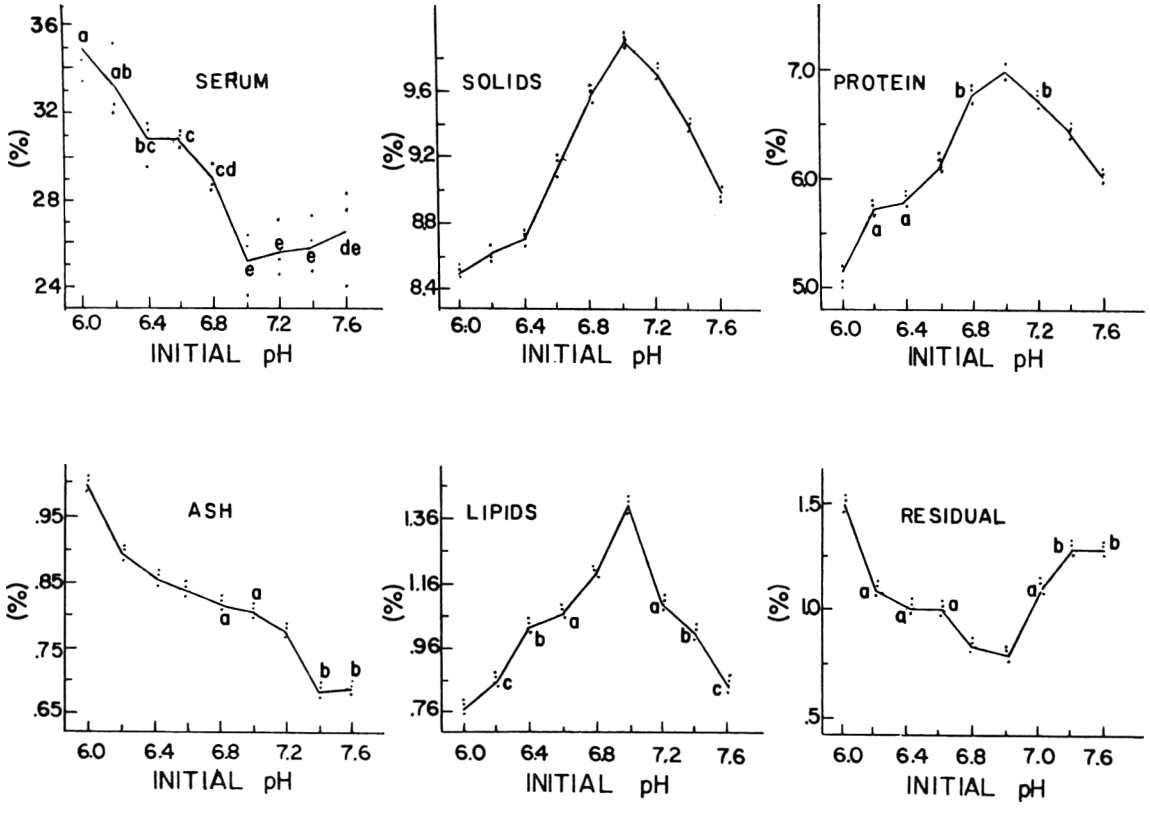

Fig. 2—Serum volume and serum solids, protein, ash, lipids and residual levels with increasing pH expressed as % of original scrambled egg volume.

RESULTS & DISCUSSION

Proximate analysis

The percent serum loss, total solids, protein, ash, total lipids, and residual levels between pH 6.0 to 7.6 serum are shown in Fig. 2. Serum loss and ash content were both maximal at pH 6.0. Serum solids, lipids and protein levels were highest at pH 7.0. The residual material (other than protein, lipid or ash) was maximal at pH 6.0 and 7.6 with a minimal level at pH 7.0.

Most of these components were lower in the serum than in the original liquid whole egg (Table 1). Note that most of the lipids are retained in the coagulated scrambled egg while the ash content of the serum was more nearly the same as the original whole egg. Since the serum is essentially a water extract this relationship is to be expected.

Elemental analyses

The effect of pH on the chloride, iron, phosphorus, potassium and sodium content of the serum is shown in Fig. 3. Sodium and potassium levels were little affected by pH. Increasing the pH increased the amount of chlorine in the serum but decreased the amount of phosphorus. Iron content was maximal at pH 6.2 and at 7.0, the latter being a much broader peak.

The water soluble ions (chloride, potassium and sodium) were higher in the serum than in the original liquid whole egg (Table 1). Iron was slightly lower in the serum. Very little phosphorus was present in the serum.

Presumably most of the iron is bound to the proteins conalbumin and phosvitin and is not in a free state. The addition of 12% trichloracetic acid to the serum at pH 6.2 and 7.0 caused over a 90% reduction in iron, while only a 50% reduction in phosphorus was observed. The high level of iron at pH 6.2 corresponds with the increase in phosphorus at the same pH, suggesting that the iron at this pH is complexed with phosvitin. Also, the ratio of phosphorus to iron in the serum at pH 6.2 was 2.9:1 which is near the ratio of 1.0:1 in phosvitin reported by Taborsky (1963). However, it was not possible to electrophoretically verify this peak at pH 6.2 as phosvitin since phosvitin and ovalbumin appear at the same location on the gel. The broader peak for iron at pH 6.8–7.2 appears to be complexed with conalbumin. Note that conalbumin could be detected by gel electrophoresis in this pH range of serum, but not at pH 6.2. Also, phosphorus was greatly reduced at the higher pH levels suggesting that phosvitin was not present. The width of this peak could be due to the two fractions of conalbumin as reported by Clark et al. (1963) and Feeney et al. (1963).

Table 1—Average percent of components in serum as compared to original liquid whole egg

Components	Proportion in serum[a]
Proximate	(%)
Total solids	38[b]
Protein	52
Total lipids	10
Ash	80
Elements	
Chlorine	119
Iron	74
Phosphorus	2
Potassium	140
Sodium	137

[a] [(Percent level in serum)/(Percent level in whole egg)] X 100 = Amount in serum
[b] Average for all pH levels

Electrophoresis

Fig. 4 contains electrophoretograms of the serum from CFTR scrambled egg. The initial pH values are used as a point of reference through this discussion. The pH of the serum is shown below the initial liquid values.

In addition, Fig. 4 shows the electrophoretic patterns for native egg white (NEW), whole egg (NWE) and yolk (NEY). The protein bands for NEW and NEY are identified on the left and right sides respectively. A total of 15 bands was observed on electrophoretic separation of NEW. One band of ovomacroglobulin, 2 bands of conalbumin, 3 bands of globulin G_2, 5 bands of globulin G_3, 3 bands of ovalbumin and 1 band of flavoprotein were observed as previously reported by Galyean and Cotterill (1979). The electrophoretic pattern of NEY separated in 18 bands as reported by McBee and Cotterill (1979). Lipovitellin, 3 bands of γ-livetins, α and β livetins and 3 bands of phosvitin were identified. NWE electrophoretic separation consisted of a combination of NEW and NEY components. Those easily observed were ovomacroglobulin, lipovitellin, conalbumin, globulins (G_2, G_{3A}, G_{3B}), livetins (B), (A_1, A_2, A_3) and flavoprotein.

Many of the egg white and yolk proteins in the CFTR serum were still electrophoretically mobile. The ovalbumins are apparent throughout all the pH ranges. Several bands were still present in the globulin and livetin areas. Chang et al. (1970) and Dixon and Cotterill (1981) demonstrated that α, β and δ livetins were heat labile. However, it is difficult to identify them individually because of overlapping within these protein groups.

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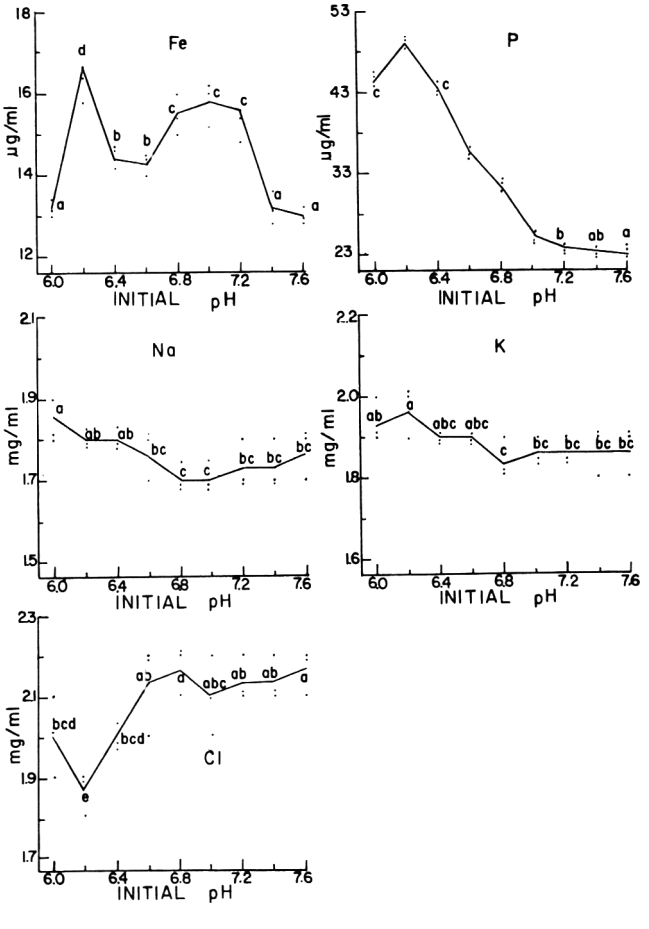

Fig. 3—Serum iron, phosphorus, sodium, potassium and chlorine content with increasing pH.

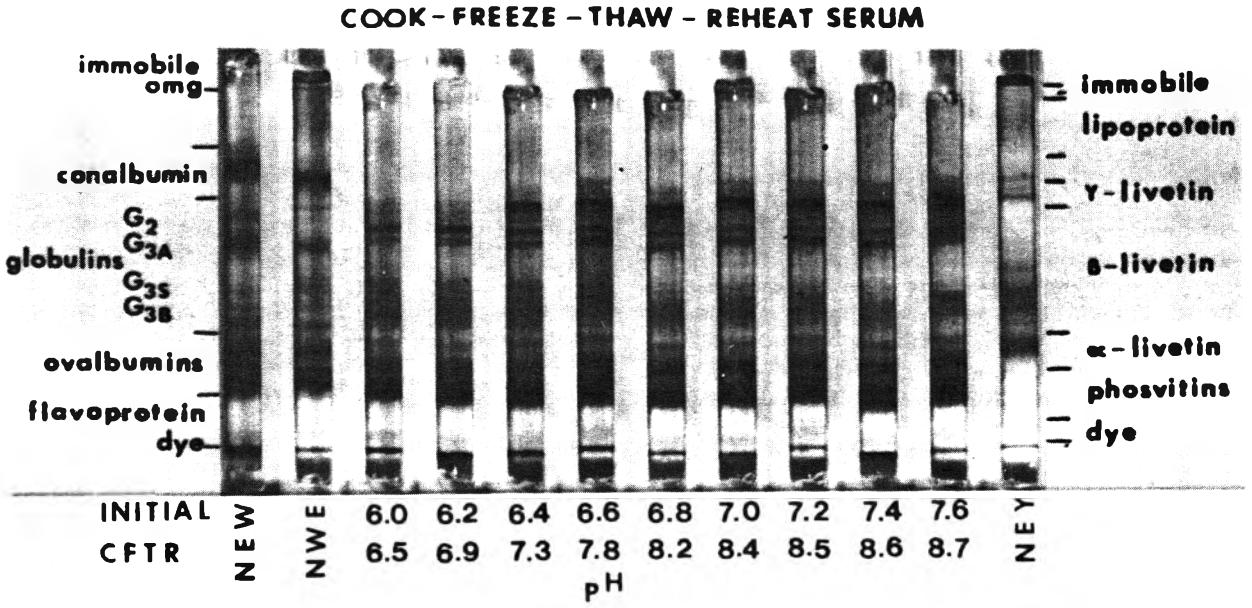

Fig. 4—Electrophoretogram of cook-freeze-thaw-reheat serum with increasing pH treatments.

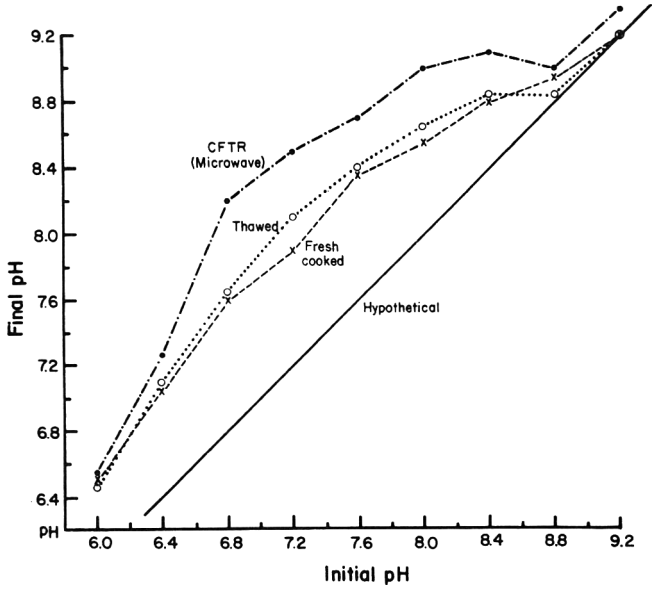

Fig. 5—Increase in pH of scrambled egg due to cooking and the serum after thawing and reheating.

Ovomacroglobulin and lipovitelln were absent throughout all the CFTR samples. Ovomacroglobulin, as expected, was absent due to its heat sensitivity (Miller and Feeney, 1966; Donovan et al. 1969). lipovitellin appears to be retained in the pressed coagulated egg. Recall that, the serum contains only about 10% as much lipid as the NWE. The G_{3A} globulins which appear in NEW and less so in NWE are not apparent in gels from pH 6.0–7.6. This must be due to its heat sensitivity. The heat lability of conalbumin was apparent at pH 6.0 and 6.2. This was to be expected as previously reported by Seideman et al. (1963).

The sharpened bands in the G_2 region may be ovomucoid since heating improves the dye binding capacity of this protein. Ovomucoid has an isoelectric point at 3.9–4.3 (Powrie, 1977) and these bands are more intense at lower pH levels.

Phosvitin appears in the gel from NEY but was not apparent in NWE or in any of the CFTR samples. This is due to ovalbumin masking the phosvitin band. Both the gels from NWE and NEY had a large immobile band which was not present in NEW or any of the CFTR gels. Apparently this immobile protein was retained in the pressed coagulated egg.

pH Change in CFTR system

Fig. 5 shows the change in pH of the serum from CFTR scrambled egg. The liquid WE had been adjusted to pH levels between 6.0 and 9.2 at intervals of 0.2 pH units with 2M citric acid and 1M NaOH. The pH was observed immediately after cooking, after freezing and thawing, then after reheating in a microwave oven for 5 min. Slightly over half of the pH increase occurred during cooking. Freezing and thawing caused only a slight change in pH. During reheating the pH further increased, particularly in the pH 6.8–8.4 range. The largest change occurred when the NWE was adjusted to pH 6.8, which subsequently increased to 7.6 after cooking, freezing and thawing, then to 8.2 when reheated in the microwave oven. Very little pH change occurred at pH 8.8–9.2. Likewise, the increase was greatly reduced at pH 6.0.

Since the pH changes during cooking and reheating, it is difficult to relate a specific pH to composition or electrophoretic differences. Hence, the effect of heat on a specific protein must be considered throughout these respective ranges between the initial and final pH.

The above curves differed from those reported by Davis et al. (1952) for cooked-frozen egg white. Their observations extended between pH 6.5–10.5, with no data in the 6.0–6.5 range. Hence, they failed to note the reduced pH change in this region but implied that even a greater change should occur.

An explanation for these changes in pH due to cooking and reheating is not readily apparent. At least three buffer systems must be considered; (1) carbonate, (2) citrate, and (3) egg proteins. Carbon dioxide is easily lost and could account for major pH changes due to the limited buffering capacity of proteins in the pH 7–9 region. Carbon dioxide loss is responsible for the change in pH for egg white in shell eggs during aging. Also, its loss is presumably responsible for the increase in pH of whole egg during dehydration. Since the highest pK for citric acid is 5.4, only the lowest pH levels might be influenced by any special buffering effects by this acid.

The green discoloration observed in scrambled eggs which is caused by the formation of FeS is related to the pH change during cooking and reheating. The initial pH of liquid whole egg ranges between 7.0–7.6. An increase in pH favors the dissociation of -SH groups, release of H_2S and formation of FeS upon heating (Tinkler and Soar, 1920; Baker, et al., 1967; Germs 1973, and Gossett and Baker, 1981).

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Effect of Hydrogenation on the Chemical Composition of Canola Oil

J. D. BANSAL and J. M. deMAN

ABSTRACT

Canola oil was hydrogenated under selective and nonselective conditions with commercial nickel catalysts. The composition of the hydrogenated oils was characterized by iodine value, fatty acid composition, *trans*-isomer content and *cis,cis*-methylene interrupted (CCMI) polyunsaturated fatty acids. Selectivity ratios and hydrogenation rates were calculated. Selective conditions resulted in high levels of *trans*-isomers. Loss of CCMI fatty acids occurred in the early stages of the hydrogenation process. Hydrogenation rates were higher under selective than under nonselective conditions.

INTRODUCTION

THE PROCESS of hydrogenation is an essential step in the production of margarines and shortenings from vegetable oils. The nickel catalyzed commercial hydrogenation of oils results in saturation of double bonds and also produces a variety of *trans*-isomers and positional isomers. The hardening effect of hydrogenation, therefore, results from both increased saturation as well as *trans*-isomer levels. The composition of margarines has been the subject of several recent studies (Ottenstein et al., 1977; Perkins et al., 1977; Heckers and Melcher, 1978; Beare-Rogers et al., 1979; Sahasrabudhe and Kurian, 1979).

In 1980, an ad hoc committee (Davignon et al., 1980) on the composition of special margarines submitted a report to the Health Protection Branch of the Canada Department of Health and Welfare. One of the recommendations of the committee was that industry should be encouraged to seek ways to increase th linoleate content while reducing the *trans* fatty acid content of Canadian foods. Although the effect of *trans*-isomers on human health remains controversial, one precent paper reported that feeding elaidic acid resulted in a decreased survival rate of tumor-bearing mice (Awad, 1981). Because of the questions raised about *trans* fatty acids, it is important to determine how conditions of hydrogenation influence the formation of *trans*-isomers. Canola oil is now the major source of oil for Canadian margarine and shortening production and this oil was, therefore, selected for this study.

Rapeseed cultivars have been systematically modified by plant breeding to improve production and nutritional characteristics. The original rapeseed oils were unique among fat sources because of their high content of erucic acid. Canadian plant breeding research has resulted in the development of a series of improved rapeseed cultivars with very low erucic acid content. In 1978, the rapeseed industry adopted the name Canola to identify rapeseed cultivars which yield oil low in erucic acid and meal low in glucosinolates. There is little published information available on the hydrogenation behavior of these low erucic acid Canola oils. Temperature, hydrogen pressure, agitation, catalyst concentration and catalyst type are important variables influencing the composition and properties of hydrogenated products (Coenen, 1976). Hydrogenation of edible oils is generally carried out at relatively selective or nonselective conditions. A series of recent studies (El-Shattory et al., 1981a, b) dealt with the effect of temperature and pressure on the hydrogenation of Canola oil from the cultivar Zephyr. The hydrogenation reaction rate increased with temperature as well as pressure but the selectivity ratios for the hydrogenation reaction were more influenced by pressure than by temperature; the highest selectivity ratios were observed at the lower pressure. El-Shattory et al. (1981c) studied the influence of catalyst concentration on the hydrogenation of Canola oil (Tower) and observed that hydrogenation was carried out without difficulty with a catalyst concentration of 0.2% and increasing the level of catalyst did not result in any appreciable improvement in activity. The overall objective of this study was to find out how selective and nonselective conditions affect the properties of Canola oils and to what extent the two commercial nickel catalysts at the same concentration under similar conditions differ in their action on the hydrogenation of Canola oils.

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MATERIALS & METHODS

THE OILS USED in this study were two commercially refined and bleached Canola oils obtained from CSP Foods Ltd., Saskatoon. The first oil was obtained from the mixed seed of three cultivars, Regent and Tower (*Brassica napus*) and Candle (*Brassica campestris*) in the approximate proportions of 50:30:15 respectively. The second oil was obtained from the Regent cultivar of *B. napus*. Two commercial nickel catalysts were used, a dry reduced catalyst (Harshaw Nysel N-3201F) and a wet reduced catalyst (Food Specialties Company Ltd., Ajax, Ontario). These catalysts are designated H and F respectively. Hydrogenation of the oils was done under selective 200°C and 48 kPa hydrogen pressure) and nonselective (160°C and 303 kPa hydrogen pressure) conditions using 0.2% catalyst by weight. Hydrogenation was carried out in a Parr Pressure Reaction Apparatus by the AOCS Recommended Practice Cd. 17-76 (1974) using a 2 liter bomb and a charge of 1 kg of oil. Samples were taken during hydrogenation through the sampling valve at regular intervals. Iodine values were determined by the Wijs method (AOCS Cd 1-25, 1974). Fatty acid composition of the oils was determined on the methyl esters prepared by the method of Shehata and deMan (1970). The methyl esters were analyzed by GLC using

Table 1 – Catalyst activity for selective and nonselective hydrogenation of oils using catalysts H and F

Canola oil	Reaction time min	Reaction conditions	Activity ΔIV/min	
			Catalyst H	Catalyst F
Mixed	20	Selective	0.79	1.10
	40	Selective	0.74	0.79
	60	Selective	0.61	0.57
Regent	20	Selective	1.35	1.32
	40	Selective	1.27	1.11
	60	Selective	0.99	1.03
Mixed	40	Nonselective	0.34	0.77
	80	Nonselective	0.42	0.50
	120	Nonselective	0.39	0.44
Regent	40	Nonselective	0.43	0.92
	80	Nonselective	0.50	0.62
	120	Nonselective	0.46	0.57

a Varian model 1400 instrument equipped with flame ionization detector. Column length was 125 cm, diameter 2 mm, carrier gas flow 30 ml/min and column packing was 15% DEGS on Chromosorb RZ 60-80 mesh, operated at 185°C. *Cis* and *trans* isomers were separated on a 6m column of 15% OV-275 on Chromosorb P 100-120 mesh (Supelco Inc.) operated at 220°C (Perkins et al., 1977).

Total isolated *trans* isomer content was determined by infrared spectrophotometry, AOCS tentative method Cd. 14-61 (1974) using a Beckman model 4300 infra-red spectrophotometer. *Cis,cis*-methylene interrupted fatty acids were determined enzymatically using lipoxidase type I (Sigma #L7127) by the method of MacGee (1959). The activity of lipoxidase was checked before use with cottonseed oil which contains approximately 50% *cis-cis* linoleic acid.

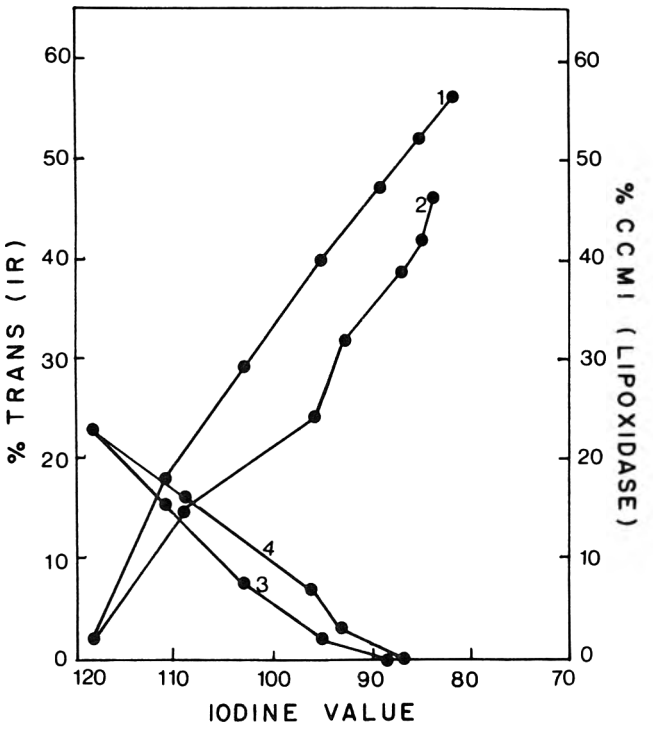

Fig. 1—Trans and cis,cis-methylene interrupted (CCMI) fatty acids as a function of iodine value in selectively hydrogenated Canola oil (mixed seed) using catalysts H and F:1—Trans isomer formation using catalyst H; 2—Trans isomer formation using catalyst F; 3—CCMI using catalyst H; 4—CCMI using catalyst F.

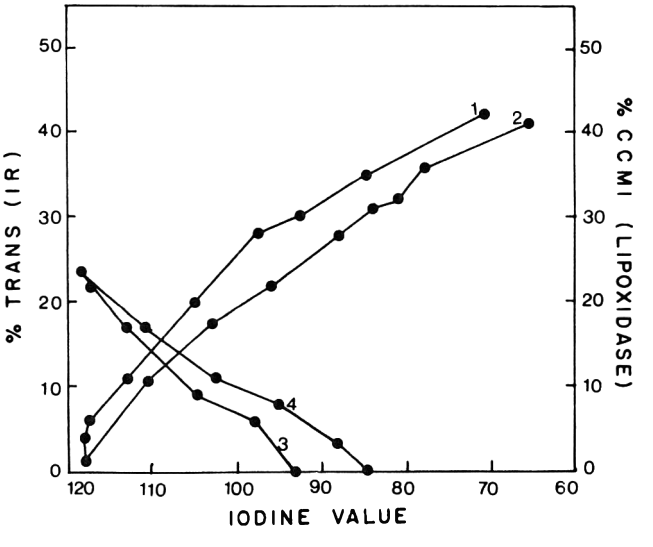

Fig. 2—Trans and cis,cis-methylene interrupted (CCMI) fatty acids as a function of iodine value in nonselectively hydrogenated Canola oil (mixed seed): 1—Trans isomer formation using catalyst H; 2—Trans isomer formation using catalyst F; 3—CCMI using catalyst H; 4—CCMI using catalyst F.

RESULTS & DISCUSSION

THE CHANGE IN IODINE VALUE as a function of hydrogenation time was used to measure the activity of catalysts H and F during the hydrogenation of the two Canola oils. The activity of the catalysts was higher under selective conditions than under nonselective conditions. Catalyst F was found to be slightly more active than catalyst H under selective and nonselective conditions of hydrogenation of the two Canola oils (Table 1).

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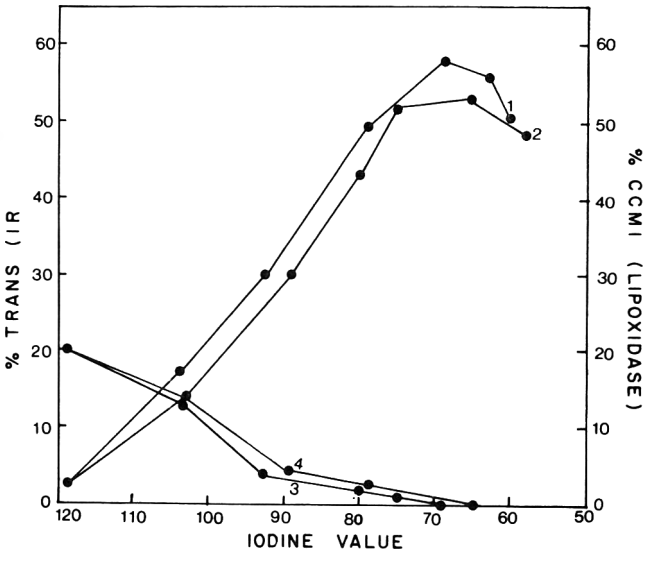

Fig. 3—Trans and cis,cis-methylene interrupted (CCMI) fatty acids as a function of iodine value in selectively hydrogenated Canola oil (Regent) using catalysts H and F: 1—Trans isomer formation using catalyst H; 2—Trans isomer formation using catalyst F; 3—CCMI using catalyst H; 4—CCMI using catalyst F.

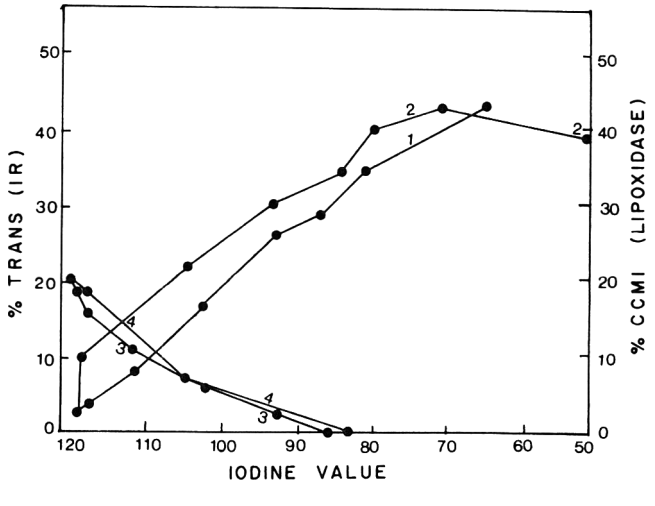

Fig. 4—Trans and cis,cis-methylene interrupted (CCMI) fatty acids as a function of iodine value in nonselectively hydrogenated Canola oil (Regent) using catalysts H and F: 1—Trans isomer formation using catalyst H; 2—Trans isomer formation using catalyst F; 3—CCMI using catalyst H; 4—CCMI using catalyst F.

Recent developments of very polar stationary phases on packed columns (Ottenstein et al., 1977; Perkins et al., 1977) have facilitated the separation of *cis* and *trans* fatty acids by gas chromatography; OV-275 was used in this study and resulted in good separation and recoveries of isomers. The values of total *trans* fatty acids obtained by gas liquid chromatography on OV-275 were plotted against those determined by infra-red spectrophotometry. The correlation coefficeint for the two methods was 0.989 for Canola oil (mixed seed) and 0.986 for Canola oil (Regent). The regression equation for Canola oil (mixed seed) was:

Trans by GLC = (*Trans* by IR) 0.879 + 2.458

For Canola oil (Regent) the equation was:

Trans by GLC = (*Trans* by IR) 0.924 + 2.596

Thus *trans* content by GLC was in good agreement with total *trans* determined by infra-red analysis. On comparing the data of *cis,cis*-methylene interrupted (CCMI) polyunsaturated fatty acids obtained by lipoxidase analysis and 18:2 cc by GLC, correlation coefficients of 0.966 and 0.975 were obtained for Canola oil (mixed seed) and Canola oil (Regent), respectively. The 18:2 cc content obtained by GLC was in good agreement with the CCMI determined by lipoxidase with the following regression equations:

18:2cc (GLC) = (CCMI) 0.758 + 2.632 Canola oil (mixed seed)

18:2cc (GLC) = (CCMI) 0.938 + 1.390 Canola oil (Regent)

Levels of *trans* isomers determined by IR and CCMI determined by lipoxidase found during selective and nonselective hydrogenation of the two oils with the two commercial catalysts are presented graphically in Fig. 1–4. Under selective conditions of hydrogenation, 45–56% *trans* acids were produced in the mixed seed oil in the iodine value range of about 80–85. In the Regent oil 53–58% *trans* acids were formed in the IV range of about 66–69. *Trans* acids in the 40–43% range were obtained in the IV range of about 65–70 under nonselective conditions in both oils. In Regent oil, under both selective and nonselective conditions, *trans* fatty acids after reaching a maximum level, showed a declining trend as *trans* isomers were saturated with further progress in hydrogenation. Under selective conditions of hydrogenation of both oils, *trans* isomer levels obtained with catalyst H were generally higher than those obtained with catalyst F. Under nonselec-

Table 2 – Trans content, specific isomerization index (SII) for selective hydrogenation of Canola and Regent oils using catalysts H and F

Canola oil	Hydrogenation time min	Catalyst H			Catalyst F		
		IV	% *Trans*	SII	IV	% *Trans*	SII
Mixed	0	118.4	2.3	–	118.4	2.3	–
	10	110.9	18.1	241.3	108.9	15.2	160.0
	20	102.7	29.5	187.9	96.4	23.9	108.6
	30	94.5	39.6	165.7	92.7	31.9	124.1
	40	88.8	46.5	157.1	87.0	39.3	125.2
	50	85.2	51.7	155.7	84.6	42.4	125.4
	60	81.6	55.6	151.1	84.4	45.9	135.0
Regent	0	119.4	2.7	–	119.4	2.7	–
	10	103.5	17.3	108.8	103.1	14.2	87.1
	20	92.5	30.1	111.9	89.0	30.3	99.7
	30	78.8	49.6	122.2	80.3	42.5	108.7
	40	68.7	57.8	114.0	74.9	51.8	116.4
	50	63.3	55.9	99.6	65.5	53.1	98.5
	60	59.8	50.5	84.7	57.9	48.6	79.0

Table 3 – Trans content, specific isomerization index (SII) for nonselective hydrogenation of Canola and Regent oils using catalysts H and F

Canola oil	Hydrogenation time min	Catalyst H			Catalyst F		
		IV	% *Trans*	SII	IV	% *Trans*	SII
Mixed	0	118.4	2.3	–	118.4	2.3	–
	10	118.3	4.1	–	111.1	11.0	150.7
	20	117.6	6.0	–	103.4	18.0	120.0
	30	113.2	10.7	205.8	95.5	22.0	96.9
	40	104.8	19.7	144.9	87.6	27.4	90.3
	50	97.6	27.6	132.7	84.6	31.3	84.3
	60	93.4	30.4	121.6	81.4	32.4	87.6
	80	84.9	35.3	105.4	78.3	36.2	90.3
	120	71.7	42.0	89.9	65.5	40.7	76.9
Regent	0	119.4	2.7	–	119.4	2.7	–
	10	118.9	2.7	–	118.4	9.7	–
	20	118.1	3.5	–	103.5	21.8	137.1
	30	111.2	7.6	92.7	91.8	31.0	112.3
	40	102.4	16.9	99.4	82.6	35.0	95.9
	50	91.9	25.8	93.8	81.0	36.6	95.3
	60	85.6	28.9	85.5	79.1	40.1	99.5
	80	79.6	34.8	87.4	70.2	43.4	88.2
	120	64.5	43.0	78.3	50.5	39.4	57.2

tive conditions, catalyst H resulted in the formation of more *trans* isomers than catalyst F in the mixed seed oil, whereas in Regent oil, catalyst F resulted in the formation of higher *trans* isomers than catalyst H.

The level of *cis,cis*-methylene interrupted polyunsatured fatty acids (CCMI) in the mixed seed oil was slightly higher for a given total unsaturation under nonselective conditions than under selective conditions. The opposite pattern was observed with Regent oil. In both oils, catalyst F generally resulted in a slightly better retention of CCMI than catalyst H. In each series of hydrogenations, the total unsaturation and level of CCMI decreased with the progress in hydrogenation and in all cases a complete loss of CCMI occurred before any significant amount of hardening took place. The complete loss of CCMI obtained during the early stages of hydrogenation of both Canola oils is related to the relatively low initial content of CCMI (20–23%) in these oils as compared to 54% and 64% in soybean and sunflower oils respectively (Ackman, 1977). Swindells (1970) also observed that high erucic acid rapeseed oil gives hydrogenated products which are very low in CCMI, much lower than products of equivalent solids content prepared from soybean or sunflower oils.

Specific isomerization index of hydrogenated oils

Puri (1978) has suggested that use of the specific isomerization index (SII) to characterize the *trans* isomerization during hydrogenation. The SII is defined as the number of *trans* bonds formed per unit reduction of iodine value. The SII was calculated for the hydrogenated oils from the relationship:

$$SII = (\%\ trans/IV_0) \times 100$$

where IV_0 and IV are the initial and final iodine values of the oil. The SII values are included in Tables 2 and 3. During the initial stages of hydrogenation of the mixed seed oil, a sharp increase in SII values took place (150–240) in the iodine value range of 109–113 under both conditions of selectivity. Maximum SII values were observed during selective hydrogenation of the mixed seed oil using catalyst H and decreased slightly with progress in hydrogenation until the iodine value reached 82. Selective hydrogenation of the mixed seed oil using catalyst F resulted in a specific isomerization index of 160 at an iodine value of 109 and decreased in the initial stages of hydrogenation until the iodine value reached 96. This was followed by a slight increase with further progress in hydrogenation. The specific isomerization index values during nonselective hydrogenation of the mixed seed oil decreased with increase in hydrogenation time up to an iodine value of 72 with catalyst H. Use of catalyst F during nonselective hydrogenation of the mixed seed oil also resulted in increasing SII up to an IV of 66. During selective hydrogenation of the Regent oil, SII values increased slightly with a decrease in iodine value to 79 using catalyst H. Selective hydrogenation of Regent oil using catalyst F also resulted in an increase in SII values. After reaching an iodine value of 75, the SII declined with

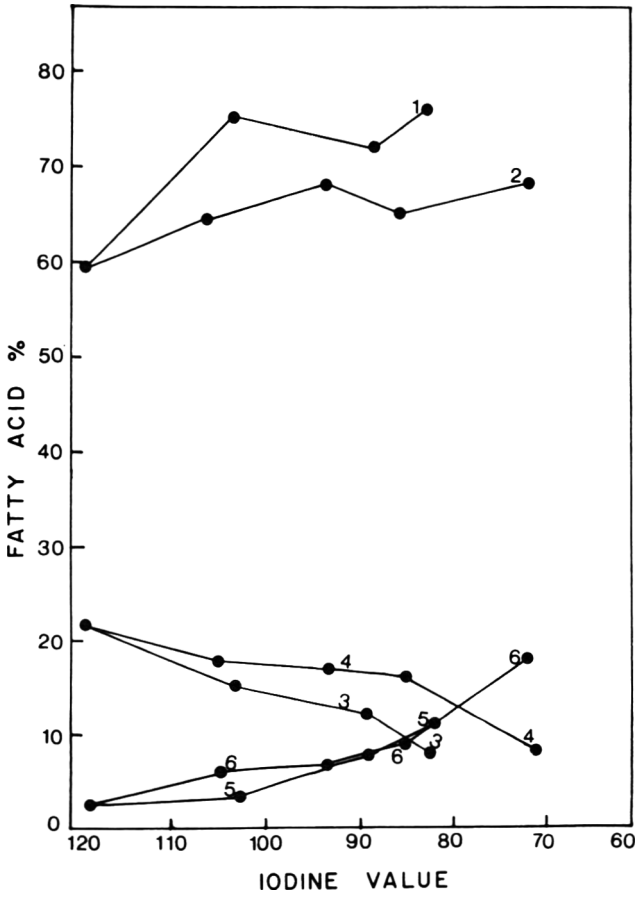

Fig. 5–C_{18} fatty acids as a function of iodine value in nonselectively hydrogenated Canola oil (mixed seed) using catalyst H: 1–18:1 fatty acids during selective conditions; 2–18:1 fatty acids during nonselective conditions; 3–18:2 fatty acids during selective conditions; 4–18:2 fatty acids during nonselective conditions; 5–18:0 fatty acids during selective conditions; 6–18:0 fatty acids during nonselective conditions.

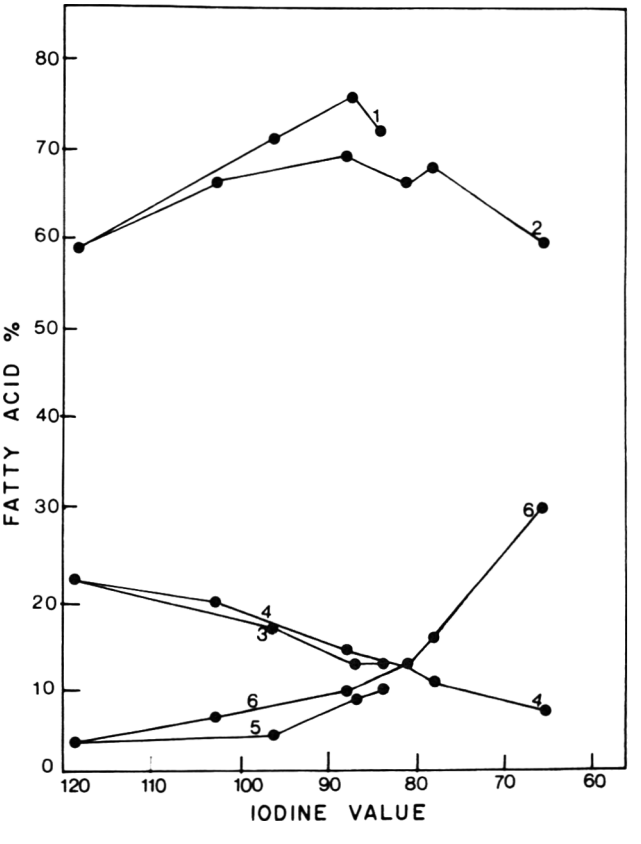

Fig. 6–C_{18} fatty acids as a function of iodine value in selectively and nonselectively hydrogenated Canola oil (mixed seed) using catalyst F: 1–18:1 fatty acids during selective conditions; 2–18:1 fatty acids during nonselective conditions; 3–18:2 fatty acids during selective conditions; 4–18:2 fatty acids during nonselective conditions; 5–18:0 fatty acids during selective conditions; 6–18:0 fatty acids during nonselective conditions.

both catalysts. The SII values during nonselective hydrogenation of Regent oil with catalyst H decreased until the iodine value reached 50. With catalyst F, the SII values were variable and no consistent pattern was observed. These data on specific isomerization index indicated that catalyst H generally resulted in higher SII values than catalyst F during selective hydrogenation of the two Canola oils. During nonselective hydrogenation, catalyst H resulted in higher SII values than catalyst F in the mixed seed oil, whereas in Regent oil, the opposite trend was observed and higher SII values were obtained with catalyst F than with catalyst H. These results indicated that in addition to selectivity of the catalysts, another important factor to be considered in catalyst selection is the *trans* isomer formation.

Fatty acid composition of hydrogenated oils

Fig. 5–8 indicate changes in the levels of C_{18} fatty acids during the progress of hydrogenation of the two oils. The C_{18} fatty acids constitute 90–92% of the total fatty acids. Selective conditions of hydrogenation of both oils resulted in slightly more saturation of linoleic acid and produced more 18:1 and less 18:0. The opposite pattern was observed under nonselective conditions. The two types of catalysts used produced different levels of these fatty acids during hydrogenation of both oils. From the change in fatty acid composition during selective hydrogenation of th two oils, selectivity ratios were determined according to the procedure of Allen (1978) and these are listed in Table 4. The selectivity ratio is defined as K_2/K_3 where:

$$K_2 = 1 - L/L_o$$

and $$K_3 = S - S_o$$

L_o and S_o represent the linoleic and stearic acid content of the oil and L and S the levels in the hydrogenated samples. Catalyst H was more selective than the catalyst F during hydrogenation of both oils (Table 4).

Fig. 9–12 indicate the percentage of 18:1t and 18:2tt fatty acids as a function of iodine value for the hydrogenation of the two oils using catalysts H and F. *Trans* monoenes (18:1t) were the most abundant *trans* fatty acids, whereas *trans* dienes occurred only at low levels from 0 to 3.8%. The presence of *trans-trans* diene fatty acids has received considerable attention lately because of their possible health implications. Questions have been raised (Jackson et al., 1980) about the composition of the isomers covered by the "*trans-trans* diene" peak obtained by separation on OV-275 columns. These authors suggest that the "*trans-trans*" peak includes *cis-cis*, *trans-cis* and *cis-trans*

Table 4 — Selectivity ratios for selective hydrogenation of oils

Canola oil	Hydrogenation time min	Selectivity ratio	
		Catalyst H	Catalyst F
Mixed	20	46.7	29.8
	40	9.8	8.7
	60	8.5	5.5
Regent	20	41.4	28.0
	40	6.3	5.5
	60	3.3	3.4

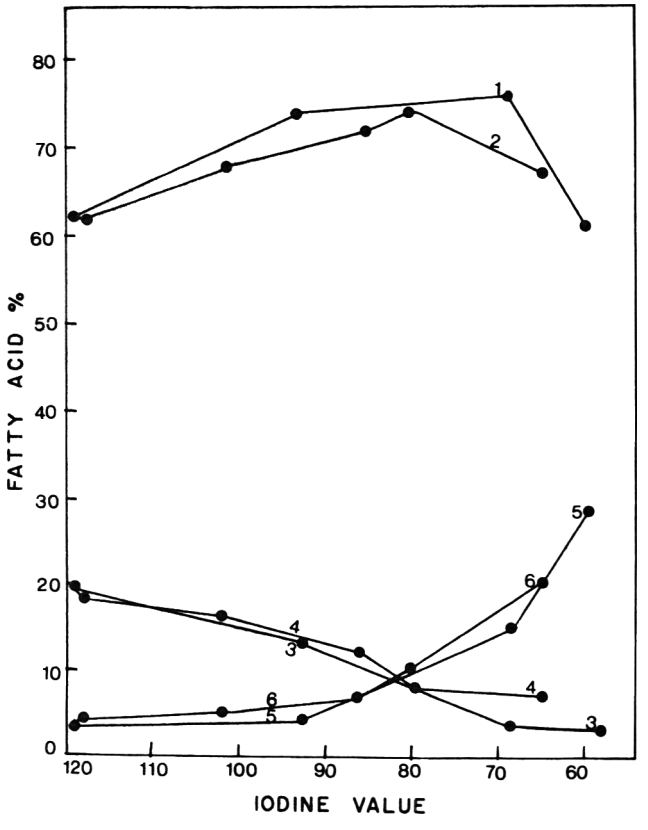

Fig. 7–C_{18} fatty acids as a function of iodine value in selectively and nonselectively hydrogenated Canola oil (Regent) using catalyst H: 1–18:1 fatty acids during selective conditions; 2–18:1 fatty acids during nonselective conditions; 3:18:2 fatty acids during selective conditions; 4–18:2 fatty acids during nonselective conditions; 5–18:0 fatty acids during selective conditions; 6–18:0 fatty acids during nonselective conditions.

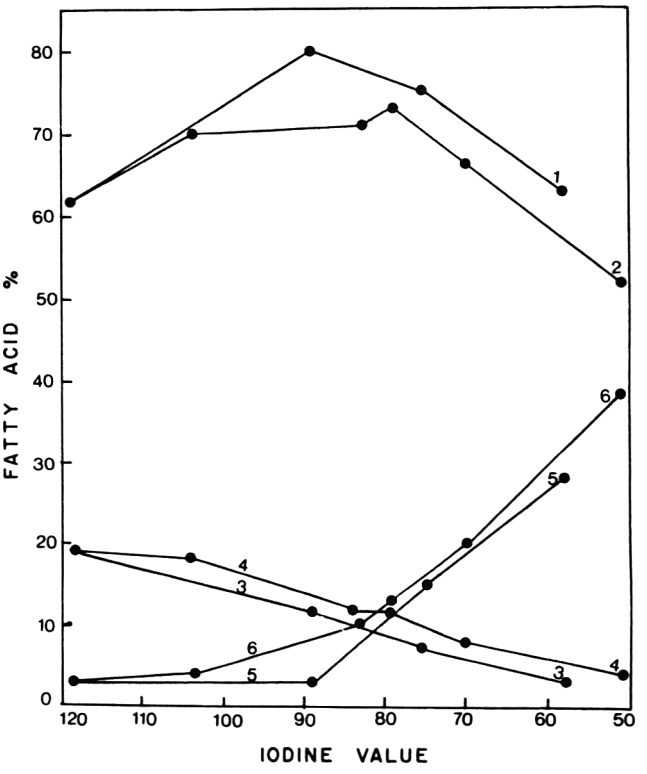

Fig. 8–C_{18} fatty acids as a function of iodine value in selectively and nonselectively hydrogenated Canola oil (Regent) using catalyst F: 1:18:1 fatty acids during selective conditions; 2:18:1 fatty acids during nonselective conditions; 3–18:2 fatty acids during selective conditions; 4:18:2 fatty acids during nonselective conditions: 5–18:0 fatty acids during selective conditions; 6–18:0 fatty acids during nonselective conditions.

positional isomers as well. Chromatograms obtained in our laboratory show distinctly separated peaks for *cis-trans* and *cis-cis* isomers. However, until this controversy has been resolved, the importance of the *trans-trans* levels should be interpreted with caution.

Selective hydrogenation of the two oils generally resulted in the formation of more *trans* monoenes than non-selective conditions and the two catalysts behaved different in the formation of *trans* monoenes. There was very little difference in *trans,trans*-diene formation (18:2tt). Selective hydrogenation of the mixed seed oil with catalyst H resulted in the formation of more *trans* monoenes than with catalyst F but both catalysts were identical in the formation of *trans* monoenes during non-selective hydrogenation of the mixed seed oil. With Regent oil, use of catalyst H generally resulted in the formation of more *trans* monoenes than with catalyst F during selective hydrogenation, whereas during non-selective hydrogenation, the opposite pattern was observed. Hydrogenation of the mixed seed oil with catalyst H resulted in the formation of slightly more 18:2tt than with catalyst F under both selective and nonselective conditions. During hydrogenation of Regent oil, catalyst F resulted in the formation of slightly more 18:2tt than with catalyst H under both selective and non-

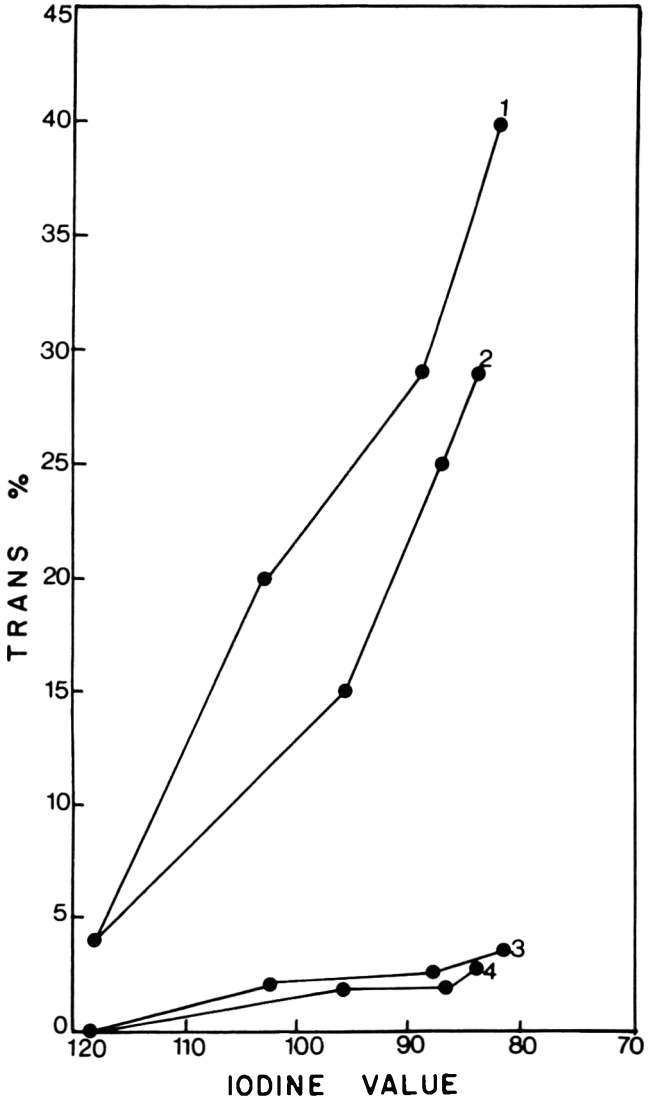

Fig. 9—18:1t and 18:2tt fatty acids as a function of iodine value in selectively hydrogenated Canola oil (mixed seed) using catalysts H and F: 1—18:1t fatty acids using catalysts H and F: 1—18:1t fatty acids using catalyst H; 2—18:1t fatty acids using catalyst F; 3—18:2tt fatty acids using catalyst H; 4—18:2 fatty acids using catalyst F.

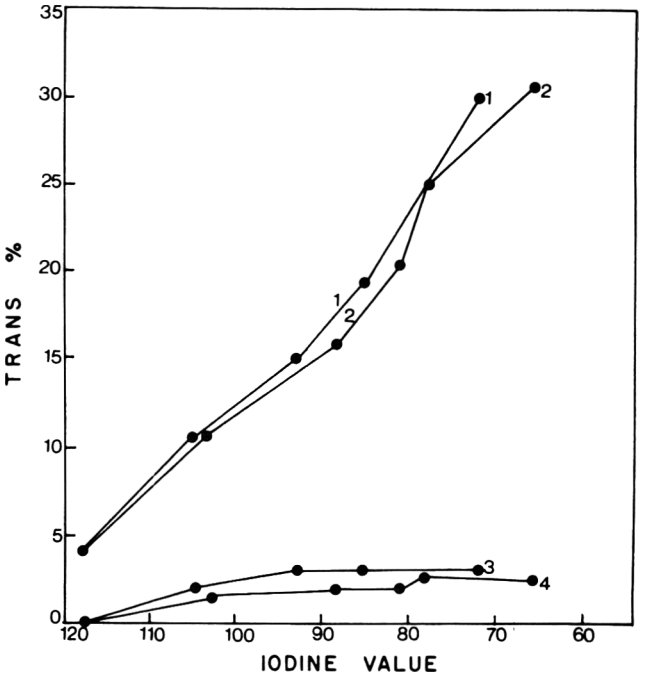

Fig. 10—18:1t and 18:2tt fatty acids as a fucntion of iodine value in nonselectively hydrogenated Canola oil (mixed seed) using catalysts H and F: 1—18:1t fatty acids using catalyst H; 2—18:1t fatty acids using catalyst F; 3—18:2tt fatty acids using catalyst H; 4—18:2tt fatty acids using catalyst F.

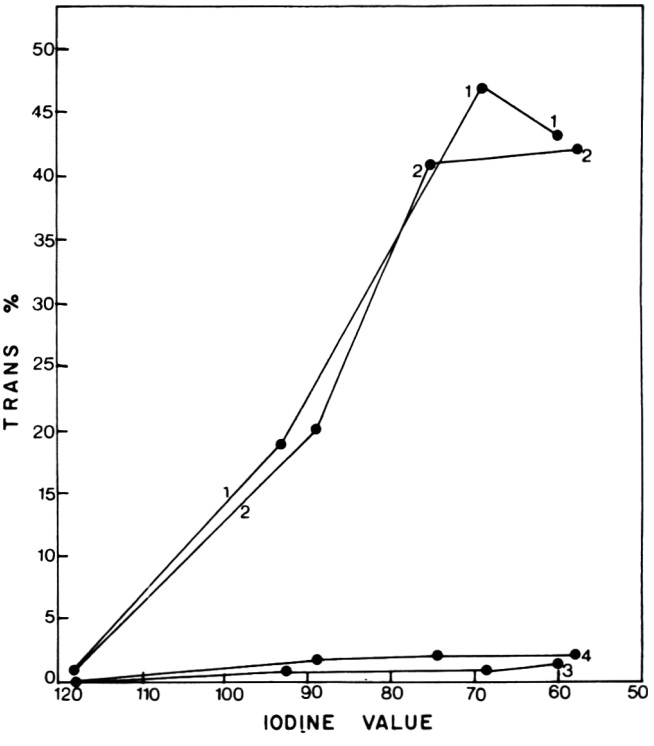

Fig. 11—18:1t and 18:2tt fatty acids as a function of iodine value in selectively hydrogenated Canola oil (Regent) using catalysts H and F: 1—18:1t fatty acids using catalyst H; 2—18:1t fatty acids using catalyst F; 3—18:2tt fatty acids using catalyst H; 4—18:2tt fatty acids using catalyst F.

selective conditions. The different values obtained from the analysis of *trans* isomers in both hydrogenated oils indicate that the type of catalyst is an important factor influencing the formation of *trans* monoenes as well as *trans,trans*-dienes during hydrogenation of Canola oils.

The results obtained in this study of hydrogenated oils under two sets of hydrogenation conditions of unequal selectivity using two types of commercial catalysts showed that selective conditions resulted in a higher level of *trans* isomers. The loss of CCMI occurred in the early stages of the hardening process with both selective and nonselective conditions. There was also a difference in the fatty acid composition and specific isomerization index. The two commercial catalysts differed in their action on both oils under selective as well as nonselective conditions. The activity of the catalysts was higher under selective conditions of hydrogenation of both oils. Different selectivity ratios as well as *trans* isomerization characteristics were obtained with both catalysts under selective and nonselective conditions of hydrogenation.

The hydrogenation of edible oils is an important step in the processing of oils into plastic fat products such as margarine and shortening. By controlling hydrogenation conditions, the food technologist can influence the chemical composition of the resulting products. In addition, the physical properties of the hydrogenated fats are of utmost importance, since these relate to such consumer concerns as spreadability, mouthfeel and shortening properties. The effect of hydrogenation conditions on physical properties of Canola oil will be the subject of a later report.

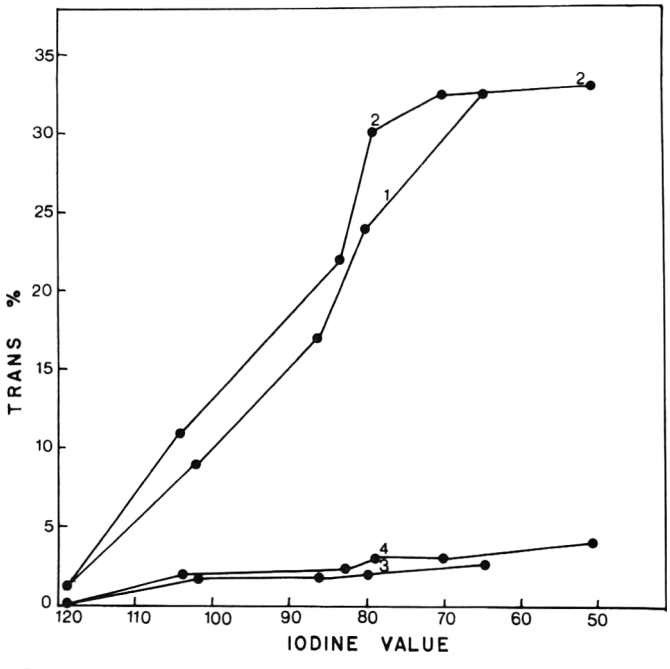

Fig. 12—18:1t and 18:2tt fatty acids as a function of iodine value in nonselectively hydrogenated Canola oil (Regent) using catalysts H and F: 1—18:1t fatty acids using catalyst H; 2—18:1t fatty acids using catalyst F; 3—18:2tt fatty acids using catalyst H; 4—18:2tt fatty acids using catalyst F.

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Inactivation and Injury of a Hemolytic Radiation-Resistant Micrococcus Isolated From Chicken Meat

SHIEH-TE TAN and R. B. MAXCY

ABSTRACT

The effects of environmental factors on a highly radiation-resistant hemolytic micrococcus isolated from chicken meat were studied. NaCl tolerance and gamma radiation resistance of the cells were growth phase related. The cells were resistant to injury from drying or freezing/thawing. Under certain conditions, cells in the frozen state required approximately 5 Mrad to inactivate 90% of the population; 0.2 Mrad injured an equivalent proportion. Survival curve of the cells heated at 60°C showed a unique pattern which was in three distinct phases. Heat-stressed cells were much more sensitive to radiation inactivation than unheated cells. When suspended in fresh m-Plate Count Broth (PCB), the injured cells repaired without multiplication during incubation at 32°C. The repair process in this bacterium, however, was slower compared to thermally injured organisms studied by other workers.

INTRODUCTION

HIGHLY RADIATION-RESISTANT MICROCOCCI that were hemolytic on blood agar have been isolated from chicken meat (Welch and Maxcy, 1979). The occurrence of these bacteria in radurized food creates a concern for the commercial acceptability of the process, because bacteria capable of producing hemolysis on blood agar are commonly considered of public health significance (Bailey and Scott, 1974). Though highly radiation-resistant bacteria are not present in great numbers (Maxcy et al., 1976), their presence in the residual flora must be considered. Radurization might selectively favor the highly radiation-resistant bacteria during any subsequent opportunity for growth, because most of the preirradiation contaminants would be destroyed.

An early review paper (Bridges and Horne, 1959) discussed the many environmental factors that influence the destruction of bacteria by ionizing radiation. The emphasis, however, was on sensitive vegetative cells and endospores. In many ways the response of highly radiation-resistant vegetative cells is not predictable from data on either sensitive cells or endospores (Keller, 1981; Ma and Maxcy, 1981).

Welch and Maxcy (1979) reported the striking NaCl intolerance of some highly radiation-resistant hemolytic micrococci. No attempt was made, however, to study the factors affecting the NaCl tolerance of these bacteria. Also, little research has been done to determine the response of these micrococci to environmental factors.

In this investigation, a representative isolate of the hemolytic micrococci (Welch and Maxcy, 1979) was used to study the impact of salt, drying, freezing/thawing, heating, and irradiation on these bacteria. Consideration was also given the potential role of the injury and repair processes.

MATERIALS & METHODS

Organisms and culture conditions

Isolate C-7 from the work of Welch and Maxcy (1979) and *Escherichia coli* were propagated on Plate Count Agar (PCA; Difco) at 32°C and stored at 5°C. Cultures were rejuvenated by inoculating into m-Plate Count Broth (PCB; Difco) and incubated in a shaker-incubator. Cultures in the early stationary phase, unless specified in the results, were used for each test.

Fate of cells on stainless steel surfaces

Cultures were inoculated onto stainless steel, dried, stored for timed intervals, and subsequently evaluated for colony forming units (CFU). The detailed procedure was described by Maxcy (1975).

Freezing/thawing and lyophilization

Cultures were quick-frozen by immersing the tubes in an acetone-dry ice bath for 5 min. Some of these frozen cultures were lyophilized with a Virtis Model NO. 10/145 MRBA lyophilizer. Frozen and lyophilized cultures were held at −20°C for further observations. Frozen cultures were thawed by immersing the tubes in water at room temperature.

Heat destruction of cells

For heat sensitivity studies, PCB cultures in 10 x 70 mm tubes were heated in water bath for timed intervals. Following heating, tubes were immediately placed in 5°C water for holding prior to plating for cell enumeration.

Irradiation

A Cobalt-60 irradiator of the design described by Teeny and Miyauchi (1970), providing a gamma-radiation dose of approximately 5 Krad per minute, was used. PCB cultures in tubes were quick-frozen and irradiated at subfreezing temperature (−30 ± 10°C) by utilizing dry ice in the sample carrier.

Enumeration procedures

The general procedures for cell enumeration were those outlined by Speck (1976). Plating was made with PCA or PCA plus salt. For Isolate C-7, counts expressed as CFU were determined after 5–9 days of incubation at 32°C. The longer incubation time was needed because inclusion of NaCl in PCA and/or irradiation-induced cell injury delayed visible colony formation. For *E. coli*, plates were incubated at 37°C for 24 hr.

Determination of cell injury and repair

PCA containing NaCl was used to estimate thermal injury in this study. As will be seen in the Results, Isolate C-7 cells harvested at or before early stationary phase were able to form colonies on both PCA and PCA plus 1.2% NaCl (PCAS). Based on these results, cell injury of Isolate C-7 was measured by the difference in colony counts with PCA and PCAS. The gain of ability to form colonies in PCAS was a measure of repair.

Each experiment described in this paper was repeated at least two times, but always until logically acceptable reproducible results were obtained. The results shown represent the average of data from each experiment.

RESULTS

Response of Isolat C-7 cells to NaCl

Cultures of Isolate C-7 in the early stationary phase were diluted and inoculated into PCB containing 0, 0.8, 1.0, 1.2, or 1.4% of NaCl. Care was taken to ensure a homogeneous initial cell population (approximately 100

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CFU/ml) among flasks. The cultures were incubated in a shaker-incubator at 32°C and sampled for plating with PCA at timed intervals. The results expressed in the form of growth curves are shown in Fig. 1. High concentration of NaCl in media results in a longer lag phase, a slower exponential growth rate, and a reduced maximum population.

PCB cultures of Isolate C-7 were also studied after 13, 27, 49, and 72 hr of incubation to determine the effect of physiological age on tolerance to NaCl. As shown in Fig. 1, cells harvested at these four times were at exponential phase, early stationary phase, stationary phase, and late stationary phase, respectively. The results show that the progression of incubation renders Isolate C-7 cells less tolerant to NaCl (Fig. 2).

In another experiment, NaCl was added to fully-grown PCB cultures of Isolate C-7 at four levels (0, 1.2, 1.6, and 10%) and incubated at 32°C. Tubes were sampled at intervals for survivor estimation by plating with PCA. As shown in Fig. 3, the level of NaCl tolerance was between 1.2 and 1.6%.

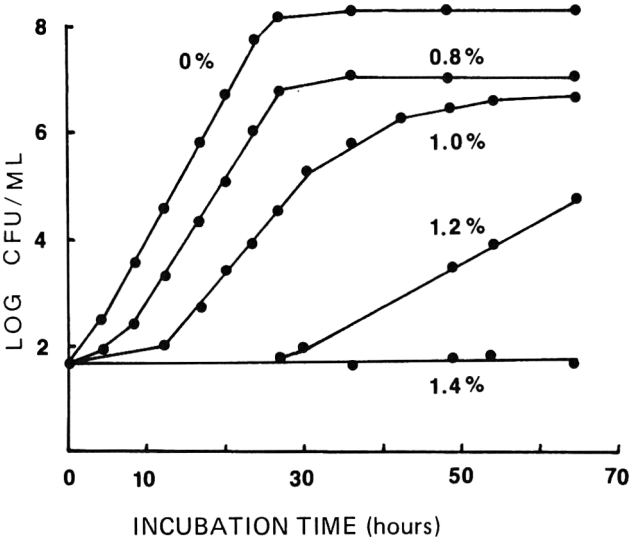

Fig. 1—Growth of Isolate C-7 in PCB with various concentrations of added NaCl.

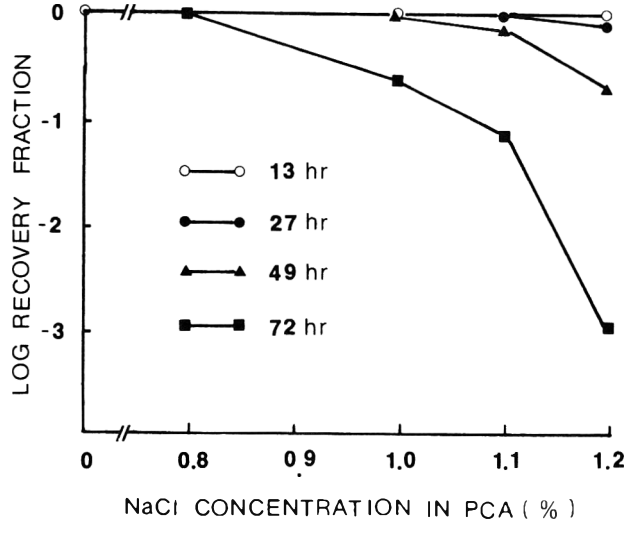

Fig. 2—Effect of physiological age on recovery of Isolate C-7 cells in PCA with various concentrations of NaCl.

The tolerance level to NaCl for some cells was dependent on previous stress. For example, Isolate C-7 cells survived 1.5 Mrad of gamma radiation without significant numbers being inactivated as determined by plating with PCA. However, part of the population failed to grow in PCA when NaCl was added at 1.0% or more (Fig. 4).

Resistance to drying and storage

To determine the fate of Isolate C-7 and *E. coli* on food processing equipment, cultures suspended in PCB were inoculated onto stainless steel and allowed to dry at 25°C. Numbers of survivors were determined for up to 3 wk. The data in Fig. 5 show no apparent change in numbers of Isolate C-7 while the numbers of *E. coli* decreased more than 3 logs in 3 wk.

For further comparison, cultures of Isolate C-7 and *E. coli* were harvested and washed twice in distilled water, then suspended in distilled water for transfer onto stainless steel. This treatment was to simulate removal of nutrients in a washing process. Results in Fig. 5 show washing of Isolate C-7 had no apparent subsequent effect on longevity on stainless steel. Washed cells of *E. coli*, however, were exceedingly vulnerable under the same conditions. *E. coli* cells lost viability during visible drying within the first 2 hr.

Effects of freezing/thawing and freeze-drying on viability

Fig. 6 shows that neither freezing/thawing nor freeze-drying reduced the CTU obtained with PCA. Likewise, neither freezing/thawing nor freeze-drying caused injury of cells of Isolate C-7, because there was no apparent difference between counts on PCA and counts on PCA plus NaCl at various levels up to 1.4%. When the freezing/thawing process was repeated 10 times on the same cells, there was no detectable effect on the viability. Slow-freezing of the cells at −20°C for 1 hr instead of quick-freezing in an acetone-dry ice bath during the freezing/thawing cycles did not result in any difference.

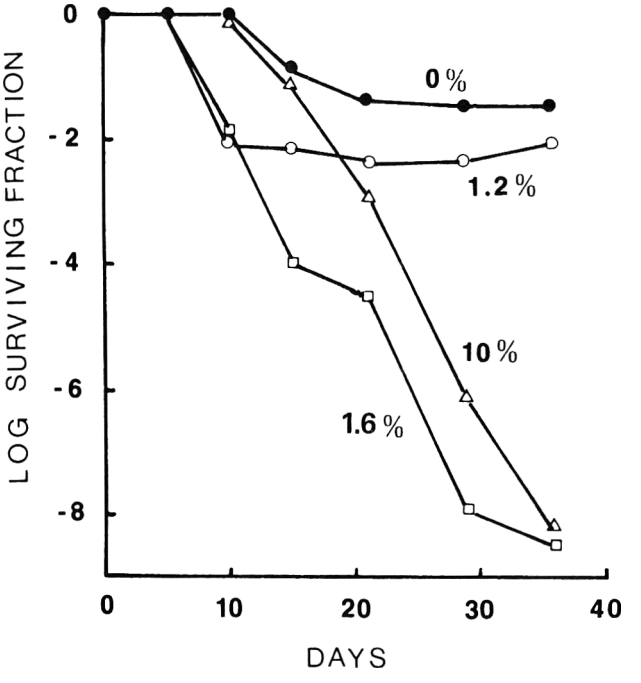

Fig. 3—Survival of Isolate C-7 at 32°C in PCB with various concentrations of NaCl.

Effect of mild heating on Isolate C-7

The survival curve of Isolate C-7 at 60°C is shown in Fig. 7. The curve can be divided into three distinct segments which might be suggestive of two or more populations with widely different heat sensitivities. The first segment shows the culture to be quite sensitive to heat, while the remainder of the curve shows that a much longer exposure at 60°C is required before there is apparent destruction.

Attempts were made to obtain a population to show only the resistant segment by subculture of survivors of a heat treatment for 40 min at 60°C. Five cycles of heating and subculturing of survivors did not change the pattern of the survivors shown in Fig. 7.

While the heating process at 60°C was destructive during the first 10 min, there was a secondary effect in the form of injury as measured by failure to grow on PCAS (Fig. 8). Injury was apparent even before the medium reached 60°C; more than 99.9% were injured in 5 min. This injury phenomenon applied to those cells of high sensitivity accounting for the first phase of the survival curve. Cells which survived during the second phase, however, were much more resistant to injury; more than 20% of the cells remained noninjured.

Effect of physiological age on radiation resistance of Isolate C-7

Fig. 9 shows the radiation resistance of Isolate C-7 cells in various growth phases. Cells in the early stationary phase appear to be the most resistant, while those at the later part of the exponential phase were among the least resistant. Growth phase-related variation in survival increased significantly as the dose of radiation was increased.

Although Isolate C-7 cells are highly radiation resistant, they are easily injured by a low dose of radiation. The progression of injury with increased dose is shown in Fig. 10.

Radiation susceptibility of thermally stressed Isolate C-7 cells

To study the combined effect of heat and radiation on Isolate C-7, cultures were heated at 60°C for 3 min. Approximately 70% of the cells were inactivated and the survivors were extremely sensitive to subsequent exposure to radiation (Fig. 11).

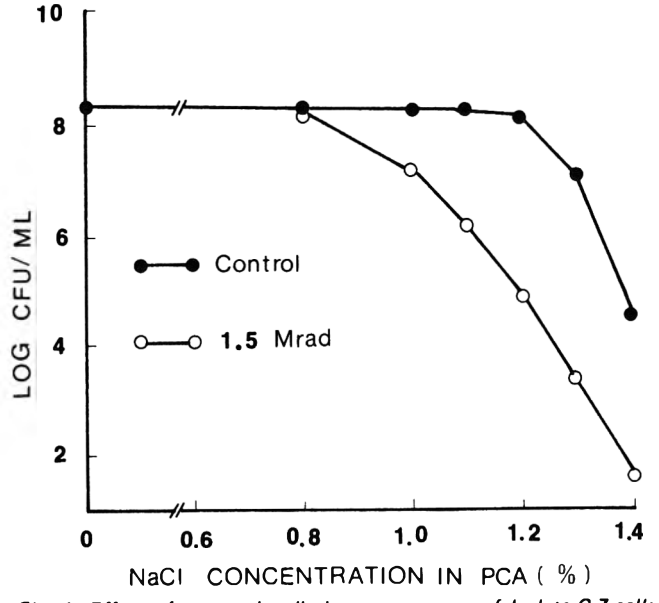

Fig. 4—Effect of gamma irradiation on recovery of Isolate C-7 cells in PCA with various concentrations of NaCl.

Repair of thermally injured Isolate C-7

Cultures of Isolate C-7 were heated at 60°C to reduce the total population by approximately 70%. The stressed cultures were then subcultured in fresh PCB and incubated at 32°C. Samples were taken at intervals for plating with both PCA and PCAS. As shown in Fig. 12, Isolate C-7 cells repaired during the incubation as evidenced by the increased numbers of CFU on PCAS while the numbers on PCA remained constant. When the initial number of stressed cells (total survivors) in PCB was adjusted to approximately 10^2 CFU/ml, the same repair phenomenon prevailed irrespective of the dilution process (data not given).

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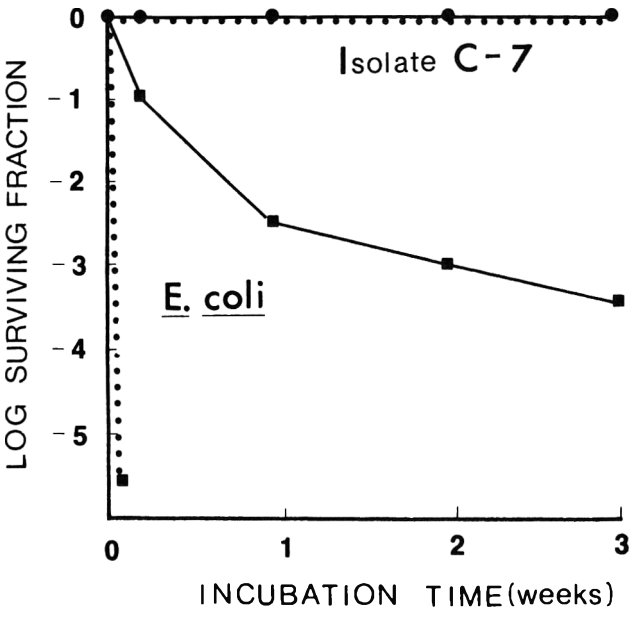

Fig. 5—Survival of Isolate C-7 and E. coli on a stainless steel surface: ●, Isolate C-7, 27-hr old, 2 x 10^8 CFU/ml; ■, E. coli, 20-hr old, 10^9 CFU/ml; —, Cells suspended in PCB; · · ·, Cells washed and suspended in distilled water.

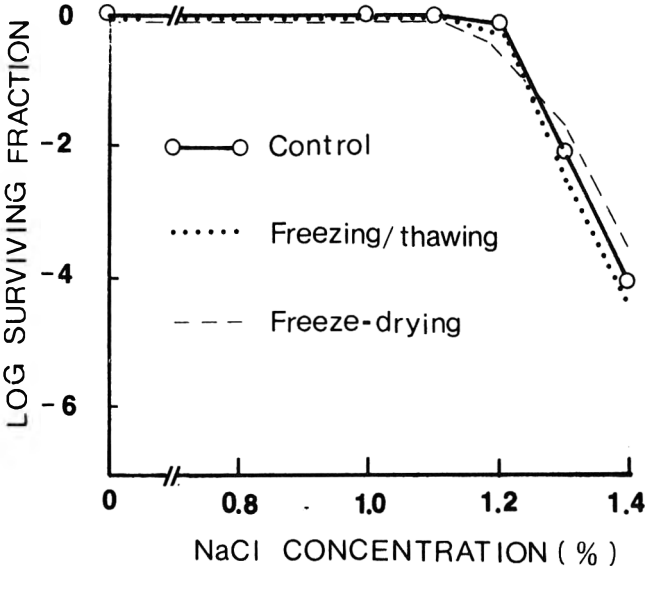

Fig. 6—Effects of freezing/thawing and freeze-drying on the recovery of Isolate C-7 in PCA with various concentrations of NaCl.

DISCUSSION

IT IS GENERALLY RECOGNIZED that certain bacteria common in food are quite resistant to radiation (Ingram and Farkas, 1977). Their significance in irradiated food is not known, but it is recognized that the process of irradiation would selectively favor radiation-resistant elements of the flora. Their fate in irradiated food would depend on their physiological characteristics.

Some radiation-resistant vegetative bacteria have rather unique physiological characteristics; e.g., the radiation-resistant hemolytic micrococci isolated by Welch and Maxcy (1979). These bacteria were unexpectedly sensitive to NaCl in comparison to other common *Micrococcus* sp., but the level of NaCl tolerance was comparable to another highly radiation-resistant vegetative bacterium, *Micrococcus radiophilus* (Lewis, 1971). The latter was isolated from salt water fish. The low salt tolerance suggests these bacteria might be sensitive to the killing and injurious effects of drying and storage. The *Micrococcus* sp. used in our work, however, was extremely resistant to drying and storage. An ongoing experiment indicates this bacterium suspended in PCB and allowed to dry on stainless steel survives well over 20 months at room temperature because there was a reduction on count of only 1 log cycle. The relation between high radiation resistance and resistance to drying is not known though it has been observed in other highly radiation-resistant bacteria (Sanders and Maxcy, 1979).

The great resistance to radiation is related to available water, because either freezing or drying increases markedly radiation resistance. This is a major factor with the highly radiation-resistant bacteria (Bruns and Maxcy, 1979; Ma and Maxcy, 1981). Thus, it is apparent that a process of irradiation of food must consider the effect of available water.

Consideration must also be given physiological age and unique tolerance of the radiation-resistant microflora of concern. In our studies on a radiation-resistant micrococcus, growth phase-related variation was observed in (1) NaCl tolerance, (2) injury and killing by heat, and (3) injury and killing by radiation. Any system of evaluating injury is somewhat arbitrary, but the system used in this work relates to a_w, which is in turn associated with salt concentration of the medium. Thus, physiological age as a factor influencing NaCl tolerance of the cells must be considered in the detection of injury. By the same token, data related to inactivation must be interpreted with the effect of culture age in mind. It is interesting to point out that, for different bacteria, cells in the exponential phase can be either more or less radiation sensitive than those that have reached stationary phase. The micrococcus of this study and *M. radiodurans* (Duggan, 1961; Serianni and Bruce, 1968) exemplify the higher sensitivity of exponential phase, while a *Moraxella-Acinetobacter* strain represents the opposite (Keller, 1981).

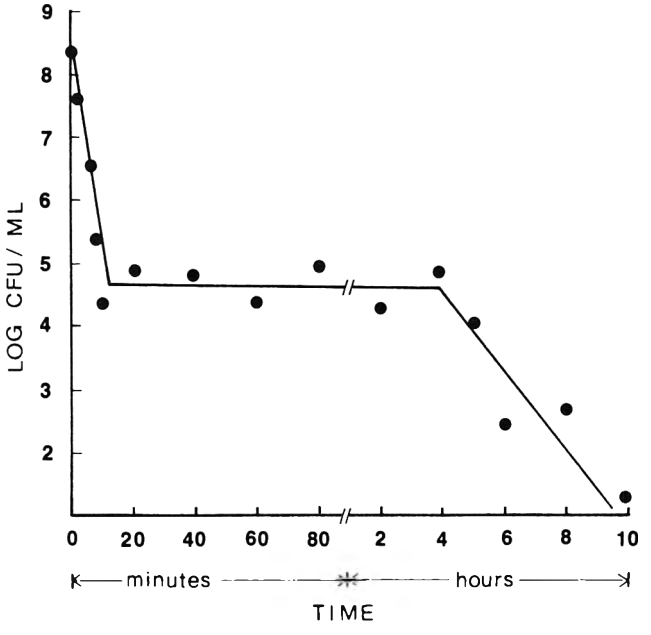

Fig. 7—Survival curve of Isolate C-7 when heated at 60°C. Heating time included 2.5 min to reach 60°C.

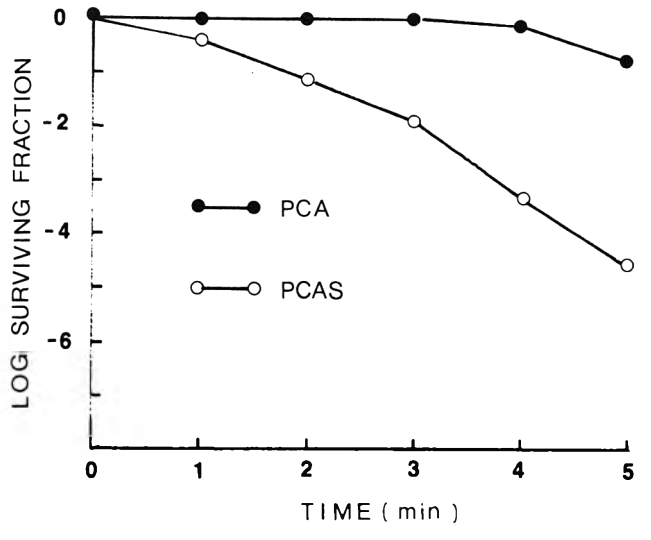

Fig. 8—Injury of Isolate C-7 cells by heating at 60°C. Heating time included 2.5 min to reach 60°C.

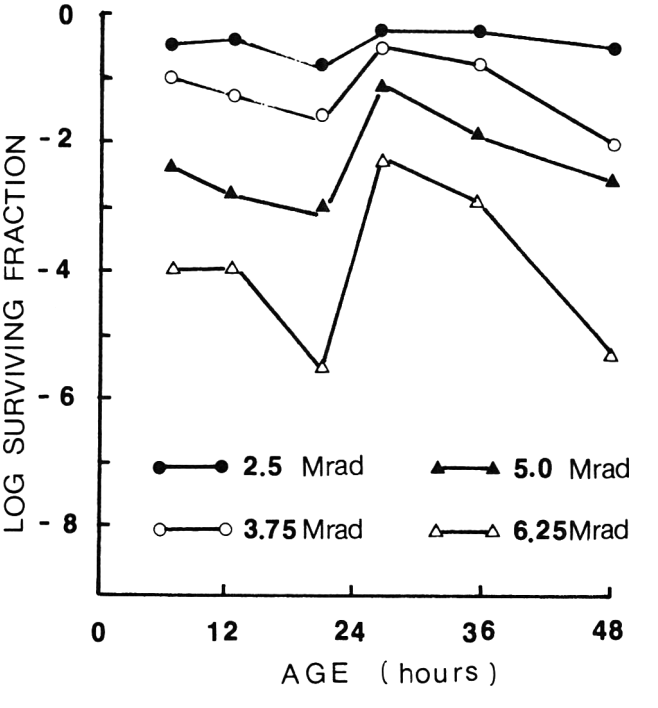

Fig. 9—Effect of physiological age on gamma radiation-resistance of Isolate C-7 cells. Refer to Fig. 1 for culture conditions.

Stapleton (1955) explained the abnormal sensitivity of *Escherichia coli* cells at the logarithmic growth phase by suggesting that, when rapidly dividing, there is no sufficient time for a cell to repair the genetic damage which is generally considered to be the major effect of radiation. Results on *E. coli* and on *Micrococcus* sp. in our study, however, show tremendous variation in resistance of cells at various sections of logarithmic growth phase during which cells multiply at a constant rate. Therefore, factor(s) other than dividing rate must be considered for an explanation of the abnormal sensitivity of some cells in the logarithmic growth phase.

To enhance the effectiveness of food irradiation, it is worthwhile to explore the factors to which the radiation-resistant flora are tolerant. Heat treatment is one of the factors. It is generally observed that radiation-resistant vegetative bacteria are quite heat sensitive and their radioresistance can be significantly reduced by a combined application of heat and radiation (Kitayama and Matsuyama, 1973; Maxcy and Rowley, 1978; Firstenberg-Eden et al., 1980). In this study, a pre-heating treatment, which inactivated only 70% of the micrococcal cells, eliminated the shoulder in the radiation survival curve and strikingly sensitized the cells to radiation destruction (Fig. 11). This is in agreement with the work with *Moraxella-Acinetobacter* by Maxcy and Rowley (1978) and Firstenberg-Eden et al. (1980). However, the presence of a heat-resistant phase in the heat killing curve of the micrococcus, as shown in Fig. 7, should be of some concern. Further investigation is needed to determine whether cells responsible for this heat-resistant phase can be sensitized to radiation by a heat treatment. The unique survival curve suggests a population of different heat resistance in a single culture of bacteria (Moats et al., 1971). Since subcultures of survivors of this micrococcus were no more resistant to heat than parent culture, the variation in resistance observed must be considered a physiological rather than a genetic effect. This observation is unique compared to other reports (Corry and Roberts, 1970; Duitschaever and Jordan, 1974).

When the microenvironment is favorable, thermally stressed cells of the micrococcus are able to regain their tolerance to 1.2% NaCl, and repair is nearly completed before cell multiplication is observed (Fig. 12). After the repair treatment, the cells would be expected to respond as a young growing culture.

The high resistance of the micrococcus to freezing/thawing and freeze-drying (Fig. 6) is in agreement with the general observations for Gram positive cocci (Ingram and Mackey, 1976; Bousfield and Mackenzie, 1976).

The factors discussed in the report must be considered in establishing criteria for the process of irradiation of foods. No evidence indicates that this bacterium will pose particular problems as a member of the surviving microflora in irradiated food.

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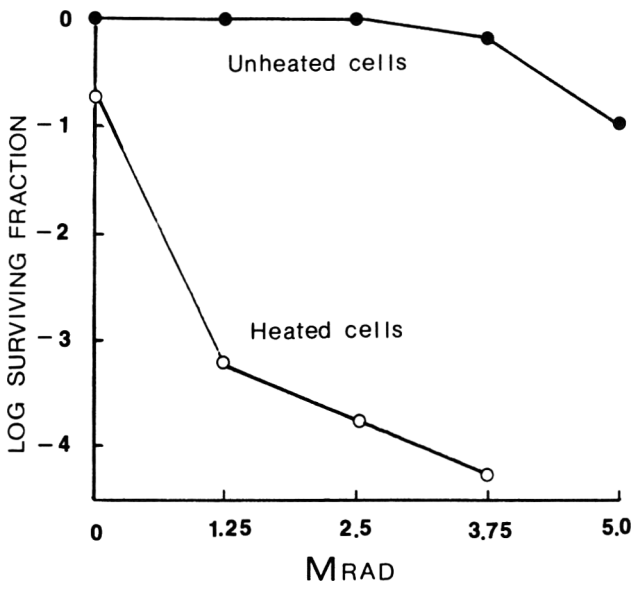

Fig. 11—Effect of thermal-stress on the radiation inactivation of Isolate C-7 cells.

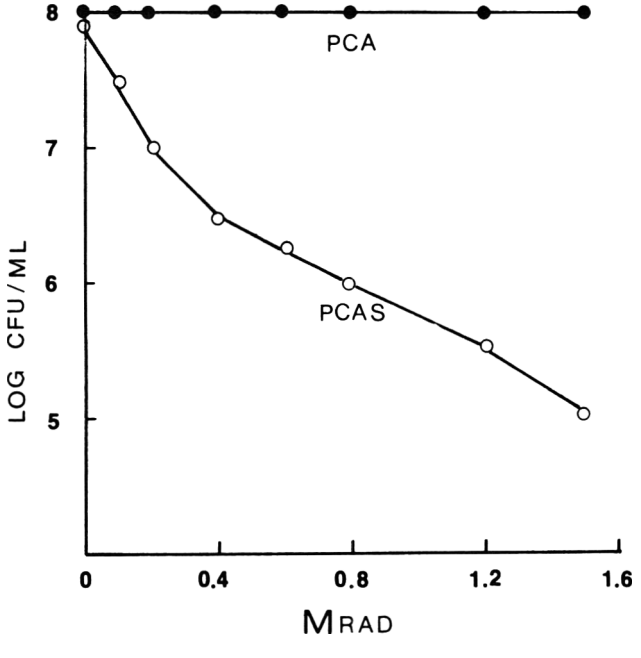

Fig. 10—Injury of Isolate C-7 by gamma radiation.

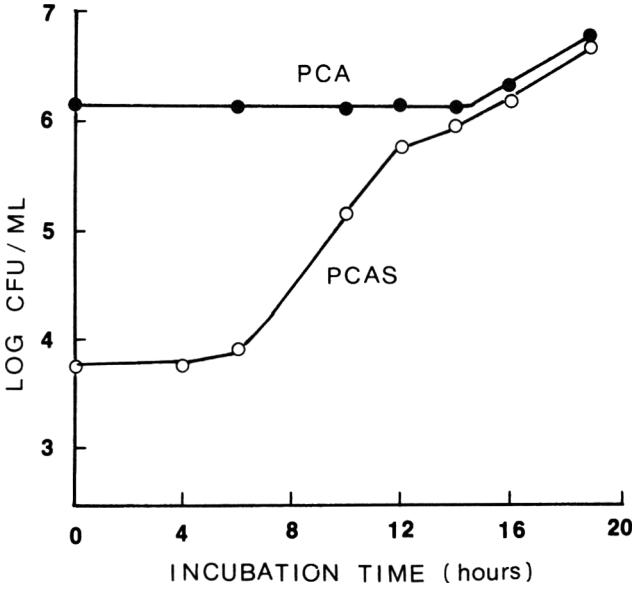

Fig. 12—Effect of incubation time at 32°C on repair of heat-injured Isolate C-7 in PCB.

Concentration of Creatine Phosphate, Adenine Nucleotides and Their Derivatives in Electrically Stimulated and Nonstimulated Beef Muscle

C. R. CALKINS, T. R. DUTSON, G. C. SMITH, and Z. L. CARPENTER

ABSTRACT

The concentration of creatine phosphate (CP), adenosine tri-, di- and monophosphate (ATP, ADP and AMP, respectively), inosine monophosphate (IMP) and inosine in longissimus muscle removed from electrically stimulated (ES) and nonstimulated (NS) beef sides was enzymatically determined. After aging the carcass 7 days, steaks were removed for flavor evaluation. R values (absorbance ratios) were obtained from muscle samples removed over time from both sides. Data indicate more rapid catabolism of CP, ATP, and ADP in ES samples, with subsequent fluctuations in IMP and inosine concentration. At 12 and 24 hr post-stimulation, ES samples had more inosine; however, this difference did not exist after aging. Flavor differences were not observed after the 7-day aging period. R values parallel the degradation of adenine nucleotides and indicate rapid onset of rigor mortis in ES muscle.

INTRODUCTION

THE MECHANISMS by which electrical stimulation (ES) induces changes in muscle are complex and not fully understood. Primarily, stimulation of pre-rigor muscle appears to accelerate glycolysis as indicated by: rate and extent of pH decline (Chrystall and Devine, 1978; Forrest and Briskey, 1967; George et al., 1980; Hallund and Bendall, 1965; Shaw and Walker, 1977); concentration of lactic acid (Bendall, 1976; Bendall et al., 1976; Forrest and Briskey, 1965); and, altered amounts of creatine phosphate (CP) and adenosine triphosphate (ATP) in muscle (Bendall et al., 1976; Bowling et al., 1978; Gilbert and Davey, 1976; George et al., 1980).

Creatine phosphate is readily available for regeneration of ATP in muscle. This ATP can be sequentially degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), and inosine. Both IMP and inosine have been implicated as compounds which contribute to flavor perception and desirability. Wood (1961) reported that IMP imparts a "meaty" flavor when heated. Dannert and Pearson (1967) found better desirability ratings for frankfurters when IMP was included in the formulation. Inosine was implicated as a possible flavor precursor by Batzer et al. (1962). Since ES alters the rate of glycolysis, and ATP and CP breakdown, it is possible that differences in the concentration of these flavor compounds may be induced.

Flavor differences have been observed between ES and NS muscle. Savell et al. (1981) found a significant advantage in flavor desirability for ES steaks aged only 1 day, but that aging of the steaks for periods longer than 1 day negated this difference. Davey et al. (1976) reported higher flavor acceptability scores for strip steaks obtained from ES sides 24 hr after slaughter; however, after an additional 2 days of aging, no difference in the sensory scores of ES and NS samples was found. McKeith et al. (1981) found more desirable flavor in beef loin steaks that were ES and aged 24 hr prior to removal of the steaks; this was true regardless of the location in the slaughter-dressing sequence at which ES was applied. Thus, it appears that flavor differences may exist initially, but not after the aging period.

The objectives of the present study were to measure changes in the concentration of CP, the adenine nucleotides and their derivatives, as influenced by ES and time postmortem, to determine when and if different amounts of these compounds exist, and to evaluate R value as a measure of postmortem metabolism.

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EXPERIMENTAL

Source of muscle

Nine grain-fed steers (carcass weight, 279–375 kg; fat thickness at 12th rib, 8–14 mm) were slaughtered during a 5-wk period. Right sides were ES (550V, 2–6 amp, 17 impulses, 1.8 sec on and 1.8 sec off per impulse) immediately following longitudinal splitting (within 45 min of exsanguination) while the left sides were used as NS controls. Entire carcasses, except for excised samples, were conventionally chilled in a 1°C cooler for 1 wk.

Quality assessments

Each side was ribbed 24 hr postmortem and evaluated for USDA (1975) quality and yield grade factors, lean color (8 = very light pink, 1 = extremely dark red) and the presence of "heat-ring" (5 = none, 1 = extremely severe).

Sensory evaluation

After a 7-day postmortem aging period, two steaks (3.2 cm in thickness) were removed from the anterior end of each shortloin, wrapped in polyethylene-coated freezer paper, frozen at −30°C and stored at −18°C for 10 wk or less. Steaks were thawed for 24 hr at 1°C prior to cooking to an internal temperature of 70°C (monitored by copper-constantan thermocouples) on open-hearth broiling units. An experienced, eight-member sensory panel, selected for their ability to detect differences in flavor intensity and the presence of off-flavor, evaluated each steak for flavor desirability, flavor intensity and presence of off-flavor (8 = extremely desirable, extremely strong flavor, no off-flavor; 1 = extremely undesirable, extremely weak flavor, extreme off-flavor, respectively).

Enzymatic assays

Samples (10g) were removed from the longissimus muscle at 2 hr (between the 10th and 11th ribs), 6 hr (between the 8th and 9th ribs), 12 hr (between the 6th and 7th ribs), 24 hr (from the posterior end of the wholesale rib at the 12th and 13th rib interface), and at 7 days (6.4 cm posterior to the 12th and 13th rib interface on the shortloin) post-stimulation. Samples were stored in liquid nitrogen until assayed. Enzyme assays were conducted to quantitate CP, ATP, ADP, AMP, IMP, and inosine. For the 7-day samples only, the concentration of hypoxanthine (HX) was also determined. Nucleotides (and their derivatives) were extracted from 4g of fragmented, liquid nitrogen-cooled muscle by adding 20 ml of 0.9M perchloric acid (room temperature) and immediately homogenizing at full speed for 1 min with a Virtis Macro Model '45' homogenizer. Homogenates were then centrifuged for 10 min at 3,000 x *g* and the supernatants were decanted and filtered through Whatman No. 1 filter paper. Filtrates were adjusted to pH 6.0–6.5 using 2M potassium hydroxide. The neutralized extracts were held on ice for 45–60 min prior to filtering for use in the assay mixtures.

CP and ATP were determined using the procedures of Lamprecht

et al. (1974) while ADP and AMP were quantitated using the methods of Jaworek et al. (1974). Hypoxanthine, inosine and IMP concentrations were determined by combining the procedures of Jorgensen (1974), Coddington (1974) and Grassl (1974). This combined procedure involved the addition of 2.95 ml of 0.05M triethanolamine buffer (pH 7.6) with 50 μl of sample extract and 10 μl of 0.1M EDTA. The sequential addition of 10 μl (0.04 U) xanthine oxidase, 20 μl (2.5 U) of nucleoside phosphorylase and 50 μl (30 U) of alkaline phosphatase (incubated 20 to 30 min) allowed for calculation of the amount of the above compounds. In all assays, 0.05M triethanolamine hydrochloride (pH 7.6) was used as the reaction buffer. Each assay was conducted in duplicate.

Absorbance ratios (R values)

The nucleotide extract prepared for enzymatic assay (0.1 ml) was added to 2.9 ml of 0.1M potassium phosphate buffer (pH 6.5) and the absorbance at 250, 258, and 260 nm was recorded. R values were calculated as the ratio of the absorbance readings at 250 nm: 260 nm, or as the ratio of absorbance values at 250 nm:258 nm. These values are related to the ratio of the relative concentrations of the adenine nucleotides to IMP and inosine, and to rigor onset (Honikel et al., 1981).

Statistical analyses

Taste panel results, nucleotide concentration and R values were statistically analyzed using the paired t-statistic (Steel and Torrie, 1980) to identify differences between NS and ES treatments. In addition, changes in nucleotide concentration and R values over time were evaluated by use of Duncan's multiple range test as contained in the Statistical Analysis System of Barr et al. (1979).

RESULTS & DISCUSSION

MEAN VALUES for NS and ES sides for USDA quality grade, USDA yield grade, lean maturity, lean color and "heat-ring" are presented in Table 1. ES sides had significantly higher quality grades, more youthful lean maturity and color, and less severe "heat-ring" than did the NS sides. These data indicate that ES in this study was sufficient to induce visual differences in muscle quality at 24 hr postmortem.

Table 1—Mean carcass traits for electrically stimulated and nonstimulated beef sides

Trait	NS[a]	ES[a]
USDA quality grade[b]	Gd^{48}	Gd^{79}
USDA yield grade	2.96	2.98
Lean maturity	A^{88}	A^{52}
Lean color[c]	5.1	6.9
Heat-ring[d]	3.6	4.9

[a] Abbreviations: NS = nonstimulated; ES = electrically stimulated beef sides.
Means for the same trait underscored by a common line are not different ($P > 0.05$).
[b] Gd = USDA Good. Quality grade factors were evaluated 24 hr after slaughter.
[c] Based on an 8-point rating scale (8 = very light pink, 1 = extremely dark red).
[d] Based on a 5-point rating scale (5 = no "heat-ring," 1 = extremely severe "heat-ring").

Changes in the concentration of CP, the adenine nucleotides and their derivatives were determined in relation to time post-stimulation and ES (Table 2). Significantly more CP and ATP were found in NS samples than in ES samples at 2 and 6 hr post-stimulation; however, these stores were depleted by 12 hr post-stimulation in both NS and ES samples. ADP concentration was higher in NS muscle samples at all times except 24 hr. Levels of ADP generally decreased, and AMP levels remained fairly constant during the aging period. No significant differences were found in AMP levels between NS and ES samples except at 6 hr (Table 2). The relatively constant amount of AMP at differing times post-stimulation coincides with the observation of Honikel and Fischer (1977) that AMP "is deaminated quite instantaneously" to IMP.

Levels of IMP were not different between ES and NS samples at 2 and 6 hr post-stimulation; however, at 12 hr and beyond, ES samples contained lower IMP concentrations than did the counterpart NS samples (Table 2). While the 7-day samples (regardless of treatment) had less IMP than did the 2 hr samples, the levels did fluctuate between these two points. These data show that IMP levels after 24 hr did not differ from those at the 2 hr sampling period, but that a decrease in IMP had occurred after aging the beef for six additional days. A similar trend was observed by Dannert and Pearson (1967) who found levels of IMP at 24 hr postmortem equal to levels of zero hr samples. The results of the present study do not indicate the increase in IMP concentrations at 12 hr which the previous researchers found. The trend of decreased IMP content after 7–10 days of aging has been reported in several previous studies (Dannert and Pearson, 1967; Rhodes, 1965; Dvorak, 1958). Terasaki et al. (1965) found that IMP levels in pork peaked and then began to diminish after 3 days of aging.

The concentration of inosine (Table 2), in general, reflected the degradation of IMP. No difference in the level of inosine was detected between ES and NS samples at 2 hr post-stimulation, but ES samples had more μmoles of inosine per gram than did NS samples at 6, 12, and 24 hr. This higher concentration was not evident after 7 days of aging. It is possible that the higher amounts of IMP observed earlier postmortem were catabolized beyond HX, since the concentrations of HX did not differ at the 7-day sampling period (Table 2). Some very interesting results were obtained during the sequential determination of inosine and

Table 2—Concentration of creatine phosphate, adenine nucleotides and their derivatives in electrically stimulated and nonstimulated beef muscle at various sampling times

Sampling time post-stimulation	Concentration (μmoles/g)													
	CP[a]		ATP		ADP		AMP		IMP		Inosine		HX	
	NS	ES	NS	ES	NS	ES	NS	ES	NS	ES	NS	ES	NS	ES
2 hr	1.34^{b}	0.22^{b}	3.07^{b}	0.60^{b}	0.67^{b}	0.52^{b}	0.26^{b}	0.21^{b}	5.50^{b}	5.46^{b}	0.19^{d}	0.28^{d}	–	–
6 hr	0.28^{c}	0.09^{c}	1.66^{c}	0.19^{c}	0.69^{b}	0.39^{bc}	0.32^{b}	0.13^{b}	4.99^{cd}	4.85^{c}	0.25^{d}	0.56^{c}	–	–
12 hr	0.01^{c}	0.01^{c}	0.21^{d}	0.09^{c}	0.50^{bc}	0.33^{c}	0.26^{b}	0.09^{b}	4.62^{de}	4.28^{d}	0.53^{c}	0.79^{c}	–	–
24 hr	0.01^{c}	0.01^{c}	0.10^{d}	0.08^{c}	0.32^{c}	0.33^{c}	0.19^{b}	0.10^{b}	5.15^{bc}	4.88^{c}	0.49^{c}	0.77^{c}	–	–
7 day	0.01^{c}	0.02^{c}	0.07^{d}	0.07^{c}	0.35^{c}	0.30^{c}	0.17^{b}	0.18^{b}	4.33^{e}	4.09^{e}	1.21^{b}	1.15^{b}	0.17	0.18

[a] CP = creatine phosphate; ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; IMP = inosine monophosphate; HX = hypoxanthine; NS = nonstimulated muscle; ES = electrically stimulated muscle.
[b,c,d,e] Means in the same column bearing a common superscript letter are not different ($P > 0.05$). Means for each compound for NS and ES muscle within each sampling time underscored by a common line are not different ($P > 0.005$).

IMP. At 2 hr post-stimulation, a very large difference in the rate of the reaction converting IMP to inosine (using added enzymes) was observed, with the ES samples reaching 50% completion in about 1.5 min while NS samples required over 8.5 min to reach this stage. This difference in assay reaction rates occurred even when NS samples had the same or greater concentrations of IMP than the ES samples. At 6 hr, the difference in reaction completion time was decreased (ES = 1.5 min; NS = 3.5 min) and at 12 hr, the rates were the same (ES = 1.2; NS = 1.2). This would suggest that some compound was present in the NS muscle samples which inhibited the rate at which the added enzyme (alkaline phosphatase) acted on the substrate or that aging and/or ES produced an activator for this reaction. It is quite possible that one of the adenine nucleotides (e.g., ATP), may compete with IMP as a substrate for the alkaline phosphatase. Further studies are needed to more accurately define and characterize the nature of this phenomenon.

An overview of all of the data on concentrations of CP, ATP, ADP, AMP, IMP, and inosine in the present study suggests that ES does increase the rate of catabolism of CP and the high-energy adenine nucleotides. The prolonged effect on reaction rates observed after ES of pre-rigor muscle may be a result of altered substrate and inhibitor concentrations.

Table 3 presents the results of sensory panel evaluation of steaks aged 7 days. No significant differences were found between ES and NS samples for flavor desirability, flavor intensity and the presence of off-flavor. Previous research, however, has indicated that differences exist in flavor attributes 1–2 days postmortem (Savell et al., 1981; Davey et al., 1976; McKeith et al., 1981). This response corresponds to changing concentrations of inosine during the postmortem aging period. Investigations should be conducted to determine if the apparent relationship between inosine concentration and flavor desirability is real or coincidental.

Since acceleration of glycolysis and breakdown of ATP and CP (by ES), and, therefore, hastening of the onset of rigor mortis, has a favorable effect on meat tenderness (McKeith et al., 1981; Savell et al., 1978, 1979; Smith et al., 1979), a simple, rapid and inexpensive method for detecting early onset of rigor mortis could be of value as a tool to predict tenderness. Khan and Frey (1971) reported a simple technique to follow the time-course of rigor mortis in beef. They obtained a perchloric acid extract of muscle and created a ratio of the absorbance at 258 nm and the absorbance at 250 nm. The intent was to measure the disappearance of the adenine nucleotides (based on a decrease in the absorbance at 258 nm) and the increase in IMP (based on an increase in the absorbance at 250 nm. Similarly, Honikel (1976) defined the term "R value" as a ratio of the absorbance of a perchloric acid extract at 250 nm and at 260 nm. He suggested that R values should be low in normal, pre-rigor muscle due to the high content of adenine nucleotides, while R values for PSE (pale, soft, and exudative) and DFD (dark, firm and dry) pork should be high as a result of high concentration of IMP, inosine and HX. Honikel and Fischer (1977) were later successful in relating R values to PSE and DFD pork. Honikel et al. (1981) reported that an R value of about 1.10 was equivalent to approximately 1.0 μmole of ATP per g of muscle. These researchers identified this set of criteria (R value = 1.10; ATP = 1.0 μmole per g; pH = 5.9) as the conditions indicative of rigor mortis in beef neck muscles, based on the decrease in extensibility of muscle fibers. In the present study, for NS muscle, an R value of about 1.12 was indicative of 1.0 μmole of ATP per g of muscle. For the first 12 hr postmortem, the relationship between R value and ATP content appeared to be linear. Therefore, R value may be useful as an indicator of the early onset of rigor mortis and, as such, may be related to ultimate meat tenderness if the nucleotides are extracted before 12 hr postmortem.

Table 4 displays the ratio of adenine nucleotides (ATP, ADP and AMP) to inosine compounds (IMP and inosine) and mean R values for NS and ES beef muscle at various sampling times. As rigor mortis progresses and glycolysis continues, the total amount of adenine nucleotides decreases, and the total amount of inosine plus IMP remains fairly constant. Thus, the advancement of rigor mortis is reflected by a decrease in the ratio of adenine nucleotides to inosine compounds (Table 4). R values chage in a similar, but inverse, fashion over time, which indicates that changes in nucleotide concentration can be observed by this measurement. Both absorbance ratios (250 nm:260 nm and 250 nm:258 nm) have the greatest magnitude of change over time and the largest difference between NS and ES samples from 2 to 12 hr post-stimulation. Sampling at 24 hr and beyond resulted in no significant difference between the stimulation treatments (ES vs NS). This coincides with changes which occur during rigor onset and suggests that, for R value to be useful as an indicator of early rigor onset (and, therefore, correlate to tenderness), samples must be prepared earlier than 12 hr postmortem. Additional research is needed to determine the relationship between R values and meat tenderness.

Table 3—Mean sensory panel ratings for flavor attributes of steaks from electrically stimulated and nonstimulated beef sides after 7 days of aging

Attribute	NS[a]	ES[a]	Level of significance
Flavor desirability[b]	6.12	6.33	$P < 0.15$
Flavor intensity[b]	6.29	6.27	$P < 0.44$
Presence of off-flavor[b]	6.69	6.85	$P < 0.24$

[a] Abbreviations: NS = nonstimulated; ES = electrically stimulated beef sides.
[b] Scored on 8-point rating scales: 8 = extremely desirable, extremely strong flavor, or no off-flavor; 1 = extremely undesirable, extremely weak flavor or extreme off-flavor.

Table 4—R values and ratios of adenine nucleotides to inosine compounds for electrically stimulated and non-stimulated beef muscle at various sampling times

Sampling time post-stimulation	R value[a] 250/260 nm		R value[a] 250/258 nm		Adenine nucleotides/inosine compounds[b]	
	NS	ES	NS	ES	NS	ES
2 hr	0.88^{c}	1.26^{c}	0.88^{c}	1.19^{c}	0.70^{e}	0.23^{d}
6 hr	1.04^{d}	1.34^{cd}	1.00^{d}	1.24^{c}	0.51^{d}	0.13^{c}
12 hr	1.21^{e}	1.32^{cd}	1.18^{e}	1.25^{c}	0.19^{c}	0.10^{c}
24 hr	1.26^{ef}	1.33^{cd}	1.22^{e}	1.26^{c}	0.11^{c}	0.09^{c}
7 day	1.36^{f}	1.35^{d}	1.26^{e}	1.26^{c}	0.22^{c}	0.10^{c}

[a] R values were calculated as the ratio of absorbance at different wavelengths (250: 258 or 260 nm).
[b] The ratio of the concentration of the adenine nucleotides (adenosine tri-, di-, and monophosphate) to the concentration of inosine compounds (inosine monophosphates and inosine). NS = nonstimulated and ES = electrically stimulated beef muscle.
[c,d,e,f] Means in the same column bearing a common superscript letter are not different ($P > 0.05$).
Means for NS and ES muscle within each ratio and sampling time underscored by a common line are not different ($P > 0.05$).

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A Comparison Between Two Different Shear Systems Used for Measuring Meat Tenderness

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ABSTRACT

A comparison has been made between two Warner-Bratzler (WB) shear devices, differing in their method of operation, used in measuring tenderness of meat samples treated to produce a wide range, in both myofibrillar and connective tissue structural strength. Estimates of parameters measured from the shear deformation curves obtained from the two systems were highly correlated and both systems showed similar and significant responses to the treatments used to modify the structural components of meat.

INTRODUCTION

IN STUDIES of the factors which influence meat tenderness it is desirable to be able to assess the likely individual structural contributions from both the myofibrillar and connective tissue structures. Such estimates have been made from various measurements of mechanical properties (Bouton and Harris, 1972a; Bouton et al., 1975a, b). The most commonly used measurement is that obtained with the Warner-Bratzler (WB) shear device (Szczesniak and Torgeson, 1965). Recently the WB shear force deformation curves have been analysed and interpreted by comparing changes in these shear curves produced by various treatments known to affect either the myofibrillar or connective tissue structures (Bouton et al., 1975b, c, 1977a, b; Møller, 1981). The WB shear device used (Bouton et al., 1975b, c; 1977b), however, was not the same as the commercial device (which uses a triangular hole in the shear blade and cylindrical shaped samples), since it had a square hole in the shear blade, one cutting edge, and used samples of rectangular cross-section. These workers (Bouton et al., 1975b, c, 1977b) suggested that initial yield force values were primarily dependent on myofibrillar strength while the difference between initial yield force and peak force values gave an indication of the contribution of the connective tissue structure to the shear force values.

Møller (1981) used the triangular shear blade of the conventional WB shear device but used it with samples of a square cross section, which were easier to cut to shape than the conventional cylindrical cores. With this system it was found that changes in initial yield force corresponded well with the expected changes in myofibrillar strength with cooking. More importantly it was found (Møller, 1981) that the yield point which occurred when the apex of the triangular shear blade was about to enter the slit gave a better indication of changes in connective tissue strength than did measurements of the peak force.

In this present work the results obtained using the square type shear blade (as used by Bouton et al., 1975b, c; 1977) have been compared with those obtained using a conventional WB shear blade (as used by Møller, 1981).

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The main purpose of the present work, therefore, was to compare the response of two shear systems, differing in the mode of application of the shearing force and the change in geometrical shape of the samples under stress, to different treatments affecting the structural components of cooked meat.

MATERIALS & METHODS

Experimental materials

Four pairs of deep pectoral (DP) muscles from 2–4 yr old steers obtained within 1 hr of slaughter, were used in each experiment. The muscles were trimmed of all extraneous fat and connective tissue.

The material used in these experiments was subjected to a number of treatments designed to affect myofibrillar or connective tissue strength. A pressure-heat treatment (150 MNm^{-2} at 60°C for 30 min after a preheat at 45°C for 30 min) was used to reduce myofibrillar strength without accompanying large changes in connective tissue strength (Bouton et al., 1977a; Ratcliff et al., 1977). Other samples were cooked at 60 or 80°C for 1 or 24 hr since (a) it was known from earlier work (Draudt, 1972) that myofibrillar hardening occurred when the cooling temperature was raised from 60 to 80°C, and (b) cooking for extended times at 80°C greatly reduced connective tissue strength. Muscle samples restrained from thermal shortening during cooking were used in experiment 4 as a treatment selectively affecting the structural strength of connective tissue (Bouton et al., 1978).

Experiment 1. The muscles were stretched by about 50% and maintained in this stretched condition by nailing the ends on a wooden board covered with polyethylene, then stored at 0–1°C for 48 hr. After storage six sub-samples of approximately equal size (4 x 3 cm) cross-section and 12 cm long alonng the fiber direction were taken from each pair and assigned at random to cooking at 80°C for 1, 2, 4, 6, 8 or 16 hr. These cooking times were chosen (as were the 1 and 24 hr cooking times used in Experiments 2 and 4) to produce a progressive decrease in the connective tissue contribution to shear force values.

Experiment 2 and 3. One of each pre-rigor muscle pair was cold-shortened by placing in a polyethylene bag and totally immersing in ice slush for 48 hr. The other muscle was stretched by about 50%, as described in Experiment 1 and stored at 0–1°C for 48 hr. After storage, four muscle pairs were assigned to Experiment 2 and the remainder to Experiment 3. For both experiments each cold-shortened and stretched muscle was divided into four approximately equal sub-samples. For Experiment 2 these were assigned at random to cooking at 60 or 80°C for 1 or 24 hr. For Experiment 3 two sub-samples were randomly assigned to be pressure-heat treated (150 MNm^{-2} at 60°C for 30 min after preheating at 45°C for 30 min (Bouton et al., 1977a) and two assigned to be controls. One pressure-heat treated and one control sample were then assigned to cooking at either 60 or 80°C for 1 hr.

Experiment 4. In this experiment the four pairs of DP muscles were stretched and stored as in Experiment 1. Each pair was divided into eight approximately equal sub-samples, four of which were assigned at random to be cooked without restraint i.e. free (F) while the others (R) were placed on racks and stretched about 30% before cooking (Bouton et al., 1978). The F and R samples were further assigned at random to cooking at 60 or 80°C for 1 or 24 hr.

Cooking methods

The sub-samples weighing about 150–200g were placed in close fitting polyethylene bags, fastened with metal clips and cooked totally immersed in a water bath thermostatically controlled

(± 0.5°C) at 60 or 80°C for 1 hr or 24 hr. The restrained (R) samples were cooked on their racks by direct immersion in the heated water; the corresponding free (F) samples were cooked in the polyethylene bags as before, but about 150 cc of water from the bath was added to each bag to approximate the conditions for the restrained (R) samples. After cooking the samples were cooled in cold running water for 30 min, dried and stored in polyethylene bags at −18°C for a maximum of 8 wk. When required for mechanical measurements the samples were thawed in cold running water.

Shear measurements

Two different WB shear systems were investigated. The modification of the WB shear device using a shear blade (hereinafter called the S-blade with a square hold and rectangular sectioned samples has been decscribed in detail elsewhere (Bouton and Harris, 1978). This device was mounted on the crosshead of an Instron Universal Testing Machine (Type TM-M) and used with a crosshead speed of 5 cm/min and chart speed of 50 cm/min. The samples of cooked meat, sheared at right angles to the fiber axis, were 4–6 cm long with a rectangular cross-sectional area of 1 cm^2 (1.5 x 0.67 cm). The fibers lay parallel to the long axis of the samples, the thickness actually sheared through was 0.67 cm and about six to eight observations were made from each sub-sample.

The second WB shear system was a conventional one in which a blade (hereinafter called the T-blade) with a triangular slot was pushed through the sample. The samples were similar in length and fiber orientation to that used in the first system and the cross-sectional area was 1 cm^2 but the dimensions were 1 x 1 cm. In experiment 1 the conventional cylindrical cores obtained using a cork borer (diameter 1 cm), were also used for comparison. This system was mounted on an Instron Universal Testing Machine (Type 1122) and used with crosshead and chart speeds of 5 and 20 cm/min respectively. The crosshead was set so that the apex of the triangular hole in the shear blade was just entering the slit. The crosshead was then moved up 1.8 cm so that the sample was just touching the edges of the shear blade. This point was then set as the zero and the crosshead was set to cycle between this zero and 2.5 cm below.

Parameters measured from force-deformation curves

The parameters measured from the WB (S-blade system) deformation curves have been described by Bouton et al. (1975c). They were:

(a) the force at initial yield which was the first major inflection on the force-deformation curve;

(b) the peak force which represented the greatest force measured on any particular force-deformation curve;

(c) the initial-yield distance defined as the distance travelled by blade from the first registering of force to the initial-yield point;

(d) the final-yield distance defined as the distance travelled by blade between the first registering of force and the registration of maximum force;

(e) the slope at yield defined as the rate of force increase as the sample approached the initial yield and measured as the tangent to the force deformation curve as it approached the yield.

Parameters from the T-blade shear deformation curves were measured as previously described (Møller, 1981) but, additionally, yield distances and slope at yield were measured using the same procedure as Bouton et al. 1975). They were: initial yield forces (as termed WB M-force by Møller, 1981), initial yield distance, peak force, peak force distance, peak force minus initial yield, WB C-force taken as a measurement of connective tissue strength and measured as the yield or last inflection on the force-deformation curve close to the slit point, and slope at initial yield point.

Statistical methods

Analysis of variance was used to ascertain treatment and blade effects, to calculate standard errors and hence least significant (LSD) difference values at $P < 0.05$ level between means. Correlation coefficients between the two systems were calculated for all parameters measured. To best illustrate the comparison between the 2 blade the correlation coefficients were calculated over the treatment means obtained for each experiment.

RESULTS & DISCUSSION

Experiment 1

Results from the two shear blade systems are shown in Table 1. For the T-blade both cylindrical cores (1 cm diameter) as well as square samples (1 x 1 cm^2) were investigated. The three shear methods showed significant and similar response to cooking time. The cooking time x shear method interaction was significant ($P < 0.05$) for peak force and the connective tissue measurements. However, the correlations between the shear methods were very high (Table 2). Since the response obtained when using the T-blade on samples with square or cylindrical cross-section were similar only the square type samples were used in the remaining experiments.

Experiments 2, 3 and 4

Initial yield and peak force values. The results obtained for the two blade systems are shown in Table 3. Both shear blades showed similar and significant responses to the effects of the treatments applied.

The correlation coefficients between the treatment means of the parameters measured using the two different blade systems are shown in Table 4. The correlations were very high for the parameters – initial yield force and peak force – for all three experiments individually and for the three experiments combined.

Estimates of connective tissue contribution. The connective tissue contribution to meat toughness was estimated as being represented by the difference betwen peak force and initial yield force for the S-blade system (Bouton et al. 1977b; 1978) and by C-force values for the T-blade system (Møller, 1981). As these estimates were obtained from dissimilar measurements it is not surprising that correlations

Table 1—Initial yield, peak, peak-initial yield and C-force values obtained with S- and T-blade systems using samples from experiment 1

Cooking time, hr at 80°C	Parameter measured (kg) and shear blade system								
	Initial yield force			Peak force			Peak force-initial yield	C-force	
	S[b]	TC[c]	TS[d]	S	TC	TS	S	TC	TS
1	5.17	6.08	7.10	9.56	8.51	9.49	4.39	8.10	9.22
2	5.64	5.49	7.22	9.54	5.97	8.05	3.89	5.50	7.32
4	5.19	5.46	6.70	8.07	5.52	6.95	2.88	4.46	5.05
6	3.89	5.52	5.84	6.33	5.52	5.87	2.44	3.73	3.94
8	3.54	4.40	5.27	5.04	4.40	5.27	1.50	2.94	3.22
16	2.58	3.23	3.68	3.21	3.23	3.68	0.63	1.99	1.95
LSDA[a]		0.95			1.43			1.40	
LSDB[a]		0.31			0.47			0.55	

[a] LSDA, LSDB—the least significant difference at $P < 0.05$ for between cooking time and between shear system, respectively.

[b] S: S-blade used on rectangular samples (1.5 x 0.67 cm)
[c] TC: T-blade used on cylindrical samples (diameter 1 cm)
[d] TS: T-blade used on square samples (1 x 1 cm)

between them were comparatively low as seen for Experiments 2 and 3 (Table 4). However, a better correlation was obtained in Experiment 4 on account of the larger range in connective tissue strength obtained from samples cooked either free or restrained.

Initial yield and peak force distances. The results obtained for the two shear blades are shown in (Table 5). For the S-blade the distances measured represent the distance the shear blade has travelled through the sample which is 0.67 cm ± 0.05% thick. For The T-blade, however, the blade starts with the apex of the triangle 0.8 cm above the upper surface of the meat sample (1 cm x 1 cm cross-section) and with the sides of the blade just touching the sample. Assuming the sample deforms without any breakage and maintains its 1 cm^2 cross-sectional area the shear blade comes down about 0.5 cm before the area under the blade is 1 cm^2 i.e. the same as the cross sectional area of the meat sample. As the blade continues downward the sample is rapidly compressed/broken until after 0.8 cm of travel the sample area under the blade would be about 0.6 cm^2, at 1.0 cm about 0.4 cm^2, at 1.2 cm about 0.2 cm^2 and at 1.6 cm about 0.04 cm^2.

For the S-blade peak force values occur at or near the slit while for the T-blade they occur – depending upon cooking temperature and cooking time – with the apex of the triangle 5 mm or so from the slit. Though the distances travelled by the blade are of different magnitude the approximate amount the samples are compressed before yield are similar for both systems. The results obtained with the two blades show similar and significant response to the treatment effects (Table 5) and good overall correlations of 0.72 for initial yield distance and 0.87 for peak force yield distance (Table 4).

Slope at yield values. These values (Table 5) show very similar changes with the various treatments for both blades.

Table 2–Correlation coefficients between the parameters measured by the various WB shear blade systems used in Experiment 1

Parameter	Shear blade system[a]								
	S	vs	TC	S	vs	TS	TC	vs	TS
Initial yield force		0.74			0.89			0.89	
Peak force		0.79			0.90			0.86	
Connective tissue measurement[b]		0.81			0.84			0.88	
Peak force-initial yield force		0.57			0.55			0.80	

[a] Same symbols as used in Table 1.
[b] Peak force-initial yield as measured by the S-blade. WB C-force as measured by the T-blade system. (DF = 22)
Coefficients of correlations > 0.52 are significant at $P < 0.01$.

Table 3–Initial yield, peak, peak-initial yield and C-force values obtained with S-and T-blade systems using samples from Experiments 2, 3 and 4.

Experiment, treatment, cooking °C/ time[a] (hr)		Parameter measured and Shear blade system[b]					
		Initial yield force		Peak force		Peak force-Initial yield	C-force
		S	T	S	T	S	T
Expt. 2. STR	60/1	3.32	4.95	10.71	9.17	7.39	9.17
	80/1	5.58	8.75	9.45	9.55	3.87	7.87
	60/24	1.95	3.85	6.22	6.05	4.27	5.92
	80/24	2.02	3.50	2.58	3.62	0.56	1.40
	LSDA	0.64		1.99		1.89	
	LSDB	0.35		1.55		1.51	
Expt. 2. CS	60/1	7.72	8.22	10.05	9.65	2.32	8.37
	80/1	17.41	14.72	19.33	15.27	1.91	9.32
	60/24	12.10	11.92	14.16	13.17	2.06	8.55
	80/24	6.53	8.17	7.71	8.45	1.18	3.97
	LSDA	1.47		1.69		1.05	
	LSDB	1.00		1.06		1.07	
Exp. 3. STR	60C	3.30	4.55	6.86	10.42	3.56	10.42
	80C	7.08	8.35	8.91	8.87	1.83	7.07
	60P	2.04	3.03	6.17	9.10	4.13	9.10
	80P	2.46	4.58	7.29	8.40	4.84	8.35
	LSDA	0.56		2.22		2.02	
	LSDB	0.47		1.13		1.35	
Expt. 3. CS	60C	7.40	7.40	8.56	8.27	1.16	7.00
	80C	14.77	12.50	15.81	12.97	1.08	7.07
	60P	1.61	2.68	3.43	6.00	1.82	6.00
	80P	1.88	3.15	4.06	5.40	2.18	5.40
	LSDA	1.30		1.48		0.91	
	LSDB	0.68		0.92		0.88	
Expt. 4. F	60/1	3.08	3.88	9.30	9.20	6.23	9.20
R	60/1	4.00	6.15	16.08	16.07	12.08	15.90
F	80/1	5.97	7.00	8.88	8.45	2.91	7.42
R	80/1	7.61	10.58	18.09	14.90	10.47	13.92
F	60/24	2.50	3.50	5.91	4.65	3.42	4.25
R	60/24	4.04	7.30	14.11	10.50	10.07	9.45
F	80/24	3.46	4.30	3.98	4.45	0.52	2.17
R	80/24	4.57	6.70	5.30	6.92	0.73	3.07
	LSDA	0.61		1.52		1.33	
	LSDB	0.30		0.71		0.76	

[a] LSDA — the least significant difference at $P < 0.05$ level between temperature-time or control and pressure-heat means.
LSDB — the least significant difference at $P < 0.05$ level between shear blades means.
[b] All means are in kg.

The correlations are all very high (Table 4) both individually and for the combined results. The values obtained for the S-blade were, however, substantially larger (see Table 5).

CONCLUSIONS

THE RESULTS (Tables 3, 4 and 5) show that in spite of appreciable differences in their modus operandi the two WB shear systems yield very similar results for initial yield force values and for the peak force values. They responded similarly to the different treatment effects since there was no significant blade x treatment interaction. The peak force-initial yield force values (S-blade) and C-force values (T-blade) were not so well correlated (Table 4) and were of markedly different magnitude. The former values are regarded (Bouton et al., 1977b, 1978) as an indicator of the connective tissue contribution to WB peak force values. They are not, as evidenced by taste panel and other work (Bouton et al., 1972a; Cross et al., 1973) likely to give any real indication of the contribution of the connective tissue to the toughness of any particular sample. Muscles with low shear values but high connective tissue strength are still found to be tough by a taste panel (Ratcliff et al., 1977; Bouton et al., 1975).

The C-force values could give a better indication of the connective tissue contribution to subjectively determined tenderness but there is only limited evidence at present to support such a contention (Møller, 1981). However, it is obviously useful in so far that it could be used, instead of more elaborate measures such as adhesion (Bouton and

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Table 4—Correlation coefficients between treatment means of various parameters measured using the WB shear device in either its S-blade or T-blade modes of operation

Parameter measured	Exp. 2[a]	Exp. 3[a]	Exp. 4[a]	Combined[b]
Initial yield force	0.975	0.984	0.928	0.953
Peak force	0.983	0.877	0.950	0.934
Peak force-initial yield vs WB C-force	0.532	0.667	0.941	0.796
Peak force-initial yield	0.930	0.808	0.844	0.766
Initial yield distance	0.919	0.670	0.656	0.716
Peak force distance	0.970	0.794	0.897	0.867
Slope at yield	0.996	0.981	0.914	0.911

[a] For n = 6 df correlation coefficients ($r > 0.71$ significant at $P < 0.05$; ($r > 0.83$ significant at $P < 0.01$.
[b] For n = 22 df correlation coefficients ($r > 0.40$ significant at $P < 0.05$; ($r > 0.52$ significant at $P < 0.01$.

Table 5—Initial yield and peak force distances and slope at yield values obtained with S- and T-blade systems using samples from Experiments 2, 3 and 4.

Experiment, treatment, cooking°C/time (hr)		Parameter measured/Shear blade system					
		Initial yield distance (cm)		Peak force distance (cm)		Slope at yield kg/cm	
		S	T	S	T	S	T
Expt. 2. STR	60/1	0.25	0.85	0.75	1.49	31.5	21.6
	80/1	0.35	1.00	0.64	1.20	30.9	25.0
	60/24	0.22	0.87	0.73	1.47	12.7	13.9
	80/24	0.20	0.77	0.47	0.84	10.7	9.9
	LSDA	0.05		0.11		1.9	
	LSDB	0.03		0.07		1.5	
Expt. 2. CS	60/1	0.42	0.95	0.60	1.20	52.4	44.5
	80/1	0.50	1.07	0.57	1.14	120.5	92.8
	60/24	0.45	1.06	0.57	1.21	76.0	62.3
	80/24	0.36	1.00	0.56	1.07	24.3	17.7
	LSDA	0.05		0.06		13.4	
	LSDB	0.02		0.04		11.4	
Expt. 3 STR	60C	0.34	0.77	0.84	1.39	25.7	21.0
	80C	0.40	0.97	0.64	1.10	39.2	26.1
	60P	0.35	0.75	0.87	1.37	12.2	12.6
	80P	0.32	0.92	0.81	1.58	14.0	13.9
	LSDA	0.05		0.05		4.3	
	LSDB	0.03		0.03		3.6	
Expt. 3. CS	60C	0.44	0.85	0.53	1.07	62.9	34.3
	80C	0.51	1.06	0.56	1.12	124.9	52.8
	60P	0.31	0.82	0.76	1.42	9.0	7.8
	80p	0.25	0.82	0.75	1.58	11.5	8.4
	LSDA	0.05		0.07		17.6	
	LSDB	0.03		0.08		8.3	
Expt. 4 F	60/1	0.33	0.82	0.94	1.42	21.5	17.8
R	60/1	0.27	0.77	0.83	1.47	26.2	29.6
F	80/1	0.41	0.91	0.64	1.23	34.6	24.4
R	80/1	0.28	0.85	0.67	1.36	52.0	45.0
F	60/24	0.34	0.86	0.73	1.25	13.6	12.4
R	60/24	0.21	0.83	0.72	1.43	32.0	35.9
F	80/24	0.31	0.89	0.48	0.95	15.9	10.8
R	80/24	0.23	9.82	0.43	0.89	25.7	19.3
	LSDA	0.03		0.06		3.43	
	LSDB	0.02		0.06		1.83	

[a] LSDA — least significant difference between temperature time or control and pressure-heat means at $P < 0.05$.
LSDB — least significant difference between shear blades means at $P < 0.05$.

Calcium Activated Neutral Protease Hydrolyzes Z-Disc Actin

P. NAGAINIS and F. H. WOLFE

ABSTRACT

Fractionation of myofibril proteins using Hasselebach-Schneider solution (H-S) and a potassium iodide solution (KI) revealed that an unusual form of actin existed in the H-S and KI extracted myofibrils which could be easily hydrolyzed by calcium activated neutral protease (CANP). That actin was similar in amino acid composition to normal thin-filament actin but differed in isoelectric pH, solubility in KI, and antigenicity in mice. Indirect immunofluorescence using antiserum prepared against the unusual actin established that the actin resides in the Z-disc. The hydrolysis of actin in the Z-disc by CANP may explain the dissolution of the Z-disc, considered responsible for the tenderization of meat during postmortem aging.

INTRODUCTION

THE Z-DISC has been described as the myofibrillar organizational center and the anchor point for the contractile filaments (Franzini-Armstrong, 1973; Gard and Lazarides, 1979). While the function of the Z-disc has been recognized and defined, until recently little was fully understood of its composition and organization. Electron microscopy (Franzini-Armstrong, 1973; Ullrick et al., 1977), extraction and reconstitution experiments (Goll et al., 1969; Stromer and Goll, 1972; Stromer et al., 1969), and fluorescent antibody techniques (Masaki et al., 1967) have shown that the Z-disc is composed of branching thin filaments overlaid with an amorphorus matrix of α-actinin. Other proteins reported to be associated with the Z-disc include actin (Yamaguchi et al., 1978), desmin and vimentin (Granger and Lazarides, 1979), and filamin (Gomer and Lazarides, 1981). Interactions of these proteins are thought to be responsible for Z-disc structure (actin + α-actinin) and the joining of adjacent Z-discs (desmin + vimentin). Gard and Lazarides (1979) identified a KI insoluble form of actin in the Z-disc.

Dayton and others (Azanza et al., 1979; Dayton et al., 1975, 1976a, b; Ishiura et al., 1979) have characterized an endogenous muscle protease which has been shown to dissolve Z-discs in isolated muscle myofibrils. This protease, called calcium activated neutral protease (CANP) because of its metal ion and pH requirements, has been shown to release unhydrolysed α-actinin from the Z-disc matrix during dissolution of the Z-discs. Gard and Lazarides (1979) mentioned that brief in vitro trypsin treatment of myofibrils hydrolyzed the KI insoluble form of Z-disc actin, and also released α-actinin. Hydrolysis at the Z-disc seems to be one of the important events in postmortem muscle leading to the conversion of muscle to meat (see review by Penny, 1980).

Studies of the CANP hydrolysis of the protein components of myofibrils using SDS polyacrylamide gel electrophoresis (Dayton et al., 1975) have revealed that CANP hydrolyzes some components of the thin filament regulatory complex, but the mechanism by which CANP acts on the Z-disc remains unknown. The experiments presented in this paper were designed as an approach to amplify our knowledge of the action of CANP on myofibrillar components, and the results have led to a sound and reasonable explanation for Z-disc dissolution by CANP in both intact postmortem muscle and isolated CANP treated myofibrils, and for the increased fragility (Takahashi et al., 1967) of myofibrils in postmortem muscle.

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MATERIALS & METHODS

Myofibril and myofibrillar protein preparation

Chicken breast muscle from six to ten week old broilers (Lilydale Poultry Farms, Edmonton, Alberta) was excised immediately following exsanguination and chilled on ice. Myofibrils were prepared from the minced muscle following the procedure of Hay et al. (1973).

Extracted myofibrils were prepared by gently stirring the myofibrils in five volumes of Hasslebach-Schneider solution (H-S) (1951) that contained 0.6M KCl, 0.01M $Na_2P_4O_7$, 0.1M sodium phosphate buffer, 1 mM $MgCl_2$, pH 6.4. Between each of the three extractions, myofibrils were pelleted at 9,800 x *g* for 15 min. The extracted pellet was then extracted three times with five volumes of KI solution consisting of 1.0M KI, 10 mM $Na_2S_2O_3$, and 10 mM ATP, pH 7.5 The resultant insoluble myofibril ghosts (the residue after KI extraction) were rinsed four times by stirring in 5 volumes of distilled water and gently centrifuging the suspension at 500 x *g* for 5 min. The sticky ghosts were stored at 2°C in distilled water sometimes containing 2 mM NaN_3.

Approximately 100 ml of each of the first HS and KI extracts were dialyzed against five changes of 3.5 liters of 0.1M NaCl, 0.05 M Tris-Cl pH 7.6. Precipitated protein was dispersed using a Teflon ball homogenizer and used as a suspension in the assays.

CANP: preparation, measurement of activity, and use

CANP was purified from chicken breast muscle according to the method of Ishiura et al. (1978). The purified enzyme was mixed with glycerol (1:1) and stored in liquid N_2 until use. CANP activity was measured at 25°C in reaction mixtures containing 0.1M NaCl, 0.24% (w/v) alkali denatured casein (from Ishiura et al., 1978, prepared fresh prior to use by dissolving the casein in 0.1N NaOH, boiling, and adjusting to pH 7.8 with 0.1N HCl), 5 mM 2-mercaptoethanol, 2 mM $CaCl_2$ or EDTA, and 0.1M Tris-acetate buffer pH 7.8. Reactions were stopped with the addition of 100 mM EDTA to a final concentration of 10 mM, and the protein was precipitated by the addition of an equal volume of ice cold 10% trichloracetic acid. Complete precipitation was ensured by standing at 2°C for 1 hr, tubes were then centrifuged at 3000 x *g* for 5 min and the absorbance at 280 nm of the supernatant solution was determined against the EDTA blank. One unit of CANP activity was defined as that amount of the enzyme which catalysed an increase in absorbance of 1.0 Absorbance units at 280 nm per hour under these standard assay conditions. In the CANP treatment of protein samples, the same conditions were used as in the assay, substituting the proteins for the casein substrate.

Protein determination

Protein was determined by the biuret method (Gornall et al., 1949) or by the Lowry method (Lowry et al., 1951) using BSA as a protein standard.

Analytical gel electrophoresis

Analytical SDS polyacrylamide gel electrophoresis was performed using the technique of Porzio and Pearson (1977). Samples in 30% glycerol were applied in volumes from 5 to 150 μl, and allowed to run into the gel at 0.25 ma/tube for 30 min prior to the actual electrophoresis. Following electrophoresis, gels were stained overnight in a solution containing 0.2% Coomassie Brilliant Blue R250 in 45% ethanol and 10% acetic acid (v/v) and destained with an electrophoretic destainer (Canalco Corp., Rockville, MD) with 5% isopropanol and 7.5% acetic acid. Gels were photographed using Kodak Panatomic-X film and an orange filter for enhanced contrast.

Preparative SDS gel electrophoresis

Samples containing 400 μg KI soluble protein or 100 μg ghost protein were applied to 6 x 80 mm acrylamide gel rods and electrophoresed until the actin band had migrated 75% of the length of the gel (usually 9.5 hr at 1.25 ma/tube). The unstained gels were immediately scanned at 280 nm in a Gilford 2520 gel scanning attachment. When the actin band passed through the light path the gel transport mechanism was stopped and the gel was nickel with a sharp scalpel at that spot to mark the position of the band. The actin bands were subsequently cut out and stored at 2°C. Actin was eluted from the gel slices following the method of Lazarides (1976) and using a solution consisting of 0.1% SDS, 0.05 M Tris-acetate pH 7.8 as an elution buffer. SDS bound to the eluted protein was removed using a modification of the proceudre of Weber and Kuter (1971). Small columns of Dowex AG1-X2 (2 ml packed volume) were prepared in Pasteur pipettes and pre-equilibrated with 0.05 M Tris-acetate pH 7.8. Each protein sample was passed through a column, and followed by the 0.05 M Tris-acetate buffer. Approximately 80% of the total UV (280 nm) absorbing material was recovered from each column.

Isoelectric focusing

Purified protein samples were focused in 7.5% (40:1, acrylamide) gels with 8M urea. Each gel also contained 5% (v/v) glycerol, 1.4% pH 5–7 ampholytes (Bio-Lyte, Bio Rad Laboratories) and 0.6% pH 2–11 ampholytes, and used 0.05% TEMED and 0.0125% ammonium persulfate as polymerizing agents. Protein samples were dissolved in 10M urea and added to the gel mixture before polymerization. The lower (anode) chamber consisted of 0.02N H_2SO_4 and the cathode solution was 0.02N NaOH. Proteins were focused for 16 hr at 300V then for 1 hr at 700V. Gels were stained and destained as described for gel electrophoresis.

Amino acid analysis

Protein samples were hydrolyzed in 6N HCl in evacuated ampoules at 110°C for 24 hr, then lyophilized. The analysis was performed in duplicate on a Beckman 121 MB amino acid analyser. No corrections were made for amino acid losses during hydrolysis.

Preparation of antisera

Protein samples recovered from SDS-gels were dialyzed against 0.1M NH_4HCO_3, then lyophilized. Samples were dissolved in 0.9% saline and mixed with an equal volume of Freund's complete adjuvant (H37A, Difco). 0.1 ml samples containing 50 μg protein were injected intraperitoneally into a group of 5 BALB/cCr female mice aged 88 days. Subsequently, mice were reinjected by foot pad injection with 100 μg protein per mouse in Freunds incomplete adjuvant at intervals from 4 to 6 wk. Following five rounds of immunization, blood was collected by cardiac puncture, and allowed to clot at 37°C for 1 hr then at 2°C overnight. After centrifugation at 3000 x *g* the serum was collected, diluted 1:1 with 0.9% saline and fractionated by adding ammonium sulfate to 33% saturation. Both the precipitated serum protein and the supernatant were dialyzed for 8 hr against each of three changes of 1.8 liters of 0.9% saline. Serum fractions were tested for precipitating antibodies using Ouchterlony immunodiffusion plates (Pattern C, Hyland). Precipitating antibodies were detected in only the ammonium sulfate-soluble fraction of mice immunized with ghost actin.

Indirect immunofluorescence

Actin was localized in homogenized glycerinated muscle fibers adhering to glass coverslips following the method of Lazarides and Balzer (1978), using fluorescein isothiocyanate-conjugated ghost anti-mouse IgG F $(ab')_2$ fraction (Polysciences Inc., Warrington, PA). In some experiments, myofibrils adhering to cover slips were flooded with the usual CANP assay mixture containing Ca^{++} or EDTA and incubated at 37°C for 45 min before treatment with antiserum.

Myofibrils were viewed under Zeiss Photomicroscope III (Carl Zeiss, Oberkochen, Germany) equipped with epifluorescence optics. Pairs of phase-contrast and fluorescence photographs were taken on Ilford HP–5 film exposed at ASA 1600 and overdeveloped 50% in Ilford Microphen. Exposure times were 5 to 25 sec.

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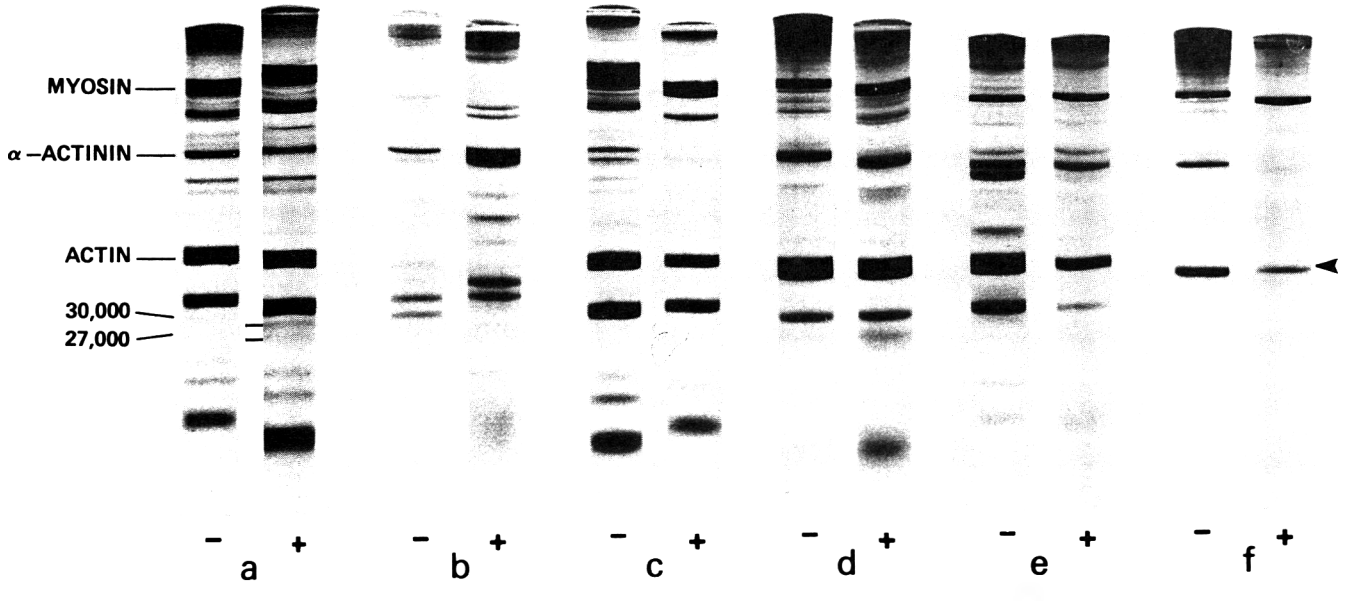

Fig. 1–SDS-PAGE of myofibrils incubated 1 hr with CANP prior to extraction with H-S and KI, showing the protein distribution in extracts. Myofibrils (12 mg/ml) were incubated with CANP (0.7 units/ml) and 5 mM $CaCl_2$ (+) or EDTA (–) at 25°C for 1 hr, and subsequently extracted with H-S and KI as described in Materials & Methods. Gels depict (a) myofibrils, 100 μg; (b) myofibril supernatant solution after CANP incubation, 10 μg; (c) protein soluble in the first H-S extraction, 100 μg; (d) H-S insoluble protein, 100 μg; (e) protein soluble in the first KI extraction, 100 μg; (f) KI insoluble residue, 50 μg. The amount of protein represents that for the control (–) gel; equivalent volumes were applied to the experimental (+) gel to facilitate comparison.

RESULTS / DISCUSSION

THE PURPOSE OF THIS RESEARCH was to determine the substrate or substrates of CANP within the myofibril which, when hydrolyzed, caused the release of α-actinin and the observed degradation or dissolution of Z-disc structure. Most previous research into the effects of CANP on the proteins of the myofibril has involved the study of complex unfractionated mixtures of myofibrillar proteins or simple isolated proteins using SDS-acrylamide gels (Dayton et al., 1975, 1976b; Azanza et al., 1979). Our approach was to simplify the gel patterns by fractionating the myofibrillar proteins before and after CANP treatment. Myofibrillar dissassembly was achieved using selective solvents to sequentially extract the components of the thick and thin filaments.

CANP-treated and control myofibrils were extracted using H-S to remove a majority of the myosin and some actin. This was followed by extraction of the residue with KI to depolymerize and solubilize the remaining actin, thus leaving the components of the Z-disc in an insoluble residue. This residue represented about 8% of the original myofibril protein. Fig. 1 displays the result of the sequential extraction of myofibrils following mild treatment with CANP. Evident in the myofibrils treated with CANP in the presence of Ca^{++} (Fig. 1a) are the 30,000 and 27,000 dalton fragments of Troponin-T (TN-T) hydrolysis and a decreased amount of α-actinin as compared to control myofibrils. The α-actinin appeared unhydrolyzed in the supernatant, shown in Fig. 1b, and has molecular weight identical to α-actinin in the intact myofibril. In myofibrils extracted with H-S, some of all of the myofibril proteins (with the exception of α-actinin) were soluble under the conditions of the extraction (Fig. 2c). Further extraction of the insoluble residue with KI resulted in the solubilization of most of the remaining protein, leaving an insoluble residue that contained only actin, α-actinin and myosin, as shown in Fig. 1f. In these paired gels of the factionated myofibril proteins, the most striking difference between the samples treated with CANP in the presence of Ca^{++} and control samples was in the amount of actin and α-actinin that remained in the KI insoluble residue. In the CANP case,

Table 1—Amino acid analysis of SDS-polyacrylamide gel purified KI soluble and KI insoluble actin

Amino acid	Number of residues (Mols/Mol)	
	KI soluble actin	Ghost actin
Lys	19.2	19.7
His	8.6	7.9
His (τ Me)	–	–
Arg	15.9	17.0
Asx	30.1	30.9
Thr	21.6	21.5
Ser	21.3	22.4
Glx	39.6	40.4
Pro	19.7	21.1
Gly	33.8	31.2
Ala	28.9	29.8
Cys	0	0
Val	16.3	17.5
Met	12.7	12.0
Ile	22.2	21.3
Leu	24.1	25.4
Tyr	12.2	10.6
Phe	10.4	10.6
Trp	–	–

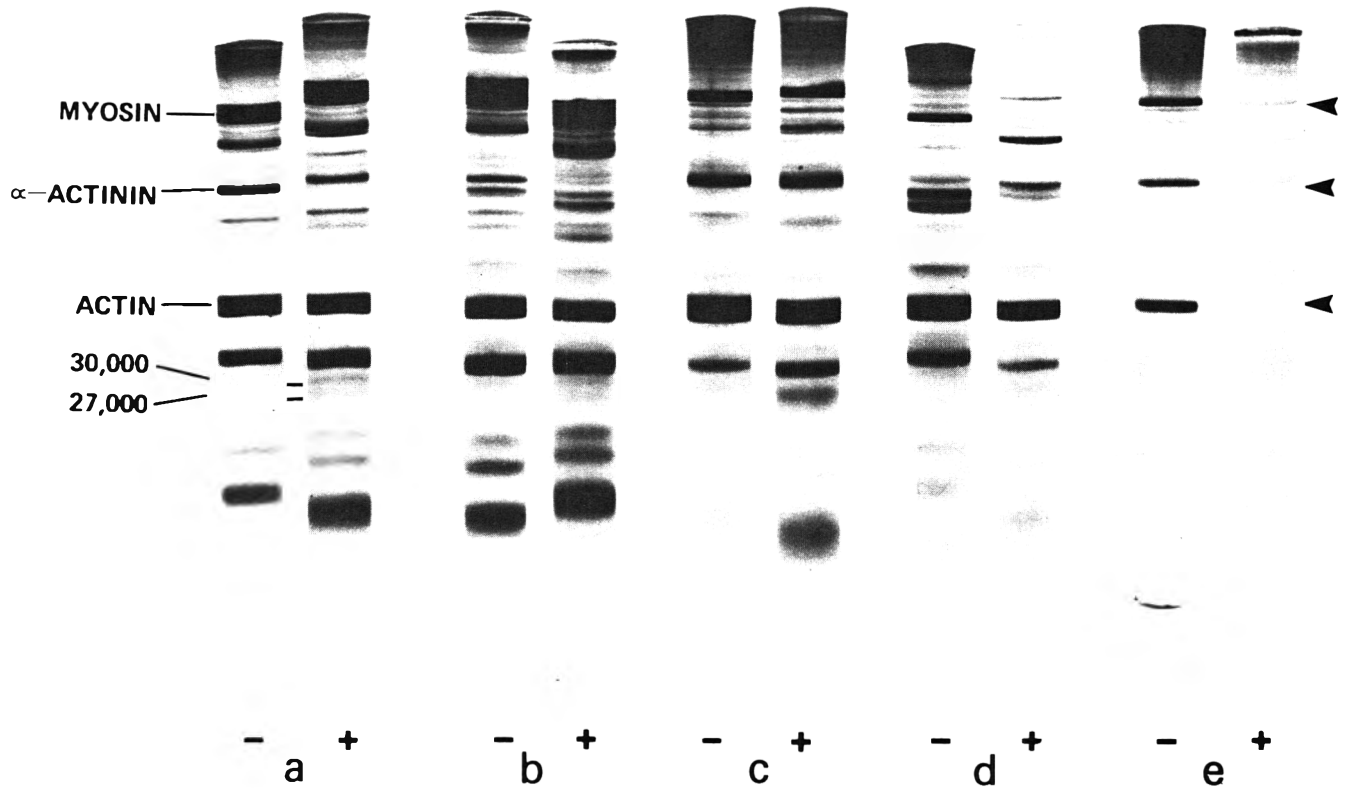

Fig. 2—SDS-PAGE of H-S and KI protein extracts and insoluble residues after incubation with CANP. Myofibrils (25 mg/ml) were extracted with H-S and KI. Protein extracts or residues were incubated with CANP (0.7 units/ml) and 5 mM $CaCl_2$ (+) or EDTA (–) for 1 hr at 25° C. (a) Myofibrils, 100 μg; (b) protein soluble in the first H-S extraction, 100 μg; (c) H-S insoluble residue, 100 μg; (d) protein soluble in the first KI extraction, 100 μg; (e) KI insoluble residue, 50 μg. Protein quantities represent those for the control (–) samples; equivalent volumes were applied to experimental (+) gels to facilitate direct comparison.

considerably less actin was evident in the residue. In experiments in which the period of incubation with CANP was prolonged, the actin in the KI insoluble residue was completely eliminated (gels not shown). Since it is understood that α-actinin is released unhydrolyzed due to CANP activity, the single important change that is evident is that actin (or a protein with a similar molecular weight) is hydrolyzed by CANP.

To determine if the protein in the KI residues could be hydrolyzed, the proteins of myofibrils fractionated with the above method were incubated with CANP. The results presented in Fig. 2 show nearly the same pattern of hydrolysis as previously shown for the intact myofibrils, and established that the 44,000 dalton protein in KI residues is hydrolysed by CANP (Fig. 2e). Both myosin and α-actinin also showed some hydrolysis both in the KI soluble and insoluble fractions (Fig. 2d and e). These results demonstrate that myosin and α-actinin were affected by exposure to KI in a way which caused those proteins to become susceptible to CANP hydrolysis. Actin in contrast, was segregated by KI into two distinct species, one that was soluble in KI and resistant to hydrolysis, and the other which remained KI insoluble and was completely hydrolyzable by CANP. Since it is understood that normal thin filament actin is refractory to CANP hydrolysis (Dayton et al., 1975), and is easily depolymerized in KI (Szent-Gyorgyi, 1951; Maruyama et al., 1965), the KI insoluble actin must be different.

To examine the properties of KI insoluble actin it first had to be purified, which proved difficult since the KI insoluble myofibril residue was highly insoluble in the aqueous buffers normally employed in common methods of protein purification. However, those proteins were soluble in solutions containing both SDS and 2-mercaptoethanol and could easily be resolved on acrylamide gels. Therefore actin in the myofibril residue was purified using preparative SDS gel electrophoresis. Actin prepared by that method showed one protein band on re-electrophoresis. Purified KI insoluble actin had a molecular weight identical to KI soluble actin prepared the same way; both actins comigrated as a single band on SDS-polyacrylamide gels.

The two actins, indistinguishable on the basis of molecular weight in SDS, differed however in isoelectric pH (Fig. 3). This result indicated a difference in the overall net charge on the protein, which might be due to a different amino acid composition or to some post-translational protein modification.

Amino acid analysis of both isolated actins is presented in Table 1. The fact that the two actins were indistinguishable by amino acid composition confirms that the protein in the KI insoluble residue is in fact actin and not a completely different protein having an identical molecular weight. Slight differences in the amino acid composition may exist, but would be distinguishable only in a more detailed sequence analysis.

Similarities between the two forms of actin were further investigated using immunological techniques. Both forms of SDS-purified actin were injected into mice to try to elicit

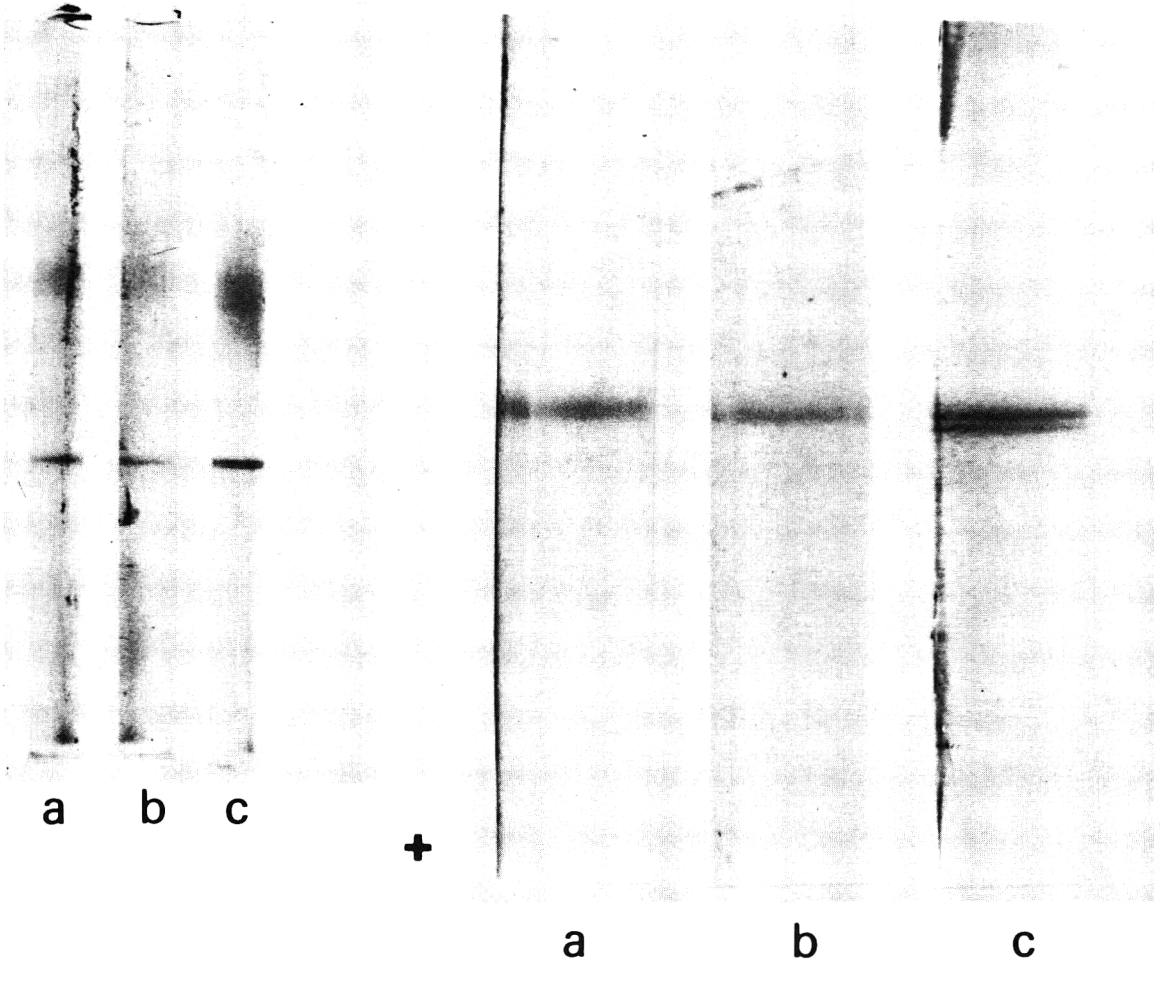

Fig. 3—Isoelectric focusing of actins isolated by preparative gel electrophoresis. (a) KI soluble actin, 10 μg; (b) KI insoluble actin, 10 μg and (c) both KI soluble and insoluble actins combined, 10 μg each. Magnified sections of the same gels are also illustrated.

an antigenic response. Precipitating antibodies to KI insoluble actin were detected after the fourth round of immunization. Mice failed to produce an antigenic response to the KI soluble actin even after five injections of the protein. Several researchers have reported that it is difficult to elicit antibodies to thin filament actin, apparently a highly conserved molecule in nature. The difference in response to the two forms of actin suggests some structural differences exist between the two molecules that maybe recognized at the level of the T-helper cells rather than the antibody producing B-cells. Ouchterlony double immunodiffusion showed that antibodies produced against KI insoluble actin recognized both actins identically, demonstrating a close homology between actins.

Indirect immunofluorescence using fractionated antiserum prepared against the KI insoluble actin established the location of that actin within the Z-disc. In Fig. 4B, intense labeling of actin occurred at the Z-disc, with weaker fluoresence observed across the entire myofibril, in comparison with non-immune serum controls shown in Fig. 4A. Weak M-line labeling was evident in some myofibrils. The cause of the differential Z-disc staining observed in these micrographs cannot be determined from these experiments. It could be due to a higher affinity of the antibody for the actin in the Z-disc, because antigenic sites on the Z-disc are more accessible to the antibody, or simply because of a greater abundance of actin at the Z-disc. In the KI extracted myofibrils shown in Figure 4D, fluorescent actin

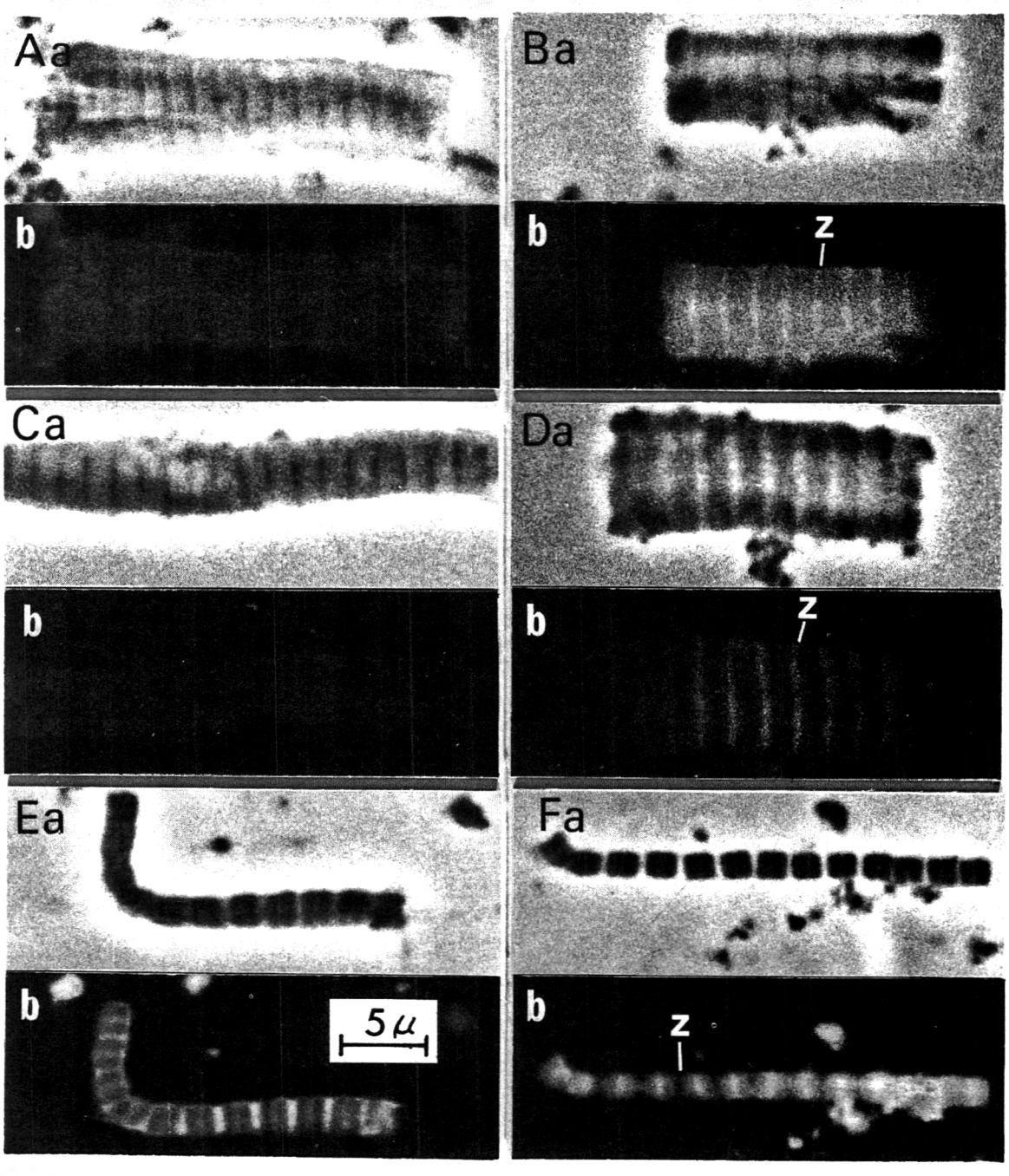

Fig. 4—Indirect immunofluorescent labelling of KI insoluble actin in myofibrils, KI extracted myofibrils and myofibrils incubated with CANP. Paired phase contrast (a) and fluorescence (b) micrographs of FITC labelled anti-KI insoluble actin (α-G) treated glycerinated muscle myofibrils and extracts. (A) Myofibrils, nonimmune serum control; (B) Myofibrils, α-G serum treated; (C) KI-extracted myofibrils, nonimmune serum control; (D) α-G serum treated; (E) Myofibrils incubated with CANP, EDTA control; (F) Myofibrils incubated with CANP and Ca^{++}.

staining at the Z-disc remained unaffected while the weaker thin filament fluorescence was completely eliminated. Clearly, KI removed only thin filament actin, leaving the actin in the Z-disc undisturbed.

Following CANP treatment of myofibrils open gaps were evident in phase-contrast micrographs between I-zones where Z-discs once existed [Fig. 4F(a)]. Fluorescent labeling at the Z-disc was completely absent [Fig. 4F(b)] while weak actin fluorescence in the residual myofibril remained as in the untreated myofibril depicted in Fig. 4E(b). These results show that CANP preferentially hydrolysed the KI insoluble actin at the Z-disc, without affecting thin filament actin.

The hydrolysis of Z-disc actin provides a useful explanation of the mechanism for the breakdown of Z-disc structure. It is now generally accepted that the breakdown of Z-disc structure is responsible for the tenderization of meat which occurs during postmortem aging. Deterioration of Z-disc structure was identified through studies of the ultrastructural changes which meat undergoes during postmortem aging. Z-discs were found to be completely removed (Fukazawa and Yasui, 1967; Fukazawa and Briskey, 1970) or to develop gaps and discontinuities (Stromer et al., 1967; Goll et al., 1970) during the aging process. The complete or partial deterioration of Z-disc structure is considered responsible for the breaks in the myofibril at the Z-disc or the I-Z junction which occur in aged muscle when stretched or homogenized (Davey and Dickson, 1970; Gann and Merkel, 1978; Parrish et al., 1973). This Z-disc fragility in aged muscle myofibrils is thought to be the basis of tenderization which develops during postmortem aging. In fact, methods using a fragmentation index to correlate the susceptibility of myofibrils to undergo breakage during homogenization with organoleptic tenderness have been successful in predicting tenderness (Takahashi et al., 1967; Moller et al., 1973). Although over a dozen years have passed since the first observations of Z-disc breakdown, the mechanism by which it occurs has remained obscure. CANP was identified as the enzyme responsible for Z-disc dissolution (Busch et al., 1972; Dayton et al., 1975, 1976a, b), and investigations of the enzyme's substrate specificity have shown that it readily hydrolyses TN-T. Although a parallel increase in tenderness has been observed with the degradation of TN-T (Olson et al., 1977; MacBride and Parrish, 1977), a direct connection with Z-disc weakening is not obvious. As well, in high concentrations CANP has been shown to partially hydrolyze troponin-I, tropomyosin, C-protein, M-protein (Dayton et al., 1975), myosin heavy chain and α-actinin (Ishiura et al., 1979; Azanza et al., 1979). However, in no case does the hydrolysis of these proteins explain the release of unhydrolysed α-actinin or the detachment of the thin filament from the orderly arrangement of the Z-disc.

α-actinin was first identified and shown to reside in the Z disc by extraction and reconstitution experiments (Goll et al., 1969; Robson et al., 1970; Stromer and Goll, 1972). In vitro, α-actinin has been shown to bind F-actin to form an insoluble gel matrix (Drabikowski et al., 1968). At physiological temperatures, α-actinin binds only the Z-disc end of the thin filament (Goll et al., 1972). These results, in conjunction with reconstitution experiments, have suggested that the branching thin filaments in the Z-disc provide a template for the assembly of α-actinin on the Z-disc site. The destruction of the orderly protein arrangement at the Z-disc could easily result from the hydrolysis of the Z-disc actin and the hydrolysis of the binding site for α-actinin would account for its release from the Z-disc. Furthermore if this Z-disc actin is continuous with the thin filaments or is in some other way responsible for holding the thin filaments together at the Z-disc, its hydrolysis might explain the detachment of the thin filaments from the Z-disc seen in electron micrographs of fragile (CANP-treated) myofibrils (Dayton et al., 1976b).

The discovery of an isoelectric variant of actin is not unprecedented. A number of isoelectric forms of actin have been identified in a wide variety of tissues (Pollard and Weihing, 1974). Several have been sequenced and found to differ by as many as twenty-four amino acid residues (Vandekerckhove and Weber, 1978). At least three different forms have been recognized in muscle (Whalen et al., 1976). It is not yet known if the KI insoluble form described here is a fourth variant.

Other evidence supporting these results has been presented by Gard and Lazarides (1979) who used guinea pig transglutaminase to label Z-disc proteins and also identified a KI insoluble form of actin residing in the Z-disc. These workers reported that brief treatment of myofibrils with trypsin (a protease that has the same effects on Z-disc structure as CANP) resulted in the complete hydrolysis of the Z-disc actin and the release of unhydrolyzed α-actinin.

The results presented here add new information to that which is currently known about myofibril structure and composition. Two kinds of actin exist in the myofibril, with the majority of the actin being found in the thin filaments and probably involved in interaction with myosin. The lesser portion of the myofibrillar actin (the isoelectric variant of actin) resides in the Z-disc and has a primarily structural role as the key element that provides the basis for the strength and continuity of the Z-disc. The hydrolysis of this Z-disc actin provides a plausible mechanism by which CANP can dissolve visible Z-disc structure and bring about tenderization during the postmortem conversion of muscle to meat.

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SHEAR MEASUREMENTS OF MEAT TENDERNESS . . . From page 1357

Harris, 1972a), to estimate the likely effects of various treatments on connective tissue.

It is of interest overall to find that two dissimilar measuring systems, both nominally called WB shear systems, yield very comparable results. This suggests that the assessments of overall treatment effects using WB shear devices should be comparable in different laboratories around the world.

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Influence of Type (Wooled or Hair) and Breed on Growth and Carcass Characteristics and Sensory Properties of Lamb

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ABSTRACT

Comparisons were made between 12 Barbados Blackbelly and 12 St. Croix ram lambs (hair types) and 10 Florida Native and 12 crossbred ram lambs (wooled types) for growth, carcass characteristics and sensory panel evaluation scores. In addition, 14 predominantly hair and 14 predominantly wooled crossbred ram lambs were compared for carcass characteristics and sensory panel evaluation scores. The hair sheep had a lower rate of gain than the wooled types. The wooled breeds and predominantly wooled crossbreds were heavier and graded higher than the hair breeds and predominantly hair types. The sensory panel found the meat acceptable (tenderness, juiciness, flavor and acceptability) from all breeds.

INTRODUCTION

HAIR TYPE SHEEP indigenous to the tropical regions of the world have a greater resistance to parasites, are more heat tolerant and more prolific than wooled breeds (Turner, 1974). Multiple births are quite common in the Barbados Blackbelly breed and it is possible to breed ewes twice a year (Devendra, 1977). These characteristics have attracted the interest of the U.S. sheep industry. There is limited information available on growth characteristics of the hair sheep breeds. Maule (1977) summarized the available data. Goode et al. (1980) have published data on the reproductive performance of crosses of Blackbelly and wool breeds. Shelton and Carpenter (1972) reported poorer gains for Barbados-sired lambs than for other exotic breeds. However, carcass quality was acceptable for lambs fed to 100 lb (45 kg).

Most research has been done on the wooled breeds. Dransfield et al. (1979) reported that sire breed (Texel, Dorset Down, Suffolk, Oxford, Cotswold or Southdown) had no effect on the sensory qualities of loin roasts from crossbred lambs. Cramer et al. (1970) reported increases in fineness of wool in the wool breeds were accompanied by increased mutton flavor. They found Rambouillet to have more objectionable mutton flavor than Targhee and Targhee more objectionable flavor than Columbia. Mendenhall and Ercanbrack (1979) reported however that weight, sex and breed had no effect on consumer acceptance of retail cuts from Rambouillet, Targhee, Columbia or crossbred lamb carcasses marketed in Utah and California. Carcass weight did not appear to influence consumer preference when retail package size was similar (Southam and Field, 1969).

Tichenor et al. (1969) reported that flavor and juiciness of roasted Longissimus dorsi muscles were not affected by slaughter weight, rate of gain or sex of lambs. The flavor of all roasts was considered acceptable, and meat from the heavy lambs was as well accepted as that from the younger, lightweight lambs. Tenderness of roasted racks from wethers increased with increasing weight. Roasts from wethers had a higher average tenderness score than roasts from rams; but, all were acceptable. General acceptability scores were highly correlated with flavor, juiciness and tenderness; but, tenderness appeared to be the most important determinant of acceptability.

The purpose of Phase 1 of this research was to evaluate the influence of hair types (Barbados Blackbelly and St. Croix) on growth, carcass characteristics and sensory panel evaluation scores. The crossbreeding effects of wooled/hair sheep on similar traits were evaluated in Phase 2.

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EXPERIMENTAL

Phase 1

Forty-six ram lambs including 12 straightbred Barbados Blackbelly, 12 straightbred St. Croix (White Hair from St. Croix Island, Virgin Islands), 10 Florida Natives and 12 Suffolk sired crossbreds were used in this study. Twenty-four of the lambs (Barbados and St. Croix) were hair type, and twenty-two of the lambs (Florida Native and crossbred) were wooled type. Florida Native sheep were chosen for this comparison for their light fleece weight and heat resistance (Loggins et al., 1975) making them more similar to the hair type sheep than other wooled breeds.

The 12 crossbred lambs were Suffolk sired from white face wooled type ewes. Seven were 1/2 Suffolk, 3/8 Finn and 1/8 Rambouillet. Two were 1/2 Suffolk, 1/4 Targhee, 1/8 Rambouillet and 1/8 Finn. One was 1/2 Suffolk, and 1/2 Targhee. The remaining lambs were 1/2 Suffolk, 1/4 Targhee, 1/4 Finn; and, 1/2 Suffolk, 1/4 Targhee, 1/8 Dorset and 1/8 Finn.

All lambs were born in housed confinement and were creep fed a high protein concentrate (CP-18%) beginning at 2 wk of age. The high concentrate diet was continued for approximately 2 months post weaning and then changed to a 15% CP diet during the last month of the trial. Lambs were removed from experiment when the live animals appeared to have a similar degree of body fatness based on a subjective evaluation of body composition. Test animals were handled for fatness at each weighing period and removed from slaughter when fat thickness over the 12-13th rib area was estimated to be between 0.65–0.74 cm.

Lambs were transported to The Ohio State University Meat Laboratory and slaughtered in a conventional manner. Carcasses were chilled at 3 ± 4°C for 72 hr prior to cutting into wholesale cuts.

Carcass evaluation

All carcasses were evaluated for marbling, conformation, leg conformation score, and quality score. A final quality grade was assigned based on conformation and quality scores. Measurements included three fat thicknesses over the Longissimus dorsi (L.D.; medial, central and lateral), quantity of kidney and pelvic fat and L.D. area.

Sensory evaluation

The lamb meat was prepared and cooked as rib chops and as rolled shoulder and evaluated for tenderness, color, juiciness, flavor, and acceptability. Six chops and one rolled roast from each lamb in each group were used for sensory evaluation. The wholesale rack was separated into two single racks. One rack was cut into 2.54 cm thick rib chops. The rib chops were randomized and six were selected for sensory panel evaluation. Chops were broiled in an electric broiler until they reached 77°C internal temperature. The L.D. was trimmed of external fat and served as 1/2 in. cubes. Untrimmed rolled shoulders were roasted at a temperature of 149°C in an electric oven until they reached 80 ± 2°C internal temperature. Chop and rolled roast sample were served warm to panel members. A six

member laboratory panel (experienced with lamb and liked lamb flavor) assessed duplicate samples at each session. The samples were scored for tenderness (1 = extremely tough, 8 = extremely tender), cooked internal lean color (1 = extremely pale, 8 = extremely dark), juiciness (1 = extremely dry, 8 = extremely juicy), flavor (1 = intense mutton flavor, 8 = intense lamb flavor) and acceptability (1 = extremely unacceptable, 8 = extremely acceptable) using an 8-point scale and an average score was calculated.

The remaining uncooked lean portion of the L.D. muscle and a center slice from the rolled shoulder were each ground, mixed and analyzed for moisture and fat. Moisture was determined on all samples by oven drying at 100°C for 18 hr, fat by Soxhlet extraction with anhydrous ether for 8 hr and calculated on a wet basis. Protein was estimated by difference assuming the ash content to be 1%.

Table 1—Distribution of lambs according to type and breed (Phase 2)

Phenotype[a]	Blood relationship	No. of lambs
1	1/2 St. Croix, 1/4 Finn, 1/4 Wooled Breeding	1
	1/2 St. Croix, 1/4 Barbados, 1/4 Finn	2
	1/2 Barbados, 1/4 St. Croix, 1/4 Finn	2
	3/4 St. Croix, 1/4 Finn	1
	1/2 Barbados 1/4 St. Croix, 1/4 Rambouliiet	1
2	3/4 St. Croix, 1/4 Finn	2
	1/2 Barbados, 1/4 St. Croix, 1/4 Finn	2
	1/2 St. Croix, 1/4 Finn, 1/4 Wooled Breeding	1
3	3/4 St. Croix, 1/4 Finn	1
	1/2 St. Croix, 1/4 Finn, 1/4 Wooled Breeding	1
4	1/2 Dorset, 1/4 St. Croix, 1/4 Rambouliiet	5
	1/4 Suffolk, 1/4 Barbados, 1/4 Dorset, 1/4 Wooled Breeding	1
	1/2 Suffolk, 1/4 Rambouliiet, 1/4 Finn	1
5	1/2 Dorset, 1/4 St. Croix, 1/4 Rambouliiet	2
	3/4 Suffolk, 1/4 St. Croix	3
	1/4 Suffolk, 1/4 Barbados, 1/4 Dorset, 1/4 Wooled Bredding	1
	3/4 Dorset, 1/4 St. Croix	1

[a] Wool Score: 1 — Hair, 2 — Predominantly Hair, 3 — 1/2 Hair and 1/2 Wool, 4 — Predominantly Wool, 5 — Wool.

Yield

Cooking yield was determined by recording the uncooked and cooked weights of the rib chops used for sensory evaluation. Cooked rolled shoulder roasts were placed on paper towels for 5 minutes prior to reweighing to remove cooking drip. Yield was calculated as a ratio of cooked weight to uncooked weight and converted to a percentage.

Analysis

The data were analyzed using the SAS implementation of the Least Squares and Maximum Likelihood General Purpose Program (Harvey, 1977) with breeds nested within lamb types.

Phase 2

Twenty-eight crossbred ram lambs were used to evaluate the influence of hair type or wooled type on carcass and sensory properties. Half the lambs were of predominantly hair and half predominantly wool breeding. The sires were either of straightbred hair or wooled breeding while the dams were half and half wooled. The distribution and breeding of the predominantly hair and predominantly wooled crossbred sheep is shown in Table 1. A visual wool-score was performed by two evaluators when the lambs were 3–4 months old.

These lambs were part of a nutrition study evaluating the effects of crude protein in the diet. The lambs were fed complete pelleted diets containing 10%, 13% or 16% crude protein.

Selection for slaughter was the same as Phase 1. The lambs were slaughtered on three slaughter dates. Lambs were conventionally slaughtered, aged 72 hr at 3 ± 4°C prior to cutting into wholesale cuts. Carcass grading and measurements, sensory panel evaluation and proximate analysis were carried out as in Phase 1.

The statistical analysis was conducted with the General Linear Model of the Statistical Analysis System (SAS Institute, 1979). Regression analysis of variance was performed using wool score as the continuous independent variable. Dietary protein level was determined to be nonsignificant and was pooled into the error term.

RESULTS & DISCUSSION

Phase 1

Average daily gain for the hair sheep was lower ($P < 0.01$) than the wooled type (Table 2). The St. Croix gained faster than did the Barbados. The crossbreds gained faster than the Florida Natives. This trend is also reflected in the age off test (started on test at an average of 2 wk of age). Since the basis for selection for slaughter was a visual

Table 2—Live animal and carcass traits for lambs (Values are least square means ± standard error)

Variable	Hair type			Wool type		
	Mean for hair type	Barbados	St. Croix	Mean for wooled type	Florida N.	Crossbred
Avg daily gain (g)	200 ± 9**	172 ±41*	222 ±27	304 ± 9	259 ±59**	349 ±73
Age off test (days)	166 ± 4**	158 ±21**	173 ±20	149 ± 5	158 ±22*	139 ±24
Slaughter wt (kg)	31.2 ± 0.9**	25.2 ± 3.8**	37.1 ± 2.5	42.8 ± 1.0	38.8 ± 5.5**	46.8 ± 5.6
Hot carcass wt (kg)	17.2 ± 0.6**	14.1 ± 2.2**	20.2 ± 1.8	23.1 ± 0.6	21.8 ± 3.7*	24.5 ± 3.2
Cold carcass wt (kg)	15.3 ± 0.6**	12.3 ± 1.9**	18.4 ± 1.8	21.7 ± 0.6	20.5 ± 3.7*	22.9 ± 3.6
Untrimmed leg (kg)	5.0 ± 0.2**	4.3 ± 0.6**	5.7 ± 0.5	6.6 ± 0.2	6.2 ± 0.9**	7.1 ± 1.0
Conformation[a]	9.0 ± 0.2**	9.3 ± 0.6**	10.8 ± 0.8	12.2 ± 0.2	12.2 ± 1.2	12.2 ± 1.1
Leg conformation score[a]	10.5 ± 0.2**	10.2 ± 0.8	10.8 ± 0.7	12.5 ± 0.2	12.3 ± 1.1	12.7 ± 1.0
L.D. area (sq cm)	8.77± 0.32**	7.80± 1.35**	9.68± 1.03	11.16± 0.32	10.90± 1.68	11.48± 1.81
Medial fat (cm)	0.20± 0.03**	0.10± 0.05**	0.28± 0.10	0.38± 0.03	0.43± 0.13	0.36± 0.18
Central fat (cm)	0.18± 0.03**	0.10± 0.05**	0.25± 0.10	0.28± 0.03	0.30± 0.13	0.28± 0.08
Lateral fat (cm)	0.28± 0.03**	0.18± 0.13**	0.38± 0.13	0.53± 0.03	0.56± 0.20	0.48± 0.18
Pelvic fat (g)	131.5 ±13.6**	81.6 ±27.2**	176.9 ±49.9	213.2 ±13.6	217.7 ±49.9	208.6 ±81.6
Kidney fat (g)	435.4 ±54.4**	258.6 ±86.2**	612.4±217.7	839.1 ±54.4	1011.5±444.5**	671.3±199.6
Marbling[b]	10.2 ± 0.6**	6.9 ± 3.2**	13.6 ± 2.2	15.3 ± 0.6	15.0 ± 2.9	14.7 ± 2.5
Quality score[a]	11.6 ± 0.3**	9.5 ± 1.3**	13.7 ± 1.4	14.2 ± 0.4	14.2 ± 1.8	14.2 ± 0.4
Final grade[a]	10.3 ± 0.2**	9.4 ± 1.0**	12.2 ± 0.8	13.3 ± 0.2	13.2 ± 1.6	13.3 ± 0.6

[a] Conformation, leg conformation, quality, final score, 15 = high prime, 14 = avg prime, 13 = low prime, 12 = high choice, 11 = avg choice, 10 = low choice, 9 = high good, 8 = avg good.
[b] Marbling score, 16 = Modest$^-$, 15 = Sm$^+$, 14 = Sm^0, 13 = Sm$^-$, 12 = Sl$^+$, 11 = Sl^0 . . . 7 = Tr$^-$, 6 = Prac. dev$^+$.

*Significantly different means ($P < 0.05$) for animal type or breed within animal type.
**Significantly different means ($P < 0.01$) for animal type or breed within animal type.

evaluation of finish, rate of finishing and age off test should be inversely related. Thus, older lambs at slaughter had a slower finishing rate. The crossbreds were younger ($P < 0.05$) when slaughtered than the Florida Natives. The St. Croix lambs were older when slaughtered than the Barbados ($P < 0.01$). The types (hair vs wooled) were significantly different ($P < 0.01$) for average daily gain and age off test.

When weights were compared, such as off test weight, slaughter weight, hot carcass weight, cold carcass weight and untrimmed leg weight, in all cases the wooled type animals were significantly ($P < 0.01$) heavier than the hair type animals. Lambs of the St. Croix breed were significantly ($P < 0.01$) heavier than lambs of the Barbados breed. Crossbred lambs were heavier ($P < 0.05$) than the Florida Native lambs.

Carcasses from the wooled lambs exhibited superior muscling. The L.D. area was significantly ($P < 0.01$) larger for the wooled types. Within the hair type, the L.D. area was significantly larger for the St. Croix than for the Barbados carcasses. Leg conformation scores followed the same trend. The leg conformation for the wooled breeds was scored high choice while the hair breeds scored low choice. This difference was significant ($P < 0.01$). The grading superiority of the wooled breeds and of St. Croix within the hair breeds continued for marbling, quality score and final grade ($P < 0.01$).

When measurements of fatness were compared, such as medial, central and lateral fat over the L.D., pelvic fat and marbling, in all cases the wooled type contained significantly ($P < 0.01$) more fat than the hair type and St. Croix was considerably ($P < 0.01$) fatter than the Barbados. When kidney fat was evaluated the same trend was noted with wooled sheep being significantly ($P < 0.01$) fatter than hair sheep and St. Croix being significantly ($P < 0.01$) fatter than Barbados.

The Florida Native carcasses had a thicker fat covering over the L.D. (medial, central and lateral), more pelvic fat and higher marbling scores than the crossbred carcasses but the difference was not significant ($P > 0.05$). The Florida Native carcasses contained significantly more ($P < 0.01$) kidney fat than the crossbred carcasses.

The chemical analysis of the raw muscle, yield and sensory panel evaluation of the cooked rib chops is shown in Table 3. The wooled type was significantly ($P < 0.01$) fatter than the hair type. This follows the same trend as measures of fatness in the carcass data. The chops from the St. Croix lambs contained more fat than the Barbados. The fat content of the St. Croix chops was more similar to either of the wooled breeds than to the Barbados. However, the design of the experiment did not permit statistical comparisons. Chops from Barbados lambs had higher ($P < 0.01$) cooking yields than chops from the St. Croix breed. This difference may be due to a possible higher bone to soft tissue ratio of these chops. Chops from the Barbados lambs were rated significantly ($P < 0.01$) less tender than chops from the St. Croix lambs. Similarly, these samples were rated lower in juiciness ($P < 0.01$) and acceptability ($P < 0.05$). The L.D. from these chops was lower in fat and marbling and this difference may also account for lower panel scores. The cooked chop internal lean color was slightly ($P < 0.01$) darker for the hair than the wooled

Table 3—Chemical analysis, yield and sensory panel scores of Longissimus dorsi (Least square means ± standard error)

	Hair type			Wool type		
Variable	Mean for hair type	Barbados	St. Croix	Mean for wooled type	Florida Native	Crossbred
% Moisture	75.59±0.18**	76.14±0.53**	75.06±0.57	74.58±0.19	74.22±1.31	74.94±1.03
% Fat	2.68±1.18**	1.94±0.43**	3.42±0.74	3.35±0.19	3.67±1.21	3.04±0.97
% Protein	21.73±0.12	21.93±0.44	21.52±0.75	22.06±0.13	22.11±0.57	22.02±0.57
Cooking yield (%)	61.96±0.87	64.97±2.07**	58.95±3.43	60.68±0.91	61.74±7.22	59.62±3.15
Tenderness[a]	5.3 ±0.2	4.8 ±0.9**	5.8 ±0.7	5.2 ±0.2	5.4 ±0.6	5.1 ±0.6
Color[b]	5.0 ±0.1**	4.9 ±0.3	5.1 ±0.2	4.8 ±0.1	4.8 ±0.3	4.7 ±0.4
Juiciness[c]	4.8 ±0.1	4.6 ±0.3**	5.1 ±0.4	4.7 ±0.1	4.9 ±0.4*	4.5 ±0.4
Flavor[d]	5.2 ±0.1	5.2 ±0.3	5.2 ±0.4	5.3 ±0.1	5.4 ±0.4	5.3 ±0.3
Acceptability[e]	5.8 ±0.1	5.5 ±0.6*	6.1 ±0.5	5.7 ±0.1	5.9 ±0.4	5.6 ±0.6

[a] 1 = Extremely tough . . . . 8 = extremely tender.
[b] Evaluation of internal lean color. 1 = extremely pale . . . . 8 = extremely dark.
[c] 1 = Extremely dry . . . . 8 = extremely juicy.
[d] 1 = Intense mutton flavor . . . . 8 = intense lamb flavor.
[e] 1 = Extremely unacceptable . . . . 8 = extremely acceptable.
*Significantly different means ($P < 0.05$) for animal type or breed within animal type.
**Significantly different means ($P < 0.01$) for animal type or breed within animal type.

Table 4—Chemical analysis, yield and sensory panel scores of rolled shoulder roasts (Least square means ± standard error)

	Hair type			Wool type		
Variable	Mean for hair type	Barbados	St. Croix	Mean for wooled type	Florida Native	Crossbred
% Moisture	61.83±1.16**	66.13±5.28**	57.53±5.87	45.91±1.22	44.99±5.59	46.84±5.99
% Fat	20.74±1.51**	15.36±6.65**	26.12±7.90	40.87±1.58	41.51±6.80	40.23±8.41
% Protein	17.43±0.39**	18.51±1.74**	16.35±1.93	12.97±0.42	12.99±1.38	12.93±2.46
Cooking yield (%)	64.27±0.61*	63.95±2.05	64.60±2.65	62.43±0.64	62.39±3.09	62.47±3.88
Tenderness[a]	6.4 ±0.1*	6.2 ±0.4*	6.6 ±0.4	6.7 ±0.1	6.6 ±0.4	6.7 ±0.3
Color[b]	4.2 ±0.1	4.2 ±0.3	4.1 ±0.2	4.1 ±0.1	4.2 ±0.3	4.0 ±0.3
Juiciness[c]	5.6 ±0.1	5.4 ±0.4*	5.7 ±0.4	5.7 ±0.1	5.8 ±0.4	5.7 ±0.4
Flavor[d]	5.4 ±0.1*	5.4 ±0.2	5.4 ±0.5	5.6 ±0.1	5.7 ±0.3	5.6 ±0.4
Acceptability[e]	6.2 ±0.1**	6.2 ±0.4	6.3 ±0.4	6.6 ±0.1	6.5 ±0.2	6.6 ±0.3

[a] 1 = Extremely tough . . . . 8 = extremely tender.
[b] Evaluation of internal lean color. 1 = extremely pale . . . . 8 = extremely dark.
[c] 1 = Extremely dry . . . . 8 = extremely juicy.
[d] 1 = Intense mutton flavor . . . . 8 = intense lamb flavor.
[e] 1 = Extremely unacceptable . . . . 8 = extremely acceptable.
*Significantly different means ($P < 0.05$) for animal type or breed within animal type.
**Significantly different means ($P < 0.01$) for animal type or breed within animal type.

Table 5—Carcass traits and grade scores for Phase 2 (Hair x Wooled Crosses) (Values are least square means ± standard error)

Variable	Wool score[a] 1	2	3	4	5
Number of lambs	7	5	2	7	7
Slaughter wt (kg)**	36.46±1.24	38.04±1.36	39.09±0.0	40.38±1.52	44.06±1.13
Hot carcass wt (kg)*	21.23±0.64	21.82±0.85	22.04±0.68	24.29±1.25	24.48±0.58
Cold carcass wt (kg)*	19.26±0.70	20.08±0.74	20.27±0.45	22.21±1.25	22.71±0.51
Conformation[b]*	11.3 ±0.3	11.8 ±0.4	11.0 ±0	12.7 ±0.3	12.6 ±0.3
Leg conformation[b]*	11.6 ±0.3	11.8 ±0.4	11.0 ±0	13.1 ±0.3	12.9 ±0.3
Quality score[b]	14.3 ±0.3	14.2 ±0.4	14.0 ±0	14.6 ±0.4	14.6 ±0.4
Final grade[b]	12.7 ±0.4	13.0 ±0.4	13.0 ±0	13.6 ±0.3	13.7 ±0.4
Medial fat (cm)	0.28±0.02	0.33±0.05	0.38±0	0.41±0.05	0.48±0.08
Central fat (cm)	0.28±0.02	0.28±0.02	0.25±0.13	0.35±0.08	0.43±0.08
Lateral fat (cm)	0.38±0.02	0.46±0.05	0.38±0.13	0.53±0.05	0.58±0.05
Marbling[c]	13.0 ±1.0	14.6 ±1.3	13.5 ±1.5	13.7 ±0.6	14.0 ±0.9
L.D. area (sq cm)	11.42±0.52	11.48±0.84	10.90±0.45	14.06±1.10	13.61±0.40
Kidney & pelvic fat (%)	1.47±0.16	1.80±0.25	1.80±0	1.58±0.31	1.40±0.08
Untrimmed leg (kg)*	5.89±0.15	6.01±0.20	6.11±0.25	6.77±0.34	6.87±0.15

[a] 1 = Hair, 2 = Predominantly hair, 3 = 1/2 Hair and 1/2 wool, 4 = Peedominantly wool, 5 = Wool.
[b] Conformation, leg conformation, quality, and final score, 15 = high prime, 14 = ave. prime, 13 = prime, 12 = high choice, 11 = ave. choice.
[c] Marbling score, 15 = Sm^{+}, 14 = Sm^{0}, 13 = Sm^{-}.
*$P < 0.05$ (Regresson analysis of variance)
**$P < 0.01$

Table 6—Chemical analysis and sensory panel scores for Longissimus dorsi for Phase 2 (Hair x Wooled Crosses) (Values are least square means ± standard error)

Variable	Wool score[a] 1	2	3	4	5
Number of lambs	7	5	2	7	7
% Moisture	74.20±.33	75.15±.35	75.34±.16	74.31±.29	74.75±.51
% Fat	3.72±.25	3.06±.23	3.48±.38	3.35±.15	3.51±.40
% Protein	21.08±.28	20.79±.22	20.18±.21	21.36±.25	20.74±.35
Tenderness[b]	5.2 ±.3	4.7 ±.4	6.3 ±.3	4.5 ±.4	4.2 ±.4
Juiciness[c]	4.5 ±.1	4.6 ±.2	5.0 ±.0	4.4 ±.2	4.4 ±.2
Flavor[d]	4.8 ±.2	5.0 ±.2	4.6 ±.1	4.8 ±.2	5.0 ±.2
Acceptability[e]	5.0 ± .2	5.2 ±.3	5.5 ±.1	4.9 ±.3	4.9 ±.2

[a] Wool score 1 = Hair, 2 = Predominantly hair, 3 = 1/2 Wool and 1/2 hair, 4 = Predominantly wool, 5 = Wool.
[b] 1 = Extremely tough . . . . 8 = extremely tender.
[c] 1 = Extremely dry . . . . 8 = extremely juicy.
[d] 1 = Intense mutton flavor . . . 8 = intense lamb flavor.
[e] 1 = Extremely unacceptable . . . 8 = extremely acceptable.

types. Since all chops were cooked to the same internal temperature, color difference should represent differences in lean color. Color of cooked lean for hair type sheep appeared similar to that of meat from older animals. The sensory panel found no other significant difference between chops from the hair and wooled types in juiciness, tenderness, flavor or acceptability. Samples from chops were rated at least slightly tender, slightly lamb flavored and slightly to moderately acceptable. Juiciness scores were lowest among sensory scores for most breeds and both types but may have been due to cooking to 77°C internal temperature.

Table 4 represents similar information for the rolled shoulder roasts. Roasts from the wooled types contained almost twice as much fat as from the hair types (40.87% vs. 20.74%, $P < 0.01$). Moisture and protein levels were higher ($P < 0.01$) in the hair than in the wooled types. Cooking yield was affected by fat content. Roasts from the hair type lambs had significantly ($P < 0.05$) higher yields than roasts from the wooled type lambs.

The sensory panel rated samples from the roasts of wooled type lambs higher in tenderness and flavor ($P < 0.05$) and acceptability ($P < 0.01$). Within type, samples from St. Croix lamb roasts were more tender ($P < 0.05$) but significance was not found for any other sensory attribute.

Phase 2

Carcass data from hair-wooled crossbred sheep were intermediate to the hair types and wooled types of Phase 1. Wool score was significantly related to weight (Table 5). Wooled type lambs were ($P < 0.01$) heavier (slaughter weight, hot and cold carcass weights and untrimmed leg weights) than lambs exhibiting hair characteristics. Carcass conformation score and leg conformation score were higher ($P < 0.05$) for lambs with increased wooled characteristics. Fat thickness and marbling were not significantly related ($P > 0.05$) to wool scores although fat thicknesses were higher for lambs with higher wool scores. Area of the L.D. was higher for lambs with increased wooled type breeding ($P < 0.10$).

Chemical analysis and sensory panel scores are presented (Table 6). None of the traits were significantly related to wool score. All sensory panel scores were within the acceptable range. The relationship between wool fineness and mutton flavor reported by Cramer et al. (1970) does not appear to be true for the hair X wooled crosses in this study.

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Incidence of *Salmonella* in Fresh Pork Sausage in 1979 Compared with 1969

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ABSTRACT

A *Salmonella* survey was conducted to determine its incidence in fresh pork sausage. Retail size samples representing different days of production were collected from 40 federally inspected plants and analyzed for the presence of salmonellae. The results obtained during the 1979 survey were compared to results obtained in a similar 1969 survey. Salmonellae were isolated from 162 of the 566 (28.6%) samples analyzed in 1969. For the samples analyzed in 1979, 74 of 603 samples (12.4%) were positive for salmonellae. The reduction of 16.2% over 1969 indicates a reduction in the incidence of *Salmonella* in this product for the firms tested.

INTRODUCTION

RECOMMENDATIONS by the United States Advisory Committee Report on *Salmonella*, (Anonymous, 1978) proposed the initiation of studies to determine the incidence of salmonellae in raw meat and poultry. According to the report, survey results would provide valuable information on the effects of changes in meat and poultry rearing and processing practices on the incidence of salmonellae.

Investigators in an earlier study (Surkiewicz et al., 1972) reported that salmonellae were isolated from 28.6% of the finished fresh pork sausage samples tested. The semi-quantitative methods employed in that study revealed that salmonellae were actually present at very low levels; most *Salmonella* positives were detected when 25g portions were tested and few positives were detected when 1.0 or 0.1 g amounts were analyzed.

The purpose of the present investigation was to respond to the Advisory Committee's recommendation (Anonymous, 1978) and (a) perform a benchmark survey to determine the incidence of salmonellae in fresh pork sausage produced in establishments under Federal inspection, (b) compare these findings to those previously reported (Surkiewicz et al., 1972) and (c) determine a trend, if any, in the *Salmonella* incidence levels in fresh pork sausage over an approximately 10-yr period.

MATERIALS & METHODS

Sampling

In the 1969 study (Surkiewicz et al., 1972), finished pork sausage samples were collected from 44 federally inspected plants. After collection, samples were frozen and shipped to Beltsville, MD, for analysis. Analysis for salmonellae were performed on 566 samples representing 40 of the above establishments.

In 1979, a second fresh pork sausage survey was undertaken where samples were submitted by in-plant USDA inspection personnel to the Food Safety and Inspection Service (FSIS) Field Service Laboratories (FSL) in Athens, GA, St. Louis, MO, and San Francisco, CA. Attempts were made to obtain samples from the same plants surveyed in 1969 (Surkiewicz et al. 1972). This was not possible since 16 of the original establishments were either no longer operating or not producing pork sausage. Sixteen new plants were chosen using the criteria that replacement establishments be as close as possible in size, production volumes, and geographic location to those studied in 1969.

Five randomly selected samples were collected from a production lot at each plant. This sampling was repeated on two additional occasions with intervals of approximately two months. All samples were promptly labeled, frozen, and shipped to one of the three designated laboratories. A total of 603 samples were collected from the 40 producers over a period of approximately 8 months. Where possible, information regarding the sources of starting materials, i.e. fresh or frozen trimmings, hot boning, etc. was also provided.

Laboratory methods

Samples were kept frozen until analyses were started, usually within 14 calendar days of receipt. Each pork sausage sample was thawed overnight at refrigeration temperatures. After thawing, a 25g portion of the sample was aseptically weighed out and used as the test sample. Each test portion was placed in a separate sterile blender jar to which was added 225 ml of sterile lactose broth containing 0.6% tergitol 7. Samples were blended for 2 min after which the entire contents were transferred to sterile containers and incubated for 24 hr at 35°C. After incubation, 1.0 ml from each jar was transferred to tubes containing 10 ml of tetrathionate (TT) broth (Hajna and Damon, 1956) and incubated 18–24 hr at 35°C. Loopfuls of the TT broth tubes were streaked onto Brilliant Green Sulfa agar (BGS) and Xylose-Lysine-Deoxycholate agar (XLD) plates and incubated for 24–48 hr at 35°C. Colonies (3–10) including both typical and marginally suspicious from the selective BGS and XLD plates were transferred to triple sugar iron and lysine-iron agar slants and incubated for a minimum of 24 hr at 35°C. Isolates were further identified as salmonellae by previously described methods (USDA, 1974). Each culture was transferred to 13 x 100 mm screw capped tubes containing nutrient agar (DIFCO) and stored at ambient temperature pending shipment to the Veterinary Services Laboratory, Animal and Plant Health Inspection Service, USDA, Ames, IA, for serotyping.

RESULTS & DISCUSSION

THE FRESH PORK SAUSAGE products sampled during the 1979 survey were prepared using several types of meat ingredients. Of 40 plants sampled, 19 prepared fresh sausage using trimmings from fresh primal cuts or carcasses, cut and trimmed on the premises. Eleven plants utilized chilled fresh trimmings or primal cuts from off-premise sources. Four plants employed pork trimmings obtained frozen from outside sources. Five plants prepared whole hog/hot hog sausage consisting of meat from slaughtered hot-boned carcasses. One plant used a combination of (on-premise) fresh and purchased frozen trimmings. In 1969, the majority of firms sampled also produced their sausage from fresh (on-premise) trimmings. Six plants produced whole hog/hot hog sausage and the remaining establishments used chilled or frozen trimming (off-premises). Due to the large number of variables involved in the starting materials, no correlation between these and the *Salmonella* findings was attempted.

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A reduction in *Salmonella* incidence levels was observed when the results from 40 plants surveyed in 1969 are compared to the 1979 data. In the 40 matched plants shown in Table 1, *Salmonella* incidence levels decreased in 20, increased in 13, and stayed the same in seven. Disregarding the seven that did not change, the probability is 2.4% that this number or more decreases would occur if there were actually no true difference. The difference of the average incidence rates per plant between the two years is 14.1%, and a 95% confidence interval on this difference is 3.4% to 24.7%. These probability statements and confidence intervals should be understood only as approximate and are dependent upon an equal probability of selection of plants.

All 40 plants originally sampled in 1969 were not available for the 1979 survey; however, a comparison of the *Salmonella* incidences involving the same 24 plants sampled in 1969 and 1979 was made. *Salmonella* levels for this group of plants are shown in Table 2. When comparing the 24 plants that were the same for the two periods, similar relationships are found. Furthermore, there was a small clearly nonsignificant Pearson correlation (−0.01) (Gibbons, 1971; Helwig and Council, 1979) of the incidence rates between the two years for the 24 plants. A test for a nonzero correlation using Kendall's-tau correlation coefficient (Gibbons, 1971; Helwig and Council, 1979) was also not significant (P = 0.36). This suggests that little or no precision is lost when estimating the difference between the two years using all 40 matched plants equally.

The incidence of *Salmonella* from plants sampled in 1969 and no longer operating or producing pork sausage in 1979 were compared to the *Salmonella* incidences determined in fresh product supplied by USDA inspectors in matched replacement plants. A decrease of 22.9% was observed in this comparison.

At the time the earlier study was initiated, serotyping of *Salmonella* isolates was not done. During the 1979 survey, however, serotyping was performed and the results are presented in Table 3. In several instances, more than one serotype was isolated from a sample. Sixteen serotypes were identified, five of which are among the ten most common human isolates as reported by the Centers for Disease Control (1980). The serotypes reported here may be useful to scientists and epidemiologists during future benchmark or epidemiological investigations.

The prime sources of human salmonellosis are foods of animal origin (Bryan, 1980; Cohen and Blake, 1977; Silliker, 1980). Human illnesses caused by these foods result from improper handling, inadequate thermal processing, cross-contamination of raw to cooked foods, time/temperature abuse, or combinations of these. Measures to lower the incidence of salmonellae have been suggested but as Silliker (1980) points out, the problems of salmonellae are still with us. The United States Department of Agriculture Advisory Committee (Anonymous, 1978) made recommendations for research, rearing practices, and processing practices designed to begin to reduce the level of *Salmonella* contamination in raw meat and poultry. As rearing or processing practices change, it becomes necessary to evaluate the effect or effects of the changes. The work reported

Table 1 — Comparison of Salmonella incidences in pork sausage collected at 40 federally inspected establishments in 1969 and 1979

State	Number of Plants/State	*Salmonella* No. positive/Total samples 1969[a]		1979	
GA	2	1/30	(3.3)[b]	7/30	(23.3)
TN	3	27/60	(45.0)	11/45	(24.4)
PA	4	4/40	(10.0)	14/60	(23.3)
NY	1	0/10	–	3/15	(20.0)
MD	1	5/10	(50.0)	0/15	–
MA	2	14/30	(46.6)	6/30	(20.0)
KY	1	1/30	(3.3)	1/20	(5.0)
NC	1	3/20	(15.0)	0/15	–
WI	2	0/30	–	0/30	–
VA	6	19/96	(19.8)	13/89	(14.6)
IL	3	26/40	(65.0)	4/45	(8.8)
IA	4	9/40	(22.5)	1/65	(1.5)
CA	6	36/80	(45.0)	6/86	(6.9)
AZ	1	5/20	(25.0)	4/15	(26.7)
MI	1	2/10	(20.0)	2/10	(20.0)
OH	1	3/10	(30.0)	0/15	–
IN	1	7/10	(70.0)	2/18	(11.1)
Total	40	162/566	(28.6)	74/603	(12.4)
Plant Avg.			(26.7)		(12.4)

[a] Surkiewicz et al. (1972)
[b] Percent positive

Table 2 — Comparison of Salmonella incidences in fresh pork sausage sampled at identical establishments in 1969 and 1979

State	Number of Plants/State	*Salmonella* No. positive/Total samples 1969[a]		1979	
GA	1	0/20	–	0/15	–
TN	3	27/60	(45.0)[b]	11/45	(24.4)
PA	1	2/10	(20.0)	5/15	(33.3)
NY	1	0/10	–	3/15	(20.0)
MD	1	5/10	(50.0)	0/15	–
KY	1	1/30	(3.3)	1/20	(5.0)
NC	1	3/20	(15.0)	0/15	–
WI	2	0/30	–	0/30	–
VA	5	17/86	(19.8)	10/74	(13.5)
IL	2	17/30	(56.7)	3/30	(10.0)
IA	3	6/30	(20.0)	0/45	–
CA	3	8/30	(26.7)	5/46	(10.9)
Total	24	86/366	(23.5)	38/365	(10.4)
Plant Avg			(22.6)		(10.5)

[a] Surkiewicz et al. (1972)
[b] Percent positive

Table 3 — Salmonella cultures isolated from fresh pork sausage samples collected during a 1979 survey of 40 federally inspected establishments[a]

Serotype	No. of isolates	
S. agona	10	(11.1)[b]
S. anatum	13	(14.4)
S. derby	21	(23.3)
S. drypool	2	(2.2)
S. heidelberg	1	(1.1)
S. infantis	9	(10.0)
S. livingstone	1	(1.1)
S. london	4	(4.4)
S. meleagridis	4	(4.4)
S. meunchen	8	(8.9)
S. newport	1	(1.1)
S. ohio	2	(2.2)
S. rubislaw	1	(1.1)
S. st. paul	3	(3.3)
S. typhimurium	8	(8.9)
S. uganda	1	(1.1)
Untyped	1	(1.1)
Total	90	

[a] Serological identification performed at the USDA Animal & Plant Health Inspection Service, National Veterinary Services Laboratories, Ames, IA.
[b] Percent of total isolates

here is the first effort of an ongoing program to track the changes that are occurring in *Salmonella* levels in pork. The reduction observed was not anticipated; however, it appears to be statistically significant. The authors do not know which rearing or processing practice or practices have contributed to this reduction. If identified, they could be recommended universally, possibly even applied to other species to further *Salmonella* control in raw meat and poultry. The data presented here indicate that *Salmonella* reduction in raw meat and poultry may not be impossible on a commercial basis as previously perceived. The initiation of cooperative *Salmonella* control programs between industry groups, governmental agencies and academic scientists may yet successfully contribute to reducing the levels of *Salmonella* in the meat and poultry supply.

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Composition of Guinea Keet Breast and Thigh Meat

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ABSTRACT

Guinea keets, reared to 12 wk on a modified turkey starter and grower diet, were processed like broilers and thigh and breast meat samples analyzed. Proximate analysis for breast and thigh meat without skin was: dry matter 25.4 and 24.0%; protein 22.7 and 19.4%; hexane extractables 0.86 and 2.2%; and ash 1.06 and 1.05%, respectively. Total cholesterol in breast meat was 40.6 mg/100g raw tissue, and 62.1 mg in the thigh. Also included were analyses for Na, K, Ca, Mg, P, thiamin, riboflavin, niacin, pantothenic acid and the fatty acid profiles of the meat and skin. Guinea meat was found to be lower in fat, sodium and cholesterol and higher in potassium, phosphorus, thiamin, riboflavin, niacin and vitamin B6 than broiler meat.

INTRODUCTION

INTEREST IN YOUNG GUINEAS, or keets, as specialty meat birds appears to be increasing in the United States (Clayton, 1976). There are little data in the literature on the composition of the meat from keets. Most of the data pertain to guinea meat without differentiation as to age or sex. USDA Handbook 8-5 (CFEI, 1979) contains only limited data on guinea meat. Ivashchenko et al. (1978) reported on the amino acid content of light and dark meat from guinea cocks and hens but did not specify age. Bulinskin and Szydlowsha (1970) found the mean total, free and found cholesterol from 10 guinea fowls to be 62.9, 27.8, and 35.2 mg %, respectively. Hughes and Jones (1980a) reported carcass yields, including giblets, of 76.8 for males and 76.9% for females from 12-wk-old keets with live weights of 969 and 911g, respectively. This percent yield is higher than normally noted for 6- to 8-wk-old commercial broilers today. Cooked yields were also very favorable, especially the percentage breast meat when compared with broilers (about 50 vs 33% of total meat). Consumers in a 40-family local study found keets comparable to chicken broilers when home-prepared by the consumers' regular broiler recipes (Hughes, 1980).

In view of the apparent growing popularity of the fryer or broiler type guinea (keet), this study was conducted in order to provide more detailed data on the nutrient composition of these birds.

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MATERIALS & METHODS

BIRDS used in this study were from the Clemson University Poultry Science Dept. random-bred farm flock. The parent stock was the same as reported by Hughes (1980). This stock had not been selected for growth rate nor meat production and would, therefore, be classified as a representative farm flock as found in the southeastern area of the United States. Birds were the Pearl variety but ranged from almost white to dark slate, with the typical guinea splashing of light and dark color.

Keets were confinement-raised on litter to 12 wk of age. The grow-out rations, protein adjusted at 4 and 8 wk (Hughes and Jones, 1980b), compared three levels of phosphorus (0.55, 0.60, and 0.65%) and three levels of calcium (0.8, 1.0, and 1.2%) fed to replicate pens of 20 birds each. Five keets from each of the 18 grow-out pens were randomly selected, then slaughtered using typical broiler processing methods. Carcasses were not chilled prior to sampling. Immediately after evisceration the right breast meat and the right thigh, each with normal skin covering, were collected. These were immediately stored in five-bird lots in plastic bags surrounded with crushed ice, then carried to our laboratory where they were held overnight in ice before further processing. In preparation for analyses, the skin was first removed from the flesh, then the flesh from the five-bird pooled sample after weighing was ground and blended in an electric food processor. Skin collected was pooled in 10-bird lots from replicate treatment pens and immediately frozen for later analysis. Meat samples not used immediately were placed in glass jars and frozen for later use. Proximate analysis was in accordance with AOAC methods for meat (AOAC, 1975) except lipids for cholesterol and fatty acid profiles were extracted from the raw flesh or skin using the "dry column" technique of Maxwell et al. (1980). The determination of cholesterol and fatty acids was then made using AOAC GLC methods (AOAC, 1975).

Analyses for the elements Na, Ca, K, Mg, and P were by atomic absorption spectrophotometric or colorimetric methods used in our laboratory (Hamm et al., 1980). Vitamins, thiamine, riboflavin,

Table 2—Mineral analysis of guinea keet breast and thigh meat, µg/g, wet weight basis (n = 18)

	K	Mg	Na	Ca	P
Breast	3440 (±57)	300 (±5)	290 (±18)	31 (±3)	4900 (±50)
Thigh	3220 (±77)	260 (±4)	540 (±36)	45 (±2)	4300 (±30)

Table 1—Proximate analysis of guinea keet breast and thigh meat and skin.

Sample[a]	Weight g	Dry matter %	Hexane extractables %	Protein %	Ash %
			wet weight basis		
1/2 breast flesh	60.6 (±5.7)	25.4 (±0.5)	0.86 (±0.39)	22.7 (±0.6)	1.06 (±0.06)
1/2 breast skin	5.1 (±0.08)	39.2 (±0.9)	21.2 (±4.2)	14.4 (±0.7)	0.73 (±0.04)
Thigh meat	42.5 (±6.2)	24.0 (±0.6)	2.2 (±0.4)	19.4 (±0.5)	1.05 (±0.08)
Thigh skin	4.8 (±0.4)	42.1 (±1.3)	22.1 (±1.2)	17.8 (±0.2)	0.88 (±0.02)

[a] n = 18 pooled five-bird lots for breast and thigh meat; 9 pooled ten-bird lots for skin samples.

Table 3—Vitamin content of guinea keet breast and thigh meat, mg/100g, wet weight basis

	Thiamine	Riboflavin	Niacin	Vitamin B6	Pantothenic acid
Breast[a]	0.107 (±0.012)	0.149 (±0.005)	13.20 (±0.59)	0.728 (±0.043)	1.11 (±0.078)
Thigh[a]	0.153 (±0.007)	0.264 (±0.021)	6.55 (±0.64)	0.387 (±0.025)	1.56 (±0.11)

[a] Analyses were made on 18 five-bird pooled samples.

Table 4—Fatty acid profiles of guinea meat and skin from breast and thighs

Fatty acid	Meat (n = 18)		Skin	
	Breast	Thigh	Breast	Thigh
	% by weight[a]			
14:0	0.75	0.93	0.68	0.71
Unk[b]	Tr	1.04	0.02	0.01
Unk[b]		Tr	0.09	0.14
16:0	24.44	19.77	20.63	20.57
16:1	1.26	2.13	1.15	2.53
Unk[b]	0.70	–	0.04	0.05
18:0	21.91	14.27	7.66	8.41
18:1	27.24	26.59	29.95	36.78
18:2	24.51	34.00	40.59	31.08
18:3	0.26	0.70	0.69	0.50
Total cholesterol mg/100g	40.64 (±19.5)	62.07 (±11.23)	168 (N.D.)[c]	137 (N.D.)[c]

[a] % weight determined by peak area normalization.
[b] Measurable peaks noted in sequence but which were not included in our standard.
[c] Only three samples of each type skin analyzed; value shown is the means.

niacin, Vitamin B6 and pantothenic acid were determined using the procedure of Ang and Hamm (1982).

RESULTS & DISCUSSION

MEAN LIVE WEIGHT for these keets was 1,065g (±40.8). Since these birds were not slush ice chilled before samples were drawn, our data do not include any absorbed water which is usually typical for analyses for commercial broilers. Proximate analysis values are given in Table 1. The weight values shown for raw breast meat are undoubtedly low since we rapidly removed the breast flesh without attempting to remove all the breast flesh from the anterior portion of one-half the breast area. The breast skin values will be representative for birds of this size since all the skin from the half breast area was collected. Values for meat and skin from the thigh are representative. Breast flesh is very low in fat. breast flesh and skin combined have a calculated fat level of 2.0% (0.39% of 60.6g plus 21.2% of 5.1g for a total fat of 1.318g per 65.7g of breast meat and skin combined), of which 82% is associated with the skin. The thigh flesh and skin have a combined fat value of 4.2%, of which just over half (53%) comes from the skin.

Values for K, Mg, Na, Ca, and P are shown in Table 2. Since we found no differences in the levels of Ca and P due to treatment levels of Ca and P, only mean values for all minerals checked are reported. Sodium content of these samples was much lower than we found in broiler breast meat (breast values of 290 vs 465, Hamm et al., 1980). Had these keets been immersion chilled, the differences would possibly be even greater since we find chilling at times lowers the sodium level of skin and meat close to the surface. Based on the same study (Hamm et al., 1980 guinea meat was higher in potassium and phosphorus content (breast 4,900 vs 2,810, and 4,300 vs 2,240 ppm).

Results of the analysis for thiamin, riboflavin, niacin, vitamin B6 and pantothenic acid are shown in Table 3. The first four vitamins were found to be higher in the guinea meat than found in broiler meat (CFEI, 1979; and Ang, 1980). Fatty acid profiles and chilesterol levels are shown in Table 4. The fatty acid profiles are more or less typical of what one would expect from turkeys fed conventional corn soya diets. Cholesterol levels in breast and thigh meats are slightly lower than in broiler breast and thigh meats (42 vs 58 mg/100g and 62 vs 83; CFEI, 1979), but are very similar when expressed as a percentage of fat for the part.

It would appear that guinea meat, because of its acceptable taste and low fat content, should find wide acceptance as a specialty meat item. Wider acceptance should follow if production factors can be altered so that keets reach market age earlier, thus reducing production costs.

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Purine Content of Raw and Roasted Chicken Broiler Meat

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ABSTRACT

Adenine, guanine and hypoxanthine were determined in raw and roasted broiler parts. The levels of adenine and guanine increased slightly when the meat was cooked. These increases were attributed to moisture and fat losses by the tissues during roasting. The level of hypoxanthine remained constant or decreased in the tissues because some of the purine was removed with the cooking juices. The cooking juices were found to contain high levels of hypoxanthine and only trace amounts of adenine and guanine.

INTRODUCTION

FOODS which elevate blood uric acid levels are often eliminated from the diets of hyperuricemic patients in order to control episodes of gout. Many foods have been shown to have this effect but in general foods high in nucleic acids tend to have the greatest influence (Smith, 1971). Recently, questions have been raised as to whether it is more important to control the dietary intake of nucleic acid or the intake of specific constituent purine bases. Clifford et al. (1976) presented evidence that normouricemic, hyperuricemic and guoty humans do not respond to all dietary purines the same. Each purine produces a different response depending on whether it is consumed by a normouricemic, hyperuricemic or gouty person. In their study, oral hypoxanthine, adenosine-5'-monophosphate (AMP), guanosine-5'-monophosphate (GMP) and inosine-5'-monophosphate (IMP) produced a greater hyperuricemic effect on subjects with gout than on normouricemic or hyperuricemic subjects. Of the purine compounds normally found in foods, only adenine and hypoxanthine significantly increased serum uric acid levels. This effect was particularly pronounced in gouty subjects.

In another report, Clifford and Story (1976) reported on studies in which they analyzed a variety of foods for adenine, guanine, hypoxanthine and xanthine. They concluded that the absolute and relative amounts of the purines were not necessarily high in the purines especially conducive to the elevation of blood serum uric acid levels, i.e., adenine and hypoxanthine.

There are very little data available on the purine content of poultry products. Unpublished data by Bauermann (1977) and by Weir and Clifford (1978) were summarized by Murphy et al. (1979) and showed consumption of mechanically deboned poultry did not appear hazardous to health because of its purine content.

More recently, Young (1980) determined the content of purines in several broiler tissues. His data indicated that the levels of total purines in light and dark meat were somewhat lower than what has been reported for other foods, but the level of hypoxanthine was higher. Total liver purines were higher than other broiler tissues, but of the purines found in liver, most were adenine and guanine. The levels of purines were relatively low in gizzards and mechanically deboned broiler tissues.

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The studies by Clifford and Story (1976) and by Young (1980) on the purine levels in foods were all conducted with raw product. Cooking might affect the purine levels due to chemical changes or to the loss in cooking juices. The purine content of raw and roasted broiler chicken tissues is presented in this paper.

MATERIALS & METHODS

Purine determination

Purines were analyzed using the method described by Clifford and Story (1976) as modified by Young (1980). Briefly, the procedure was to freeze-dry and defat the samples. The dry, fat-free samples were ground in a Wiley mill and the resultant powders were digested in perchloric acid. Then the purines were separated on HPLC using a cation exchange column and isocratic elution with 0.05 M phosphate buffer, pH 2.0. The level of xanthine was not determined in these experiments because (1) previous work has shown that this purine is only present in very small amounts in chicken tissue (Young, 1980), (2) it is relatively unimportant in elevating serum uric acid (Clifford et al., 1976) and (3) xanthine cannot always be completely resolved from nonpurine compounds using this HPLC procedure. Each sample was analyses singly since previous experiments demonstrated little improvement in precision by subsampling (Young, 1980).

Nitrogen determination

Nitrogen was determined on all samples using the macro-Kjeldahl procedure (AOAC, 1975) before and after removing moisture and fat. These nitrogen data were used to convert the purine data from a dry, fat-free basis to a wet, unextracted basis.

Experiment 1—Broiler breast meat

Twelve broilers weighing between 1200 and 1600g each were obtained from a local processor. They were packed in ice immediately after chilling and transported to the laboratory. They were split along the keel bone into left and right halves. One half of each bird was placed back into the ice and the other half roasted at 177°C to an internal temperature of 80°C (about 1.25 hr) in an electrically heated rotary-type oven. Internal temperature of the samples was monitored with candy thermometers inserted into the thickest part of the breast, care being taken to insure that the probe tips were not touching boney tissue. After roasting, the halves were cooled to about 23°C and then both the roasted and raw halves were placed in individual plastic bags. All halves were held overnight at 4°C.

The next day skin was removed from each breast and the muscle tissue separated from the bone and chopped. Samples were taken for nitrogen analysis and then freeze-dried and defatted as previously described. The dried samples were ground in a Wiley mill and the total nitrogen, adenine, guanine and hypoxanthine in the dry, fat free powders were determined.

Experiment 2—Broiler thigh meat

Sixteen broilers weighing between 1350g and 1650g each were obtained from a local processor. The left and right thigh meat of each was separated from bone. One thigh from each bird was immediately roasted exactly as were the breasts and the other held in ice. After roasting, all samples were chopped and then analyzed in the same manner as the breasts.

Experiment 3—Fate of hypoxanthine

Twenty-two broiler halves were roasted in individual pans as

previously described. Cooking juices were rinsed from the pans with 40 ml water and 40 ml of the juices plus rinse water were poured into 50 ml glass centrifuge tubes. The next day the fat was skimmed-off and the aqueous phase which remained was freeze-dried. Purine content of the resultant powders was then determined.

Compounds with elution volumes corresponding to that of hypoxanthine were collected from the HPLC and concentrated by lyophilization. The presence of an ultraviolet absorbing compound with an elution volume similar to that of hypoxanthine was confirmed by rechromatographing the concentrate. The ultraviolet absorbance of the material between 235 and 260 nm was then evaluated with a Beckman model 25 spectrophotometer and compared to that of hypoxanthine. Maximum absorbance of both the concentrate and hypoxanthine standard was found at 246 nm. This value compares with an acid λmax of 248 nm reported by Sober (1970).

Statistical treatment

The purine content of each sample was expressed on a wet weight basis and also on a basis of mg purine per 100 mg N. The latter expressions were calculated in order to determine whether differences in the purine content observed between raw and roasted wet samples were due to losses of purine or to changes in the moisture and fat content of the tissues. The data were analyzed using the paired Students *t* test (Steel and Torrie, 1960). Differences between raw and roasted samples were evaluated for statistical significance ($P \leq 0.05$).

Hypoxanthine content of the cooking juices in Experiment 3 was expressed on a basis of mg hypoxanthine/gram powder. The mean hypoxanthine content and standard error of the mean were calculated.

RESULTS & DISCUSSION

Experiment 1—Broiler breast meat

The adenine and guanine content on a wet weight basis increased in roasted breasts (Table 1). However, the differences in purine content between raw and roasted product were small when these data were expressed on a nitrogen basis. These observations indicate that very little of the adenine or guanine was lost from breast in the roasting process. In contrast, there was no difference between raw and roasted breast in hypoxanthine content expressed on a wet basis.

Table 1—Purine content of raw and roasted broiler chicken breast meat

	Mg/100 wet g tissue			Mg/100 mg		
Purine[a]	Raw[b]	Roasted[b]	S.D. (P≤0.05)	Raw[b]	Roasted[b]	S.D. (P≤0.05)
ADE	19.30	25.28	Yes	0.55	0.61	No
GUA	20.71	27.21	Yes	0.59	0.66	No
HYP	127.92	126.40	No	3.63	3.15	Yes

[a] ADE = Adenine; GUA = Guanine; HYP = Hypoxanthine.
[b] Each value represents mean of 12 samples.

Table 2—Purine content of raw and roasted broiler chicken thigh meat

	Mg/100 wet g tissue			Mg/100 mg		
Purine[a]	Raw[b]	Roasted[b]	S.D. (P≤0.05)	Raw[b]	Roasted[b]	S.D. (P≤0.05)
ADE	25.29	29.49	Yes	0.86	0.81	No
GUA	26.58	33.32	Yes	0.90	0.91	No
HYP	99.66	86.08	Yes	3.37	2.34	Yes

[a] ADE = Adenine; GUA = Guanine; HYP = Hypoxanthine.
[b] Each value represents mean of 16 samples.

On a nitrogen basis there was less hypoxanthine in the roasted tissues than in the raw tissues. These observations indicate that part of the hypoxanthine was lost from the breast during roasting.

Experiment 2—Broiler dark meat

The purine levels in the roasted and raw thigh meat were similar to those observed in raw and roasted breast meat (Table 2). On a wet basis, there were small increases in the content of adenine, and guanine, and a small decrease in the content of hypoxanthine. However, on a nitrogen basis adenine and guanine remained constant while the hypoxanthine decreased. These observations indicate that the adenine and guanine content increased due to losses in fat and moisture but that hypoxanthine was lost from the tissues during roasting. However, the data do not explain whether this loss in hypoxanthine was due to chemical change or loss in the cooking juices.

Experiment 3—Fate of hypoxanthine

The chromatogram of the drippings showed peaks corresponding in elution volumes to those of hypoxanthine, guanine and adenine. However, the latter two peaks were quite small which indicates that the drippings contained at most only a trace of guanine or adenine. In contrast, the peak corresponding to hypoxanthine typically was two to three times as great as the hypoxanthine peak in meat powders. The mean hypoxanthine content ± the standard error of the mean of the powder from the drippings was 11.11 ± 0.41 mg/gram of powder. This figure compares with hypoxanthine levels in meat powder of 3–5 mg/gram of powder. It would be difficult to extrapolate from the purine level of the dry powder to that of a sauce or gravy prepared from the drippings because the drippings are often concentrated by evaporation or mixed with other ingredients in preparing these products. Moreover, the solid content of the cooking juices might be influenced by the amount of chicken meat cooked in one pan, whether or not the pan was covered, the shape of the pan and many other factors. Nevertheless, the data do confirm that hypoxanthine was lost from the meat into the cooking juices. These findings indicate that physicians and dietetians should be cognizant of the potentially high levels of hypoxanthine in sauces, gravies or other products made from chicken drippings.

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A Research Note

Maillard Browning Reaction of Sugar-Glycine Model Systems: Changes in Sugar Concentration, Color and Appearance

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ABSTRACT

The reactivities of glucose, fructose, and sucrose with glycine (1:1 molar ratio) at 60°C and pH 3.5, were compared over 280 hr. While fructose initially browned at a faster rate, it was overtaken by glucose after 80 hr. Initially more fructose than glucose was consumed, but the reverse was true after 60 hr. Sucrose was readily hydrolyzed under these reaction conditions and underwent Maillard browning reactions, its color and appearance being similar to the glucose solutions at the later stages of the experiment. Glucose and sucrose solutions developed considerable haze while the fructose-glycine solution remained clear.

INTRODUCTION

MAILLARD BROWNING REACTIONS in foods continue to be an active area of research because of their important roles in color, flavor and nutritional quality. In addition, some of the products produced from these reactions may have toxicological significance (Adrian, 1974; Tanaka et al., 1977). The availability of crystalline fructose and corn syrups with varying proportions of glucose and fructose offers a wide range of sugars which are available for product formulation. Thus there is practical interest in the comparative reactivity of these sugars and their influence on quality. Previous workers (Bobbio et al., 1973; Shallenberger and Birch, 1975) have reported that fructose initially browns at a faster rate than glucose but is later overtaken by glucose. It was the purpose of this investigation to compare the reactivity of glucose, fructose, and sucrose with glycine under accelerated storage conditions over an extended reaction period. In addition to measuring the absorbance at 420 nm, the change in glucose and fructose concentration was determined as well as Hunter color and appearance parameters.

MATERIALS & METHODS

Sample preparation

Model systems consisted of 1.0M solutions of sugar (D-fructose, D-glucose or sucrose) and 1.0M glycine at pH 3.5. Controls containing only the sugar were used to determine the contribution to color formation by caramelization. The pH of all the solutions was adjusted with 0.1N HCl or 0.1N NaOH. Eight ml portions of the solutions were distributed in 10.0 ml screw-cap tubes, sealed fingertight, and held in a water bath at 60.0°C. Tubes were periodically removed for analyses during the experiment. Color and pH were monitored during storage; no change in pH was observed.

Spectrophotometric measurements

The absorbance at 420 nm was determined on a Beckman ACTA CIII spectrophotometer. Dilutions were made with distilled water, the maximum dilution for any determinations being 1:100.

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Hunter color measurements

Hunter L, *a*, *b* values were measured in the transmission mode using a Hunter Model D 25 P-2 Color Difference Meter. Samples were diluted 1:50 and read in cells with a 0.5 cm pathlength. Measurements were made with the specular component both included and excluded to allow calculation of percent haze. The Scofield-Hunter index $\Delta E = (L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2$ where L_1, a_1 and b_1 represent the color at time t and L_2, a_2 and b_2 represent initial color, was calculated as was the saturation index, $(a^2 + b^2)^{1/2}$.

Glucose and fructose determinations

Glucose and fructose content were determined enzymically using the glucose/fructose kits from Boehringer Mannheim Biochemicals (Indianapolis, IN), according to the manufacturer's instructions. After appropriate dilutions, aliquots were combined with reagents and the absorbance determined at 340 nm.

RESULTS & DISCUSSION

TABLE 1 shows the change in absorbance at 420 nm for glucose-, fructose-, and sucrose-glycine solutions over 280 hr. While the fructose-glycine solution initially browns at a faster rate, it is overtaken by both glucose- and sucrose-glycine solutions after approximately 80 hr. Other workers (Bobbio et al., 1973; McWeeny, 1973; Hodge and Osman, 1976) have reported similar results in comparing glucose- and fructose-amino compound solutions. There was no increase of absorbance in the model system solutions containing only the sugar, indicating that there was no contribution to color formation due to caramelization. Fig. 1 compares the loss of glucose and fructose in the glucose- and fructose-glycine solutions. Fructose concentration decreases faster than glucose initially, but the loss of glucose overtakes fructose after 60 hr. These changes are consistent with the browning rates observed in Table 1. Thus the differences in browning are not due to the chromophoric attributes of the pigment formed. Glucose and fructose concentrations were measured in the sucrose-glycine solution and a similar pattern followed in that fructose concentration surpassed the glucose concentration after 80 hr. Under different reaction conditions, Burton et al. (1963) and Newell et al. (1967) reported higher initial reactivity for fructose as compared to glucose. Wolfrom et al. (1974) studied the behavior of fructose, glucose, and sucrose with glycine at a 5:1 molar ratio at 65°C and a pH of 6.1 for 10 hr. The amount of browning in the glucose solution caught up with the fructose solution within 10 hr. There was negligible browning in the sucrose-glycine solutions under their experimental conditions. We measured over 50% sucrose hydrolysis after 40 hr at pH 3.5 and 60°C and our results show that sucrose initially browns at rates similar to glucose- and then surpasses the glucose-glycine system (Table 1). The sucrose system has a potential reducing sugar concentration twice that of the monosaccharide systems and accounts for that system's greater degree of browning during the later stages of experiment. The absorbance readings (Table 1) and visual appearance of the samples were confirmed by Hunter color measurements. The Hunter L values were much higher for the fructose-glycine solution than the glucose- and sucrose-glycine solu-

Table 1—Change in absorbance and Hunter color measurements of sugar-glycine solutions during storage at 60.0°C and pH 3.5

	Fructose					Glucose					Sucrose				
		Hunter measurements					Hunter measurements					Hunter measurements			
Time (hrs)	Absorbance (420 nm)	L[1]	ΔE[2]	SI[3]	Haze (%)	Absorbance (420 nm)	L[1]	ΔE[2]	SI[3]	Haze (%)	Absorbance (420 nm)	L[1]	ΔE[2]	SI[3]	Haze (%)
0	0.00	96.1	0	0.41	1.8	0.00	96.3	0	0.57	2.3	0.00	95.9	0	0.71	2.2
3	0.03	96.1	0	0.51	1.9	0.01	96.3	0	0.57	2.3	0.01	95.9	0	0.71	2.1
6	0.09	96.1	0	0.54	1.8	0.02	96.3	0	0.57	2.2	0.01	95.9	0	0.71	2.1
9	0.16	96.1	0.1	0.61	1.9	0.03	96.3	0	0.57	2.2	0.02	95.9	0	0.71	2.2
12	0.25	96.1	0.22	0.73	1.9	0.05	96.3	0.10	0.64	2.2	0.04	95.9	0	0.71	2.2
24	0.83	96.0	0.73	1.24	1.8	0.24	96.2	0.42	0.94	2.2	0.21	95.9	0.14	0.82	2.1
48	2.92	95.6	2.83	3.30	2.0	1.50	95.7	2.83	3.26	2.2	1.53	95.7	1.47	2.16	2.1
72	5.58	95.2	5.03	5.46	1.9	4.39	94.6	7.95	8.25	2.2	4.75	95.1	4.49	5.12	2.1
96	10.4	94.2	8.70	9.00	1.9	11.6	91.9	18.1	18.0	2.3	16.3	93.1	13.2	13.6	2.1
123	16.1	93.2	12.8	12.96	2.0	24.7	87.6	29.9	29.0	2.3	38.2	89.1	24.8	24.6	2.1
164	25.0	92.1	15.8	15.75	2.0	49.0	83.6	36.7	34.9	2.4	78.0	85.7	31.7	30.7	2.2
188	30.3	90.1	21.0	20.63	2.2	63.6	74.6	46.1	41.0	3.3	108	77.4	41.8	38.1	11.0
212	34.0	89.4	22.8	22.32	2.2	79.7	70.4	48.9	41.8	11.4	139	72.6	39.6	39.6	18.7
236	36.2	89.0	23.8	23.21	2.3	107	64.2	52.3	41.5	45.6	195	66.2	49.4	40.1	36.6
260	45.5	86.9	27.5	26.44	2.3	112	-	-	-	46.1	216	63.4	51.0	39.9	43.3
284	50.0	86.2	29.2	28.01	2.3	121	-	-	-	42.9	244	59.6	53.2	39.6	55.2

[1] L = Hunter L value
[2] ΔE = Scofield-Hunter Index
[3] SI = Saturation Index

tions during the later stages of the experiment. Fructose showed a much lower change in both the Scofield-Hunter Index (ΔE) and the saturation index (SI) than the glucose- and sucrose-glycine solutions (Table 1). The glucose and sucrose samples developed considerable haze in the later stages of the experiment (over 40% haze after 240 hr) whereas the frucose solution remained clear (Table 1). This important appearance quality factor suggests that the degree of polymerization of glucose-derived melanoidins was much greater than fructose-derived melanoidins under these experimental conditions.

These results show that glucose undergoes more browning than fructose during prolonged reaction times under these experimental conditions. They illustrate that initial reaction rates may not be predictive of eventual product formation. These findings point out another factor for consideration when selecting sweeteners for food products where quality deterioration due to browning reactions during storage may be a significant problem.

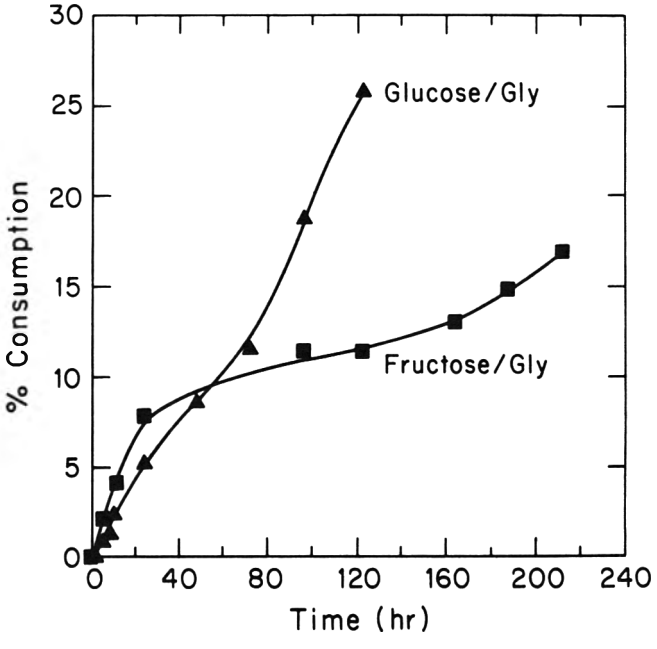

Fig. 1—Consumption of glucose and fructose in the glucose- and fructose-glycine systems during storage at 60.0°C and pH 3.5. (% consumption represents the % individual sugar lost).

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A Research Note

Effect of Extractable Lipid on the Viscosity Characteristics of Yam Tuber Flours

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ABSTRACT

Extractable lipids comprised below 2% of the flours made from yam tubers. This small amount of lipid was best extracted by water-saturated-butanol compared to 80% methanol and chloroform-methanol (2:1) mixtures. The fatty acid composition in each lipid extract was not significantly affected by the method of extraction. Defatting yielded flours which gelatinized at higher temperatures and possessed increased overall viscosities compared with undefatted flours. It is suggested that the particular uses of the yam flours may be influenced by the lipids present.

INTRODUCTION

YAMS are important starchy, staple diet item in many West African countries, especially Nigeria. The major factors that now limit the availability of yams are their relatively poor yield and large storage losses. The principal sources of loss in storage are respiration, desiccation and rotting (Coursey, 1961; 1967). It has been suggested that the most effective procedure against storage loss is to convert the fresh tubers to flour soon after harvest (Martin, 1976). In this way yams would be more available through wider use of the processed products from the flour (Misawa and Matsubara, 1965; Jarmai and Montford, 1968). Yam flours have been substituted for part of the wheat flour in bread making and pastry (Kiram and Ruiter, 1968; Lehmann and Miinzberg, 1972; Sammy, 1973). Yam flakes have been produced and the shelf life and pasting characteristics studied (Gooding, 1972; Rodriguez-Sosa, 1974).

In recent years there has been an increasing appreciation of the effects of lipids on the properties of flour and flour products. Lipids are now known to be responsible for the development of off-flavor in flour products (Fellers and Bean, 1977). Considerable problems have arisen with procedures for the removal of total lipids from flours because substantial quantities are inside impermeable cells and starch granules where they are inaccessible to solvents (Wren and Merryfield, 1970; Morrison et al., 1975). In this paper, we report the effect of extractable lipid on the viscosity characteristics of flours made from three common yam tubers.

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MATERIALS & METHODS

Dioscorea rotundata (White Yam), *Dioscorea cayenensis* (Yellow Yam, and *Xanthosoma saggitifolium* (Cocoyam) tubers were purchased in the open market. Fresh tubers were peeled, cut into flat pieces, quickly dipped into hot iso-propanol, dried with hot air, and ground into a fine powder. The flours were dried at 70°C to constant weight. Triplicate flour samples were used for lipid analysis and viscosity determinations.

Viscosity determination

A Brabender amylograph was used to determine changes in the gelatinization characteristics of the flours. Flour slurries in distilled water (10%w/v) were poured into the measuring vessel of the Viscograph at 20°C. The viscosity was recorded at a constant rotational velocity of the measuring vessel (50 rev/min) using a 1000 cmg measuring box with the temperature rising uniformly at 1.5°C/min. After reaching a temperature of 95°C, the flour paste was maintained at this temperature with constant stirring for a further 30 min. After this 'holding period' the paste was cooled uniformly at 1.5°C/min to 50°C and held there for another 30 min.

Lipid extraction

Three methods of extraction were used: (1) lipids were three times extracted with chloroform-methanol (2:1) and then chloroform; (b) lipids were extracted in a soxhlet apparatus with 85% methanol-water for 48 hr; (c) the flour was slurried in water saturated butan-1-ol (WSB) and stirred at 70°C for 100 min then recovered by filtration and the process repeated twice more with WBS. Butylated hydroxytoluene (BHT 0.005% w/v) was included as

Table 1—Lipid and fatty acid contents of yam flours

Extraction method	Total lipid[a] (% of dry wt)	Lipid phosphorus[a] (mg/100 dry wt)	Major fatty acids[b,c] 16:0	18:0	18:1	18:2	18:3
$CHCl_3$: MeOH (2:1)							
X. saggitifolium	0.63	2.9	29.4	2.8	7.1	56.1	5.4
D. cayenensis	0.77	3.5	31.6	2.0	3.0	54.2	7.1
D. rotundata	0.43	1.2	32.5	1.6	6.4	47.7	8.1
WSB							
X. saggitifolium	0.65	3.4	31.4	4.6	13.1	47.3	3.6
D. cayenensis	0.82	4.7	28.6	3.1	16.4	47.9	4.1
D. rotundata	0.51	2.2	35.6	1.1	9.1	44.4	8.8
85% MeOH							
X. saggitifolium	0.60	2.8	29.8	1.2	10.8	52.0	3.4
D. cayenensis	0.75	3.4	30.1	1.2	8.4	50.6	8.1
D. rotundata	0.42	1.1	30.6	1.1	8.5	51.6	5.2

[a] Average of triplicate analyses
[b] Percent of total fatty acids
[c] The critical values from F tables are: $F_{0.95\ (2,30)} = 3.32$; $F_{0.95\ (4,30)} = 2.69$; $F_{0.99\ (4,30)} = 4.02$; $F_{0.95\ (8,30)} = 2.27$.

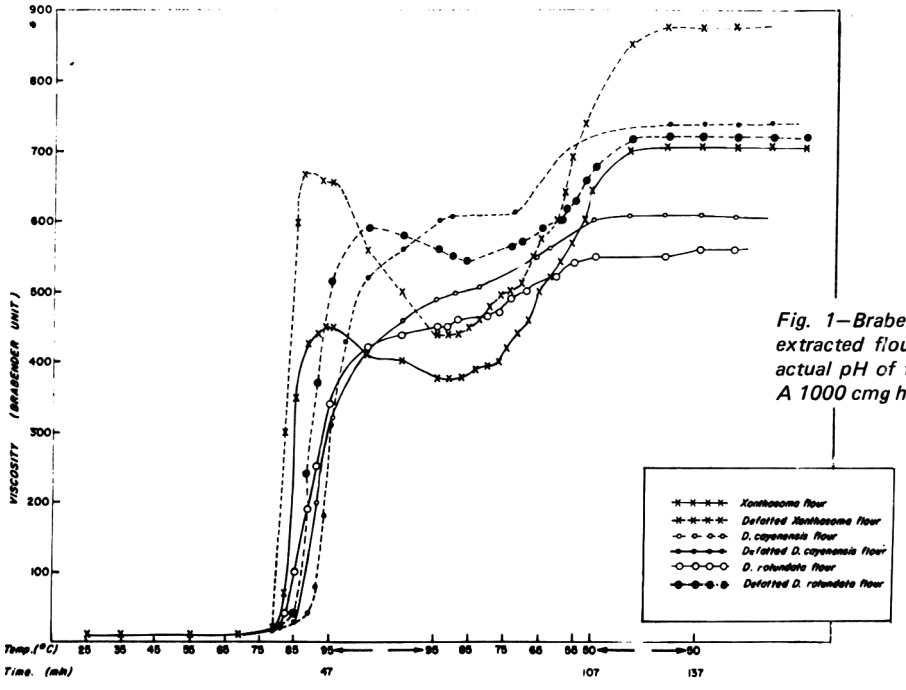

Fig. 1—Brabender amylograms for yam flours and solvent extracted flours. All determinations were carried out at the actual pH of the flour slurry (6.7—7.2); Concentration 10%. A 1000 cmg head was used.

an antioxidant in all solvents. The solvent extracts were dried by rotary evaporation at 40°C.

Lipid analysis

Aliquots of the total lipid extracts were transesterified and the fatty acid methyl esters determined by gas liquid chromatography (Opute and Osagie, 1978). Phosphorus content of the total lipid extracts was determined by Morrison's method (1964). The extracted lipids were separated into neutral, glyco- and phospholipids by column chromatography on silicic acid (Kates, 1975). Thin-layer chromatography (TLC) on silica gel (Applied Science prekotes) was used to identify the main components of the lipid extracts (Osagie and Opute, 1981).

RESULTS & DISCUSSION

TLC ANALYSIS revealed that all three extraction methods gave similar qualitative yield. WSB, however, gave higher phosphorus contents. These results suggest that WSB effected a more efficient removal of phospholipids. WSB at elevated temperatures had been previously shown to extract more phospholipids from wheat flour than other solvents (Morrison, 1978).

The composition of fatty acids obtained from the three extraction procedures are shown in Table 1. A two way analysis of variance indicated no significant difference even at the 5% level in the values for fatty acid composition among the tissues and for the different treatments. Thus the characteristic difference in properties between the flours could not be ascribed to the fatty acids.

Brabender amylograms for the control samples and the solvent-extracted flours are shown in Fig. 1. The viscosities attained by these flours were much higher than those recorded for water yam, cassava, sweet potato, and Irish potato (Osagie and Opute, unpublished observation; Leach, 1965). The high viscosities attained by these flours are of significance in the preparation of 'pounded yam' (a stiff dough made by pounding pieces of boiled yam in a mortar). It is known that *D. rotundata* and *D. cayenensis* give more satisfactory products when used for preparing "pounded yam."

The extracted flours showed an increase in both viscosity peak height and "set-back" (i.e. the change in viscosity on holding at 50°C) characteristics. The temperatures at which the viscosities started to increase were slightly higher with defatted flours. This is contrary to the findings of Furakawa et al., (1966) and Melvin (1979) who worked with cereal flours. Despite the later onset of gelatinisation, the peak width was greatly increased by defatting. This might be due to the fact that removal of lipids from the granules facilitated penetration by water molecules. These results showed that the removal of lipids from yam flours altered the viscosity characteristics although the lipids were present in very small amounts. Lipids have been previously reported to affect the gelling characteristics of starches (Medcalf et al., 1968; Melvin, 1979). Glyceryl monostearate was specifically shown to be a suitable starch granule binder when making bread from non-wheat flours (Kiram and Ruiter, 1968). The potential of yam flour in the processed food industry merits further investigation.

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A Research Note

Effect of Tannic Acid on a Low Tannin African Sorghum Variety in Relation to Carbohydrate and Amylase

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ABSTRACT

The effect of concentrations of 0–3% tannic acid on root growth, starch, soluble carbohydrate and alpha and beta amylase was determined during germination of a low tannin sorghum variety. At higher concentrations of tannic acid root growth was suppressed. Starch degradation and accumulation of soluble carbohydrates were reduced and both alpha and beta amylase synthesis was decreased. This suggests that tannins retard starch degradation indirectly by inhibiting the synthesis of starch hydrolytic enzymes during germination.

INTRODUCTION

CHAVAN et al. (1981) discussed the changes in seedling growth, starch, reducing sugars and free amino acids during the germination of one high and one low tannin sorghum cultivar of Indian origin. The authors suggested that tannins retarded seedling growth due to inhibition of starch and protein degradation, possibly caused by inactivation of hydrolytic enzymes.

Since it was possible that an unknown factor associated with tannin content might produce the effects described, the direct effect of tannic acid on the germination of a low tannin Nigerian sorghum cultivar (Ex. Bauchi) was studied. This was related to seedling growth, soluble carbohydrate, starch and amylase activity.

MATERIALS & METHODS

THE SORGHUM CULTIVAR (Ex Bauchi) was obtained from the Institute of Agricultural Research, Zaria, Nigeria.

Steeping and germination

Surface sterilization of the seeds was carried out by immersing them first in 0.1% aqueous mercuric chloride for 5 min followed by immersion in 50% aqueous ethanol for 20 min. The seeds were then washed thoroughly with distilled water (Srivastava et al., 1975). The grains were then placed into distilled water, 1%, 2% and 3% tannic acid solutions for 24 hr at 25°C. The hydrated grains were then transferred to sterile plastic Petri dishes lined with two circles of 9 cm Whatman No. 1 filter paper and containing respectively 2 ml of distilled water, 1%, 2% and 3% tannic acid solutions. The solution had been sterilized for 15 min at 15 p.s.i. pressure. The germination experiments were carried out in triplicate in the dark at 25°C for 72 hr. The filter papers were remoistened at intervals of 24 hr with the respective solutions. Root lengths were measured and the results reported in Table 1.

Determination of tannic acid equivalent

The hydrolyzable tannins of the grain were estimated using the method of Maxson and Rooney (1972). Two grams of the laboratory milled grain were first heated with 70 ml of water to gelatinize the starch. It was then cooled under the tap. Ten milligrams of amyloglucosidase (dissolved in 5.0 ml of distilled water) were added and the sample incubated at 65°C for 60 min. Then 50.0g of urea and 2–3 drops of antifoaming agent were added. The sample was then refluxed for 24 hr, cooled and made up to 120 ml with distilled water. It was then centrifuged for 15 min at 825g and filtered. To 2.0 ml of the filtrate, 5.0 ml of the ferric reagent (1.0 part FAS, 10 parts of 10% gum arabic and 89 parts of 1.0M acetate buffer at pH 4.6) were added. Five milliliters of distilled water were added to 2.0 ml. of the filtrate as a 'blank'. After 15 min absorbance for each sample was measured at 580 nm and compared with tannic acid standards (BDH Analar).

Starch, soluble carbohydrate and amylase determination

The grains were dried at 50°C for 24 hr and samples taken at 0 (ungerminated), 24, 48 and 72 hr for the determination of soluble carbohydrate and starch content. These were estimated at room temperature and in triplicate by percolation using the method of Hansen and Møller (1975).

Fifty to 100 mg of the sample were quantitatively transferred to a percolator. It was then wetted with 1 ml of 80% ethanol and any air bubbles removed by stirring to avoid channel formation. The soluble carbohydrates were then percolated for 12–15 hr with a continuous flow of cold 80% ethanol at 1.5 ml/hr from a Mariotte flask. Then the residue containing the starch was first mixed with 1 ml of 35% perchloric acid and again percolated for 12–15 hr in the same solution at 1.5 ml/hr.

Alpha amylase was determined on the 72-hr sample using the method of the European Brewery Convention (1975) and the activity expressed in dextrinizing units. This method is based upon the time at which the sample in the presence of excess beta-amylase reached the color of a calibrated standard plastic disc.

Beta-amylase was determined on the 72-hr sample also using the appropriate method of the Institute of Brewing (1977) and activity was expressed in degree Lintner. This method is based upon the volume of converted starch solution required to reduce the Fehling's solutions.

RESULTS & DISCUSSION

Seedling growth

The original grain was found to contain 0.1% tannin. Root growth was markedly suppressed at the higher concentrations of tannic acid and thus became more pronounced with increasing time (Table 1). These observations would indicate that tannic acid may be directly responsible for retarding seedling growth during the early stages of germination.

Changes in starch and soluble carbohydrate content

With the control sample (distilled water) and that in 1% tannic acid solution, starch content decreased from 62 to 55%. With increasing concentrations of tannic acid the starch content did not decrease as much (Table 1). This would indicate inhibition of starch degradation at higher tannic acid concentrations.

At low concentration of tannic acid and in distilled water soluble carbohydrate content increased with increase in germination time. At the higher concentrations (2% and 3% tannic acid solutions) soluble carbohydrate content fell after 48 hr to below the original level (Table 1). It would therefore appear that the assumption of Chaven et al. (1981) is correct namely that tannic acid directly inhibits the production of soluble carbohydrate. The fact that with high concentrations of tannic acid the soluble carbohydrate falls below its original level after germination at 48 and 72 hr may indicate that the soluble carbohydrate is being utilized.

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Table 1—Influence of tannic acid concentration on root length (cm), starch and soluble carbohydrate content (% D.B.)[a]

Tannic acid %	Root length (cm)				Starch %				Soluble Carbohydrate %			
	Germination time (hr)											
	0	24	48	72	0	24	48	72	0	24	48	72
0.0	0.0	0.5	1.6	3.0	62.0	58.0	56.0	55.0	1.4	1.4	2.0	2.1
1.0	0.0	0.5	1.6	2.1	62.0	59.0	56.0	55.0	1.4	1.4	2.0	2.1
2.0	0.0	0.3	1.1	1.2	62.0	61.5	61.0	60.0	1.4	1.4	1.0	0.9
3.0	0.0	0.2	0.8	0.9	62.0	62.0	62.0	61.6	1.4	1.4	1.2	1.0

[a] D.B. = Dry Basis

Table 2—Influence of tannic acid concentration on alpha and beta amylase activity after 72 hr germination (D.B.)[a]

Tannic acid (%)	Alpha amylase activity in seed (dextrinizing units)	Beta amylase activity in seed (degree Lintner)
0.0	16.7	13.7
1.0	16.6	13.6
2.0	13.3	10.1
3.0	11.1	9.3

[a] D.B. = Dry Basis

For the measurements of starch content in the absence of tannic acid and 1% tannic acid, the differences in results obtained after 0, 24, 48 and 72 hr, respectively, are statistically highly significant. (F = 46.0, and 48.0 respectively, $\gamma_1 = 3, \gamma_2 = 8$. The tabulated values of F for these numbers of degrees of freedom are 4.07 at the 5% level and 7.59 at the 1% level.

In 2% tannic acid, the differences in results obtained after 0, 24, 48 and 72 hr respectively, are significant at the 5% level but not at the 1% level (F = 4.70). The results obtained for 3% tannic acid are not significant ($\gamma_1^2 < \gamma_2^2$).

Depression of amylase activity

The effect of increasing concentrations of tannic acid on alpha- and beta-amylase was then studied. The effect of tannic acid concentration on the amylases in the seed was studied after a germination time of 72 hr. The alpha-amylase activity was recorded in dextrinizing units and beta-amylase activity in degree Lintner. The results (Table 2) show that both enzymes are inhibited by an increase in the concentration of tannic acid.

Tannins are known to react both reversibly or irreversibly with proteins thus cross-linking them with the formation of large agrregates and so reducing their solubility (Hough et al., 1971). It is suggested that tannins present in sorghum seeds retard the seedling growth due to inhibition of starch degradation, by inactivating the hydrolytic enzymes during germination.

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A Research Note

Processing Properties of Beef Semimembranosus Muscle as Affected by Electrical Stimulation and Postmortem Treatment

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ABSTRACT

Semimembranosus muscles from paired sides–electrically stimulated (ES) and not-stimulated (NES)–of mature cows were used to determine effects of certain postmortem treatments (state of rigor/temperature) and of ES on processing properties. ES decreased ($P<0.05$) pH values for prerigor, prerigor/frozen and postrigor/frozen muscles, but did not affect ($P>0.05$) values for total protein or juice loss during cooking. State of rigor/temperature treatment affected pH and salt-soluble protein (SSP) values more than did ES treatment. pH values differed ($P<0.05$) between each state of rigor/temperature treatment for both ES and NES samples. Salt-soluble protein values were higher ($P<0.05$) for NES prerigor than for NES postrigor/frozen samples and higher ($P<0.05$) for NES postrigor than for NES postrigor/frozen samples. SSP values for ES muscles were higher ($P<0.05$) for ES postrigor samples than for ES postrigor/frozen samples but were not different for other comparisons. Regardless of ES treatment, freezing of semimembranosus muscles increased pH values ($P<0.05$) and decreased SSP values ($P<0.05$) in three of four comparisons.

INTRODUCTION

COMMERCIAL USE of electrical stimulation (ES) for beef in the United States has been primarily to improve USDA grades for carcasses. Savell et al. (1980) reported that ES improved color of lean, hastened development of marbling, decreased incidence of "heat-ring" and improved USDA grade. Although tenderness is also improved by ES, this was not the primary reason for commercial adoption of this technology in the United States but was a primary reason for its use in other nations (Chrystall and Devine, 1980; Holt et al., 1980).

About 25% of the carcass may be used in processed products such as sausage. Current interest in accelerated slaughter and fabrication of beef (ES, hot-boning and rapid chilling) could result in production of large quantities of ES beef intended for use in manufactured products. However, few studies have determined effects of ES on beef used in processed products (Terrell et al., 1981a, 1982a, b; Whiting et al., 1981). Therefore, processing properties of ES beef should be of interest to those who manufacture the 2.4 billion kg of sausage produced annually in the U.S.A. (AMI, 1981).

Boneless manufacturing beef may be used in any of four rigor state/temperature conditions: prerigor/chilled; prerigor/frozen; postrigor/chilled; postrigor/frozen. Effects of these state of rigor/temperature conditions on processing properties of muscles were investigated during the 1960's. Saffle and Galbreath (1964) reported a decrease in salt-soluble protein (SSP) between prerigor vs postrigor vs postrigor/frozen meats from Cutter grade cows and Hamm (1960) reviewed mechanisms determining the water-holding capacity of such meat. Chemical properties (for example, SSP) are used as the basis for establishing constraints (bind values) for use in least-cost computerized sausage formulations. These values aid in making predictions of batter stability and other finished-product characteristics. Because of limited studies which have determined relative effects of postmortem conditions and of ES on SSP content, the "bind values" originally developed by Saffle (1970) may no longer be appropriate for inclusion in computer programs for sausage manufacturing. Accordingly, the objectives of this research were to determine the combined effects of ES and four state of rigor/temperature conditions on the processing properties of beef semimembranosus muscle.

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EXPERIMENTAL

FOUR MATURE COWS (two Holstein; two Jersey) were slaughtered; right sides were electrically stimulated (ES); left sides were not stimulated (NES) and served as controls. Stimulation was accomplished within 30 min postmortem by using 17 impulses (1.8 sec on; 1.8 sec off) at 550 volts and 5 amps, delivered by a Le Fiell & Co., Inc. prototype ES unit. Samples of the semimembranosus muscle were removed from each side in serial section for use in the following state of rigor/temperature treatments: prerigor, removed at 15 min post-stimulation; prerigor/frozen, removed at 15 min post-stimulation and frozen for 48 hr; postrigor, removed at 24 hr post-stimulation after storage at 3°C, and; postrigor/frozen, removed 24 hr post-stimulation after storage at 3°C and frozen for 48 hr. Immediately upon completion of their respective treatments, each sample was analyzed for processing properties (no additional storage of samples was incurred). Juice loss during cooking was determined by a modification (Terrell et al., 1981a) of the Wierbicki et al. (1957) procedure. Salt-soluble protein was determined according to the procedure of Saffle and Galbreath (1964). Total protein was determined by micro-Kjeldahl analysis (AOAC, 1975). pH was determined by direct electrode insertion into a muscle slurry containing 3% saline solution (i.e., 25g of muscle plus 100 ml of 3% saline as used for the SSP determination).

Data were analyzed by analysis of variance and paired-t distribution analysis (Steel and Torrie, 1960); mean separation was accomplished by use of a multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

MEAN VALUES for processing properties according to ES and state of rigor/temperature treatment are shown in Table 1. ES decreased ($P<0.05$) pH values for prerigor, prerigor/frozen and postrigor/frozen samples. Each state of rigor/temperature treatment resulted in muscles that had different ($P<0.05$) pH values regardless of stimulation treatment. Freezing of prerigor or postrigor samples increased ($P<0.05$) pH values above those for their respective controls (not frozen). Freezing is a means of solute concentration and is known to alter such properties as pH and osmotic pressure (Fennema, 1976). However, ES does not alter ultimate (24 hr) pH values in beef (Savell et al., 1978). Data in Table 1 confirm reports that ES accelerates pH decline (prerigor/frozen and postrigor/frozen samples) and that postrigor sample pH values for fresh (not frozen) meat are not different between ES and NES samples.

Mean values for total protein content and juice loss during cooking were not affected ($P>0.05$) by either ES treatment or state of rigor/temperature treatment. Although values for juice loss during cooking were numerically greater for ES than NES, and may confirm a trend observed in our previous work (Terrell et al., 1981), others have reported

Table 1—Mean values for processing properties of beef semimembranosus muscle stratified according to electrical stimulation treatment and state of rigor/temperature treatment[a]

State of rigor/ temperature treatment	pH[b]		Total protein, %		Juice loss during cooking, %		Salt-soluble protein, %[c]		Percentage change in SSP[h]	
	ES	NES	ES	NES	ES	NES	ES	NES	ES	NES
Prerigor	5.91[e]	6.31[e]	11.68	12.00	44.8	42.0	33.54[d]	35.88[d]	115.3	123.3
Prerigor/frozen	6.17[d]	6.43[d]	12.23	10.27	42.3	41.9	30.33[d]	26.66[ef]	104.3	91.6
Postrigor	5.57[g]	5.61[g]	11.96	12.22	41.9	40.3	30.97[d]	29.09[e]	106.5	100.0
Postrigor/frozen	5.68[f]	5.81[f]	11.75	11.68	43.5	32.5	22.98[e]	24.03[f]	79.0	82.6

[a] Means underscored by a common line are not different (P>0.05). pH, n = 122; total protein, n = 64; juice loss during cooking, n = 54; salt-soluble protein, n = 254.
[b] Determination made after adding 100 ml of 3% salt solution to 25g of muscle.
[c] Based on total protein.
[defg] Means in the same column, bearing a common superscript letter are not different (P>0.05).
[h] Means determined by dividing the percentage of SSP for postrigor NES samples into each of the other state of rigor/temperature mean values and expressing results on a percentage basis. Means were not analyzed for statistical significance.

inconsistent results for this and other processing properties of ES muscles (Whiting et al., 1981). Salt-soluble protein (Table 1) was not affected by stimulation treatment (P> 0.05) but state of rigor/temperature treatment did affect SSP values. Within ES muscle, the lowest (P<0.05) value for SSP was that for the postrigor/frozen samples. On the other hand, SSP values for NES muscles were highest (P< 0.05) for NES prerigor beef and lowest for NES prerigor/ frozen and NES postrigor/frozen samples. Our previous work (Swasdee et al., 1982; Terrell et al., 1982a,b) reported a numerical as well as a statistical trend for ES to decrease SSP values. The present data do not support these previous observations and suggest that state of rigor/temperature treatment has a greater effect on SSP than ES.

Freezing and frozen storage is known to denature proteins and to decrease SSP values (Saffle and Galbreath, 1964; Miller et al., 1980). Although Saffle and Galbreath (1964) reported that percentages of SSP associated with state of rigor/temperature treatments (relative to 100% for postrigor samples) were: prerigor = 149.6%; postrigor, 48 hr = 100.0%; and postrigor, 48 hr and frozen for an additional 48 hr = 91.0%, our data (Table 1) do not agree with these magnitudes of change in SSP. In the present study, changes in percentages of SSP (relative to postrigor NES = 100.0%) associated with state of rigor/temperature treatments were: ES prerigor = 115.3%, ES prerigor/ frozen = 104.3%, ES postrigor = 106.5%, and ES postrigor/ frozen = 79.0%; NES prerigor = 123.3%, NES prerigor/ frozen = 91.6%, NES postrigor = 100.0% and NES postrigor/ frozen = 82.6%.

Although our SSP values are lower than those reported by Saffle and Galbreath (1964) for longissimus dorsi, caput lateral triceps and semitendinosus muscles, they are comparable to those from our previous data (Terrell et al., 1982a, b). The need for measuring SSP and understanding factors which may affect this important processing property are apparent (Porteous, 1979). In that paper, the author recalculated "bind values" (originally developed by Saffle, 1970) using a larger data base and a factor for stability of emulsions, but no comparison for state of rigor/ temperature was made. Thus, more precise data (actual values or relative changes as affected by postmortem treatment) may be required in order to improve the validity of these "bind values."

Data from the present study suggest that ES does not (P>0.05) affect processing properties of postrigor (24 hr) semimembranosus muscles and only decreases pH values for prerigor, prerigor/frozen and postrigor/frozen samples. Values for SSP, total protein and juice loss during cooking were not statistically affected by ES. State of rigor/temperature treatment of semimembranosus muscles, to a greater extent than ES, affected pH and SSP values but values for juice loss during cooking were not affected (P>0.05) by state of rigor/temperature or ES treatments.

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A Research Note

Lipid Characterization of Longissimus and Biceps Femoris Muscles from Beef Animals Exsanguinated at Various Times after Stunning

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ABSTRACT

Lipid characteristics of bovine longissimus and biceps femoris muscle from 30 heifer carcasses which were exsanguinated 0 (control), 3 or 6 min after stunning were studied. Blood loss was greater when control animals were compared to animals stunned 3 or 6 min prior to exsanguination. However, total lipid, lipid phosphorus, cholesterol values and TBA numbers were similar among treatments. As time between stunning and exsanguination increased, monounsaturated and polyunsaturated fatty acids tended to increase. Delaying time between stunning and exsanguination had little influence on lipid characteristics of muscle even though large differences between lipid characteristics of muscle and blood exist.

INTRODUCTION

WORLDWIDE, a shortage of good quality protein food exists and the cost of protein foods for human consumption continues to rise (Satterlee, 1981). It may be possible to alleviate part of the shortage by utilizing more blood for human consumption since millions of tons of blood are currently wasted (Parmer et al., 1978). In some countries, slaughter blood is processed for incorporation into a number of sausage-type products (Lawrie, 1977; Swingler and Lawrie, 1979; Wismer-Pedersen, 1979; Bittel et al., 1981). Residual blood is an integral part of muscle (Wismer-Pedersen, 1979) and retaining more residual blood in muscle at the time of slaughter could increase protein yield from animals and at the same time, avoid some of the problems associated with the addition of blood to processed products. We hypothesized that if residual blood were to be increased in muscle, overall protein quality of muscle would not change but lipid characteristics might be altered due to the differences which exist in the lipid profile of skeletal muscle versus blood (Hanahan et al., 1960; Christie, 1978; Hwang et al., 1980). The objectives of this study were to evaluate fatty acid profile, cholesterol, lipid phosphorus content and degree of lipid oxidation in longissimus and biceps femoris muscle from beef carcasses which were exsanguinated at various times after stunning. In addition, viscera weights where blood may accumulate, as well as blood loss and dressing percentage were recorded.

EXPERIMENTAL

THIRTY CHOICE-GRADE cross-bred heifers, approximately 15 months of age were fed the same finishing diet and slaughtered in the University of Wyoming abattoir after an overnight stand without feed but with access to water. The heifers were stunned with a captive bolt pistol and bled following one of three experimental procedures. The control heifers were bled by severing the carotid arteries and jugular vein immediately after carcasses were stunned and hoisted. Ten additional heifers were stunned and hoisted but allowed to hang by the hind leg for 3 min before they were bled by removing the head at the atlas joint without first severing the carotid arteries and jugular vein. The remaining 10 heifers were bled in the same manner as the 3 min group except they were allowed to hang 6 min before the head was removed. Following dressing, carcass sides were chilled and aged at 4°C for 7 days. Longissimus muscle (L) cut from the thirteenth rib region and biceps femoris muscle (BF) cut from the medial portion were removed from the right side of each carcass. A portion of each muscle was trimmed of all external fat and connective tissue and ground twice through a 3.18 mm plate.

Total lipid was extracted from approximately 1g of ground L or BF (Williams, 1981). Lipid samples were purified by microfiltration (Miller et al., 1979), solvent evaporated and tared flasks weighed to the nearest 10^{-4}g to determine total lipid. Total lipid extracted was separated into portions for fatty acid analysis, cholesterol and lipid phosphorus analysis. After evaporation of hexane from the portion for fatty acid analysis, mixtures were saponified and nonsaponifiables extracted and discarded. Free fatty acids were converted to fatty acid methyl esters (FAME) using boron-tri-fluoride-methanol (MacGee and Allen, 1977) and extracted by hexane. Long chain FAME (LCFA–16–20 carbons) were separated by gas chromatography using 1.8m glass columns containing 10% SP 2330 on 100/120 mesh Chromasorb W/AW (Supelco, Inc., Bellefonte, PA), column temperature maintained at 185°C and detectors (flame ionization) at 250°C and nitrogen flow rate 30 ml/min. Detector response factors (Johnson, 1971) for quantitation of LCFA, using 21:0 as an internal standard were calculated for fatty acids using a prepared FAME mixture. Standard FAME were purchased from Supelco, Inc. (Bellefonte, PA). Detector responses were measured as peak height x retention time.

Samples and standards containing free cholesterol, cholesteryl palmitate and triolein (Miller et al., 1981) were saponified as for fatty acids but the nonsaponifiable fraction, containing cholesterol was extracted (three times) with hexane. Hexane was evaporated and colorimetric estimation of cholesterol was conducted according to the method of Zlatkis and Zak (1969). The method of Dodge and Phillips (1967) was used to determine lipid phosphorus in samples.

A 2-thiobarbituric acid (TBA) analysis (Sinnhuber and Yu, 1977) was conducted on raw and cooked L and BF. Analysis of cooked samples included boiling tubes containing ground samples for 1 hr in capped tubes. Tubes were stored, uncapped, 48 hr at room temperature. To analyze either raw or cooked tissue, reagents

Table 1—Least squares means for characteristics of heifer carcasses by treatment group

Characteristic[a]	Time between stunning and bleeding			
	0 min	3 min	6 min	SE[b]
Chilled carcass wt, kg	231.95	232.59	238.73	4.16
Dressing %	57.97	58.44	59.11	0.26
Blood loss %[c]	5.65[d]	3.96[e]	3.61[e]	0.13
Fat thickness cm	0.83	0.91	0.79	0.08
KPH fat, kg	11.31[d]	10.85[d]	13.53[e]	0.74
REA, cm^2	65.93	64.90	64.13	2.28
Liver wt, kg	5.32	5.49	5.30	0.13
Blood wt, kg	13.11[d]	9.20[e]	8.61[e]	0.54
Spleen wt, kg	0.61[d]	0.62[d]	0.75[e]	0.03
Pluck wt, kg[f]	7.20	7.44	7.40	0.16
6th rib wt, kg[g]	0.20	0.19	0.20	0.01

[a] Each value represents data from 10 animals.
[b] SE = standard error of the means.
[c] Blood loss is expressed as percentage of chilled carcass weight.
[d,e] Values in the same row with different superscripts are significantly different ($P < 0.05$).
[f] Pluck weight includes the weight of the heart and lungs.
[g] 6th rib weight is the weight of the 6th rib with all soft tissue removed.

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Table 2—Least squares means for fatty acid, percent lipid, lipid phosphorus, cholesterol and TBA numbers of longissimus and biceps femoris muscles from heifer carcasses which retained variable amounts of residual blood

Variable	Fatty acids[c]								Lipid[d]	Lipid phosphorus (mg/g lipid)	Cholesterol (mg/g lipid)	TBA number[e]	
	16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4				Raw	Cooked
Muscle[a]													
Longissimus	33.3	5.2	17.2	41.2	1.87	0.18	0.79	0.29	5.61[f]	3.37[f]	8.74[f]	1.0	2.7
Biceps femoris	35.4	5.5	16.2	39.6	1.98	0.19	0.80	0.39	4.50[g]	5.07[g]	11.17[g]	1.1	2.6
Standard error	3.2	0.7	1.7	4.1	0.18	0.02	0.09	0.04	0.25	0.27	0.50	0.1	0.2
Bleeding time[b]													
Control	35.4	5.1	17.7	38.8	1.88	0.18	0.70	0.36	5.11	4.34	9.65	0.9	2.6
3 min	34.7	5.5	16.5	40.4	1.72	0.17	0.83	0.31	5.34	3.96	9.54	1.1	2.8
6 min	33.0	5.5	15.9	42.0	2.18	0.21	0.87	0.35	4.72	4.37	10.67	1.1	2.5
Standard error	3.9	0.8	2.1	4.9	0.22	0.03	0.11	0.05	0.31	0.33	0.61	0.1	0.3

[a] Each value represents data from 30 longissimus or 30 biceps femoris muscles.
[b] Each value represents data from 10 longissimus and 10 biceps femoris muscles.
[c] No differences ($P < 0.05$) in fatty acids between muscles or within bleeding time and no muscle times bleeding time interactions were significant. Fatty acids are expressed as a percentage of the total fatty acids analyzed.
[d] Lipid is expressed as percent of fresh muscle weight.
[e] No differences ($P < 0.05$) in TBA numbers within muscles or bleeding times existed and no muscle times bleeding time interactions were significant. TBA numbers (raw) are expressed as mg malonaldehyde/g fresh tissue and TBA numbers (cooked) are expressed as mg malonaldehyde/g cooked tissue.
[f,g] Values with different superscripts within muscle or bleeding time in the same column are significantly different ($P < 0.05$).

were added to tubes containing samples after storage (or immediately after weighing for raw samples) and methods of Sinnhuber and Yu (1977) followed. Tubes were centrifuged 1 hr at 1524 x *g*. Absorbancy of the clear colored solution in the top layer was read at 532 nm.

Least squares analysis of variance of data (Harvey, 1975) was used to obtain means by muscle and treatment. The interaction between muscle and treatment was also included in the model tested. Differences between means were tested using the Duncan's Multiple Range test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

CHILLED CARCASS WEIGHT, dressing percentage, fat thickness, rib eye area (REA), liver weight, pluck weight and 6th rib weight were not different between groups of animals bled at various times following stunning indicating that these measurements were not affected by treatment (Table 1). Heifers which were exsanguinated 3 and 6 min after stunning retained greater ($P < 0.05$) amounts of blood than did heifers bled immediately following stunning, but blood loss from animals exsanguinated 3 min after stunning was not different from animals exsanguinated 6 min after stunning. While differences in dressing percentage were not statistically significant, the higher values for carcasses subjected to delayed exsanguination are suggestive of increased residual blood in carcass musculature. Von Ogielski and Wartenberg (1961) also reported increased residual blood in muscle tissue as time between stunning and exsanguination increased. The spleen was heavier ($P < 0.05$) in animals exsanguinated 6 min after stunning than in animals exsanguinated 0 or 3 min after stunning, indicating that some blood may have been diverted to the spleen in experimental animals. Fatty acid composition of skeletal muscle did not change between muscles or within bleeding times (Table 2), which supports Gillis et al. (1973) who reported that differences in fatty acids were not significant between L and BF from bovine intramuscular fat. Percentages of each fatty acid in muscle are similar to those reported by Terrell (1967), Dryden et al. (1973) and Miller et al. (1981). Increases in monounsaturated and some polyunsaturated fatty acids between control and experimental animals may suggest increased residual blood in the muscle from experimental animals since blood is much higher in unsaturated fatty acids than is muscle (Hanahan et al., 1960; Christie, 1978; Hwang et al. 1980). Percent total lipid was higher ($P < 0.05$) in the L than in the BF but did not differ among treatments for either muscle studied (Table 2). Lipid phosphorus and cholesterol were higher ($P < 0.05$) in BF than in L but differences were not significant among treatments for either muscle. Differences between muscles may be due to a higher content of red fibers in the BF than in the L (Yamauchi and Matsushita, 1980; Hunt and Hedrick, 1977). The greater percentage of red fibers with more membranous structure in the BF would be expected to be higher in lipid phosphorus and cholesterol. The higher percent of lipid in L is inversely related to lipid phosphorus and cholesterol and may simply reflect more deposition of triglycerides in the L than in the BF. No differences existed in TBA numbers between L and BF or between treatments (Table 2). TBA numbers were higher in muscles from all treatments after cooking which is in agreement with Liu and Watts (1970), Love and Pearson (1974) and Sato and Hegarty (1971), who indicate that cooking increases susceptibility of meat to rancidity. Heme iron is thought to be catalytic in fresh and in cooked meat products, due to decreased reducing conditions which develop (Greene, 1969). With increased levels of residual blood in skeletal muscle, increased heme iron present in addition to increased levels of polyunsaturated fatty acids and phospholipids could increase potential for lipid oxidation. However lipid oxidation does not appear to be a problem in muscle tissue from animals which are exsanguinated at various times after stunning.

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A Research Note

Histamine Formation in Abusively Stored Pacific Mackerel: Effect of CO_2-Modified Atmosphere

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ABSTRACT

Whole Pacific mackerel (*Scomber japonicus*) were abusively stored at 20°C in air or 80% CO_2, balance air. Samples were analyzed for amines using a modified amino acid analyzer. Following 24 hr storage, levels of histamine, tyramine, putrescine, and cadaverine increased only slightly above the low levels observed initially. During the next 24 hr, the amine content increased dramatically. Levels in the air control samples were about twice those in the modified atmosphere samples. In a separate trial, amine levels in fish stored 3 days were higher still and similar in the two atmospheres. Thus, in neither trial did CO_2-modified atmosphere storage lead to increased production of potentially toxic amines.

INTRODUCTION

INCREASING USE is being made of modified atmosphere (most often high levels of CO_2) storage of fish (Wolfe, 1980). Despite the long standing observation of greatly inhibited microbiological growth in such atmospheres, the mechanism of this inhibition is yet to be elucidated (Enfors and Molin, 1980). The effect of modified atmosphere storage of fish on several pathogenic microorganisms is of concern, since specific susceptibility to such inhibition cannot be predicted.

The problem of scombroid poisoning (histamine toxicity) has been reviewed recently (Arnold and Brown, 1978). Several microorganisms may be associated with the formation of toxic levels of histamine. The effect of carbon dioxide atmospheres on the growth of some of these microorganisms has been studied (Haines, 1933; Enfors et al., 1979). *Proteus morganii* is a frequently associated microorganism (Omura et al., 1978). One report indicates that the growth of a *Proteus* culture was not inhibited at 20°C by a 20% CO_2 atmosphere (Haines, 1933). *Proteus morganii* was reported to produce maximum levels of histamine at 20°C (Kimata and Kawai, 1953).

Of the many species of tuna and mackerel associated with scombroid poisoning (Halstead, 1967), Pacific mackerel (*Scomber japonicus*) is locally available commercially as fresh whole fish. This study investigates the effect of modified atmosphere storage on amine formation (at a temperature associated with poor product handling and significant histamine formation in these fish) to assess any potential hazard in the use of high CO_2 modified atmosphere storage under adverse conditions.

EXPERIMENTAL

Storage

Whole Pacific mackerel, about 1 kg average weight, were received in ice from Paladini Sea Food Co., San Francisco, CA. On arrival, whole fish were placed on racks in gas tight cylinders as described previously (Brown et al., 1980). The cylinders were evacuated twice, to 16 in. then to 18 in. of vacuum. The vacuum was released to atmospheric pressure with air (control samples) or with CO_2 to yield a nominal 80% CO_2 atmosphere (balance air). The cylinders were held at 20°C. Samples of each trial were taken for determination of amine levels at zero storage time, and recovery of added amines. After the 24 hour sampling, the storage atmospheres were reestablished as above.

Extraction

Five whole fish were taken for each sample. The entire skin-free filletable flesh (about 2 kg) was passed twice through a fine meshed meat grinder. Following thorough mixing, a 5.0-g sample was weighed into 45.0 ml of 0.1M HCl containing 0.94 μmol (fresh samples) or 18.8 μmol (stored samples) of α-amino-β-guanidinopropionic acid as an internal standard. Following homogenization for 30 sec using a Polytron sonicating homogenizer and centrifugation for 15 min at 20,000 × *g*, 15.0 ml of the milky aqueous phase was shell frozen and lyophylized. A 1.5 ml aliquot of the first chromatography buffer (Dierick et al., 1976) containing 3.0% (w/v) sulfosalicylic acid was added to the dry material. The clear extract obtained after centrifugal filtration through a 0.45 μm Millipore membrane filter was held frozen until analyzed.

Amine analysis

The chromatographic methodology of Dierick et al. (1976), based on a modified amino acid analyzer, was employed, using a 0.9 × 30 cm column of Durrum DC-6A resin, and a double length ninhydrin reaction coil. The resulting elution profile was as reported in Dierick's paper, except that the internal standard eluted between lysine and ammonia, and cadaverine eluted just after tyramine and was poorly resolved from it.

Between 20 and 400 μl of extract were applied for each analysis; the exact equivalent weight of fish flesh was calculated from the observed internal standard. The extraction efficiency for each amine was determined from the average recovery of 2.5 μmol of each added with the internal standard to duplicate fresh samples from each trial. Recovery of the added amines was similar to the recovery of the internal standard (96.4%) except for tyramine which averaged 58.0%.

RESULTS

RESULTS of the chromatographic analyses are summarized in Table 1. The levels of amines found in the fresh samples of both trials were low, indicating the fish had been well handled prior to delivery. The pattern of formation was similar for the four amines detected. After the first

Table 1—Amine levels in Pacific mackerel expressed as mg/100g flesh

Sample	Histamine	Tyramine	Cadaverine	Putrescine
		Trial I		
Fresh	<0.05	2.4	1.0	0.1
1 day Air	0.3	3.2	2.1	0.3
1 day 80% CO_2	1.6	3.2	2.1	0.5
2 day Air	338.0	44.9	38.8	4.1
2 day 80% CO_2	142.0	27.8	24.0	1.9
		Trial II		
Fresh	<0.05	4.7	1.0	0.1
3 day Air	377.0	16.6	40.6	22.9
3 day 80% CO_2	372.0	21.6	52.9	26.8

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24 hr of storage, little increase in amine levels was observed in either treatment group. After the second 24 hr, the levels were about twofold higher in the air control samples than in those held in 80% CO_2. In the second trial, amine levels in fish held 72 hr were higher still and similar in both treatments. Neither phenylethylamine, agmatine, nor tryptamine (other amines determined by the methodology) was observed above trace level in either fresh or these 3-day samples.

In both trials, the use of 80% CO_2 (balance air) modified atmosphere storage did not lead to increased histamine formation in Pacific mackerel. At 20°C this atmosphere mildly inhibited, at least initially, the formation of potentially toxic amines. This effect seemed uniform for all the amines observed.

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