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PUBLICATION	WHERE TO FIND IT	DEADLINES
Call for Symposia Proposals and Guidelines for Symposia Organizers	*Food Technology,* January 1984, pp. 8 & 154-160	Receipt of preliminary proposals: May 15, 1984. Notice of acceptance or rejection of preliminary proposals: July 15. Receipt of final proposals: October 1. Notice of acceptance or rejection of final proposals: November 15, 1984.
Call for Volunteered Papers and Official Abstract Forms	*Food Technology,* September 1984, pp. 97-106	Receipt of abstracts: December 1, 1984. Notice of acceptance or rejection of papers: February 1, 1984
Guidelines for Poster Presentations	*Food Technology,* September 1984, p. 96	Same as Call for Volunteered Papers
Rules Governing IFT's Graduate Paper Competitions	*Food Technology,* September 1984, pp. 94-95	Same as Call for Volunteered Papers
Call for Undergraduate Research Paper Competition Papers	*Food Technology,* September 1984, p. 95	Notify URPC chairman: January 15, 1985. Receipt of abstracts: February 15, 1985
Call for Films for Film Theater	*Food Technology,* September 1984, p. 93	Receipt of films: December 30, 1984
Call for Nominations for Achievement Awards and Fellows	*Food Technology,* September 1984, pp. 90-91 and October 1984, pp. 148-149	Receipt of nominations: Industrial Achievement Award, December 1, 1984 International Award, December 1, 1984 Appert Award, December 15, 1984 Cruess Award, December 15, 1984 Babcock-Hart Award, January 1, 1985 Prescott Award, January 1, 1985 Fellers Award, January 1, 1985 IFT Fellows, February 1, 1985
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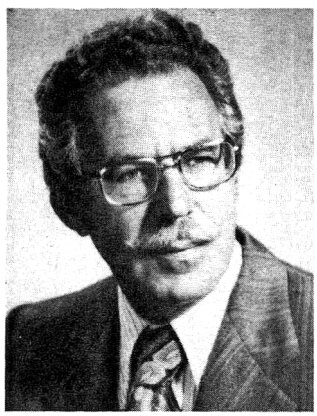

If you read the masthead of the Journal you will have noticed that there is an additional assistant Scientific Editor. Dr. Stanley Kazeniac has agreed to assist me in the processing of manuscripts. Dr. Kazeniac, formerly with the Cambell Soup Company, has had many years of experience in research and product development and I welcome his expertise.

You may have noticed that the September-October issue was considerably thinner than previous issues and you may — or may not — have wondered about this. There were just so many manuscripts available to the printer at the deadline. Submission of manuscripts continues to be as high as it has been in the last year or two, but there are several steps in the processing chain that may be contributing to the shortage of manuscripts. In the review process the manuscripts generally are returned to the author(s) within two months. However, we have no control over the length of time the authors take to revise their manuscripts. While most revisions are returned within several months, some authors take much longer, and about 5% of the manuscripts are considered withdrawn when we do not receive the revision after a year. Revised manuscripts are usually reviewed within two weeks of receipt and are either accepted for publication, sent to one of the reviewers again if significant changes have been made, or returned to the authors if further revisions are necessary. The latter two steps do impose an additional time lag in the process, but only about 30% of the manuscripts are affected. Finally, galley proofs are sent to the authors. These are supposed to be returned within 48 hours of receipt, but authors are not always able to comply with this request. Ineffective postal service is another source of delays. Thus, an accumulation of time lags can delay the availability of manuscripts.

We urge all reviewers to complete their task as quickly as possible and all authors to handle their revisions and galley proofs expeditiously, and we, for our part, will try to reduce even further the time that a manuscript is in our possession.

To make possible timely publication of a journal of this size requires the dedicated efforts of a number of people. I wish to thank Ruth Zabarsky, in my office, Dr. Kazeniac and Anna May Schenck, JFS Assistant Scientific Editors, for the time and care they have devoted. John Klis (Director of Publications), Barney Schukraft (Managing Editor) and Gladys Anderson (Editorial Department Secretary) of the IFT Publications Office have been very helpful and I am pleased to acknowledge their support and assistance.

The Editorial Board has also been very helpful, and I wish to express my thanks to the retiring Board members and welcome the new members as they begin their three year term.

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Storage Stability of a Puff Pastry Dough with Reduced Water Activity

H. K. LEUNG, J. P. MATLOCK, R. S. MEYER, and M. M. MORAD

ABSTRACT

A puff pastry dough that remained stable at refrigerated temperatures was developed. Dry heat treatment was used to partially destroy amylase and lipoxygenase in wheat flour. Water activity of puff pastry dough was reduced from 0.98-0.90 by addition of 12g sorbitol/100g flour. The adverse effects of sorbitol on baking properties of puff pastry were alleviated by addition of sodium stearoyl lactylate and gum arabic. The puff pastry dough stored at 5°C in air or nitrogen was stable for 75 days without any significant loss in specific volume or pastry height in comparison with the frozen control.

INTRODUCTION

PUFF PASTRIES are laminated, flaky products with an open structure. A puff pastry dough consists of many thin layers of dough separated by fine laminations of fat. During baking, the dough layers are expanded due to evaporation of moisture in the form of steam. The melted fat insulates the dough layers causing them to cook individually and lift or puff the pastry. As the gluten coagulates, it forms into a light, open structure with fine layers. Recipes and preparation procedures of puff pastry have been described in the bakery technology books by France (1969), Sultan (1969), Matz (1972), and Barrows (1975). However, a literature search on this subject yielded only two research articles. Lagendijk and Van Dalfsen (1965) developed a firmness meter to classify puff pastry fat based on dough firmness. Colburn and Pankey (1964) studied the effects of firmness of shortening and method of preparation on the characteristics of the finished pastries. In general, little is known about the effects of preparation procedures and ingredients on the quality of puff pastry.

Preparation of puff pastry doughs is a laborious process in which the doughs are rolled and folded repeatedly to build up hundreds of alternating layers of dough and fat. Therefore, it was not well adapted to commercial production until the Rheon Company developed an extruder to produce a cylinder of dough coated internally with margarine (Cleven and Weber, 1977). This process has been successfully applied in the automatic production of frozen puff pastry dough (Haarsgaard, 1980).

The introduction of semi-moist pet food by General Foods about two decades ago demonstrated that an intermediate moisture (IM) food product can be made shelf stable by incorporation of humectants to lower its water activity. Extensive studies have been conducted on the principles and applications of IM foods in recent years (Davies et al., 1976). The purpose of this study was to apply the IM food technology to puff pastry dough. The effect of humectants on water activity and quality of puff pastry dough was investigated. Also the storage stability of a puff pastry dough with reduced water activity (0.90) was evaluated.

Author Leung is affiliated with the Dept. of Food Science & Human Nutrition, Washington State Univ., Pullman, WA 99164-6330. Author Matlock's present address is Exxon Corporation, E. Millstone, NJ 08873. Author Meyer, formerly affiliated with Armour Co., is now with Kitchens of Sara Lee, Deerfield, IL 60015. Author Morad's present address is Dept. of Soil & Crop Science, Texas A&M Univ., College Station, TX 77843.

MATERIALS & METHODS

Materials

A commercial straight grade baker's flour with a protein content of 11.7% and moisture contents of 10-12% was used throughout this study. The Humko brand puff pastry shortening was supplied by Kraft, Inc. (Memphis, TN). The dough conditioner "Panicrust" was obtained from ITT Paniplus (Olathe, KS). Cream of tartar was purchased from a local store. Potassium sorbate was obtained from Pfizer Chemicals (New York NY). Sorbitol, gum arabic and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals used were reagent grade.

Preparation of puff pastry dough

The "English Method" described by France (1969) was modified in this study. The standard formula for puff pastry dough included 100g hard wheat flour (14% moisture basis), 30g water, 35g mix-in shortening, 35g roll-in shortening, 2g salt, and 0.5g cream of tartar. All of the ingredients except roll-in shortening were mixed for 3 min in a National Mixograph mixer (National Mfg. Co., Lincoln, NB). The dough was rolled into a rectangle of about 1.27 cm (0.5") thick and roll-in shortening was spread over two-thirds of the dough. The dough was then folded, rolled and refolded three times to build up many layers of dough laminated between thin sheets of fat. The dough was relaxed in the refrigerator for 24 hr and warmed to room temperature. It was rolled again to a 0.64 cm (0.25") thick rectangle and cut into four discs of 6.35 cm diameter and baked at 204.4°C for 20 min. In order to reduce water activity of puff pastry dough, water was reduced from 62.5 g in the original formula (France, 1969) to 30g per 100g flour. Also, the mix-in shortening was increased from 12.5g to 35g per 100g flour to allow proper mixing of the dry ingredients.

To study the effect of ingredients on dough properties and product quality, the standard formula was modified by varying water content, addition of sorbitol, dough conditioner, potassium sorbate, gum arabic and BHA, or by substituting regular wheat flour with heat-treated flour. Sorbitol, potassium sorbate and gum arabic were dissolved in water before adding to the ingredients.

Enzyme inactivation of wheat flour

Three hundred twenty grams of flour were weighed into 13" x 9" x 2" aluminum pans and heated in a laboratory oven (National Mfg. Co., Lincoln, NB). Flour was heated at 148.9°C for 0, 20, 40 and 50 min, 176.7°C for 1, 5, 10, 20 and 30 min, or 204.4°C for 0, 5, 10, 15 and 20 min. The flour was allowed to reach room temperature before storage. Moisture content of heat-treated flour was determined by the AACC Method, 44-16 (1980).

For the lipoxygenase assay, a crude extract was prepared from an untreated control and each of the heat treated flour samples by modification of the method of Flurkey et al. (1978). Six-gram flour samples and 30 mL distilled water were stirred over a magnetic mixer for 30 min at room temperature. The slurries were centrifuged at 11,400 x *g* and 10°C for 15 min in a Beckman Model J-21C centrifuge. The supernatant, which was filtered through Whatman No. 4 paper under vacuum, was used as the crude extract and kept in ice until used. Lipoxygenase activity was determined by measuring the increase in absorbance at 234 nm due to the formation of conjugated diene products according to the method of Ben-Aziz et al. (1970) as modified by McCurdy et al. (1983). One unit of lipoxygenase activity was defined as an increase in absorbance of 0.001 per min at 234 nm.

For the amylase assay, crude enzyme preparation was similar to that for the lipoxygenase assay except 0.002N glycerophosphate-HCl buffer (pH 6.0) was substituted for distilled water as the extractant. Alpha-amylase activity was determined by the 3,5-dinitrosalicylate method (Whelan, 1964). One unit of amylase activity is that amount of reducing substances released from starch equivalent to 1 mg maltose in 3 min. Lipase activity of unheated wheat flour was determined by the method of Lazo-Wasem (1961). One unit of lipase liberates an amount of fatty acid equivalent to 1 mL 0.05N alcoholic NaOH from 1 mL olive oil in 30 min.

Effect of ingredients on pastry quality

A number of baking experiments were conducted to evaluate the effects of heat treated wheat flour, sorbitol, dough conditioner (Panicrust) and gum arabic on baking quality of puff pastry dough. The dough was formulated based on the standard formula except that the specific ingredients studied were varied. The pastries were compared with controls made with the standard formula. Four pastries were prepared for each treatment and the experiment was repeated three times. Weight and height of the pastries were determined after cooling to room temperature. Pastry volume was determined by rapeseed displacement with a National Volumeter (National Mfg. Co., Lincoln, NB). Specific volume (c.c./g) was calculated from the weight and volume of pastry. The final dough formula (Table 1) was chosen based on the combined effect of all treatments.

Water activity (a_w)

To evaluate the effect of sorbitol on water activity of puff-pastry dough, standard formulations containing 0, 10, 12, 14, 16, and 18g sorbitol per 100g flour were prepared. Water activity was determined with a vapor pressure manometer (Lewicki et al., 1978), with a Hygrodynamics Hygrometer (Silver Spring, MD) and by graphical interpolation. The interpolation method of Landrock and Proctor (1951) was modified by increasing the equilibration time from 2 to 24 hr and by substituting sulfuric acid solutions with salt slurries. The salt slurries and their a_w at 24°C were: potassium sulfate (0.97), potassium nitrate (0.94), barium chloride (0.90), potassium chloride (0.84) and ammonium sulfate (0.81) (Smith, 1971; Greenspan, 1976).

For water adsorption isotherm determination, five groups of standard puff-pastry dough were prepared as usual except that the moisture contents were adjusted to approximately 18, 22, 27, 31 and 35% in each of the experimental dough systems during mixing. Five groups of the final experimental dough formula with 12g sorbitol (Table 1) were prepared similarly except that the moisture contents were adjusted to approximately 14, 18, 22, 27 and 31% during mixing. Moisture content was determined by Karl Fischer titration using a Precision Scientific Aquatrator (Precision Scientific Co., Chicago, IL). Water activity of the dough samples was determined by vapor pressure manometry (Lewicki et al., 1978).

Storage stability

Dough samples were prepared from the standard formula to serve as the experimental controls. They were stacked between layers of waxed paper in units of eight, then wrapped in plastic and stored at −40°C in airtight glass jars. Dough samples were also prepared from the final experimental formula. One-third were sealed in laminated pouches under air and stored at 5°C. Another one-third were sealed under nitrogen using a Kenfield vacuum packaging sealer and stored at 5°C, while the final one-third were sealed under nitrogen and stored at 15°C. The pouches were supplied by Armour Co. (Phoenix, AR) and were made of laminated polyester (0.05 mil), aluminum (0.0035 mil) and polypropylene (2.0 mil). Dough samples were withdrawn from storage at different time intervals for microbial analysis, total carbonyl determination and evaluation of baking properties.

Standard microbial plate counts of the puff pastry dough were performed according to Gilliland et al. (1976). Eleven-gram samples were placed in a sterile Waring Blendor with 99 mL sterile 1% peptone diluent and blended for 1 min at low speed and 1 min at high speed. Further dilutions were made from this solution. Total counts were determined using pour plate procedures with Standard Methods Agar (Difco Laboratories, Detroit, MI) and incubation at 32°C for 48 hr. Anaerobic counts were conducted in duplicate according to the pour plate procedure described by Hays and Lynt (1976). The plates were poured with Thioglycollate Agar (BBL, Division of BioQuest, Cockeysville, MD) and incubated at 32°C for 5-6 days at 32°C in BBL Gaspak systems.

Total carbonyls of the samples was determined by the method of Henick et al., (1954). Five gram samples of dough were extracted in 20 mL benzene. Five ml of extract was reacted with 5 ml 0.05% 2,4-dinitrophenyl hydrazine in a 60°C water bath for 30 min. Absorbance was measured at 460 and 430 nm. Baking quality of the dough samples was determined as described earlier. Height and specific volume of pastries were expressed as percentage of those of the frozen controls.

Statistical analysis

Analysis of variance and Duncan's multiple-range test (Steel and Torrie, 1980) was used to determine any significant differences among treatment means for specific volume and height of the pastries.

Table 1—Final formula of puff pastry dough with reduced water activity

Ingredients	Weight (g)
Heat treated flour[a] (176.7°C, 10 min.)	91.5
Water	39.0
Mix-in shortening	35.0
Roll-in shortening	35.0
Sorbitol[b]	12.0
Salt	2.0
Dough conditioner (Panicrust)	1.0
Cream of Tartar	0.5
Gum Arabic[b]	0.5
BHA	0.016
Potassium sorbate[b]	0.010

[a] Moisture content of flour = 6%
[b] Ingredients dissolved in water before mixing

RESULTS & DISCUSSION

Heat treatment of wheat flour

The purpose of heating wheat flour was to partially inactivate enzymes which may cause potential problems in puff pastry dough during storage. Moisture content of wheat flour decreased with increasing heating time and temperature. Browning of flour was visible after heating for 20 min at 176.6°C or 10 min at 204.4°C.

The effect of heat treatment of lipoxygenase activity in wheat flour is shown in Fig. 1. Lipoxygenase activity decreased rapidly during the first 20 min of heating at the three temperatures. Ten minutes of heating at 176.7°C

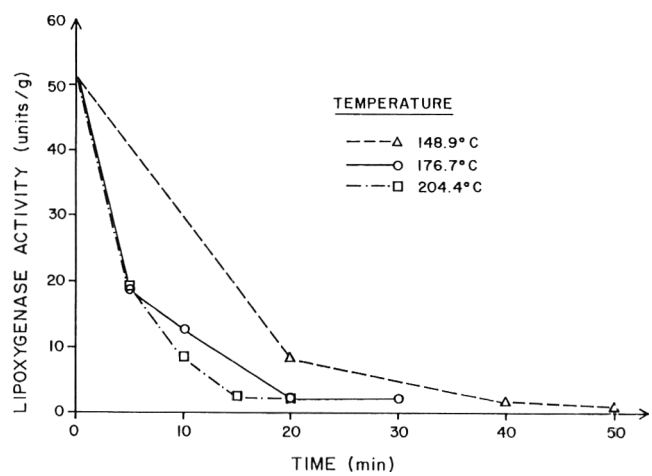

Fig. 1—Effect of heat treatment on lipoxygenase activity of wheat flour.

caused a decrease of about 75% in activity. Alpha amylase was more heat resistant than lipoxygenase in wheat flour (Fig. 2). The most severe heating (20 min at 204.4°C) caused only about a 70% decrease in amylase activity. Lipase activity in wheat flour before any heat treatment was negligible (0.04 unit/g flour) and, therefore, was not a concern in puff pastry dough. Widhe and Onselius (1949) also showed that lipase activity in wheat flour dough is insignificant.

Heat treatment was not only detrimental to enzymes, but also baking properties of wheat flour. Fig. 3 shows the effect of heating wheat flour on specific volume of puff pastry. The adverse effect of heating was probably due to denaturation of gluten. The 176.7°C-10 min treatment was used throughout this study because it provided partial inactivation of lipoxygenase (75%) and amylase (40%) without excessive loss of puff pastry quality.

Effect of sorbitol

Water activity of puff pastry dough decreased with increasing concentration of sorbitol (Table 2). Water activity values determined by vapor pressure manometer (VPM), electric hygrometer and graphical interpolation were generally in good agreement with each other. Addition of 10% sorbitol (flour basis) lowered a_w of puff pastry dough from 0.98 to 0.92. Higher sorbitol concentration resulted in less changes in a_w. Sorbitol can be added to baked goods at a maximum level of 30% according to good manufacturing practice (Friedman, 1978).

The effect of sorbitol on a_w of pastry dough was further demonstrated in Fig. 4. Addition of 12% sorbitol (flour basis) to the dough resulted in marked changes in the adsorption isotherms. Without sorbitol, a decrease in moisture from 52 to 22% (dry basis) resulted only in a 7% reduction in a_w of the dough. Sorbitol was more effective in lowing a_w of puff pastry dough at lower moisture content.

While sorbitol was effective in lowering a_w of puff pastry dough, it was detrimental to dough handling properties and pastry quality. With 14% or more sorbitol, the dough became greasy, weak and difficult to handle. Table 2 shows the adverse effect of sorbitol on specific volume and height of puff pastry. Increasing sorbitol concentration from 10 to 12% did not cause any significant decrease in specific volume ($P<0.05$). However, a further increase in

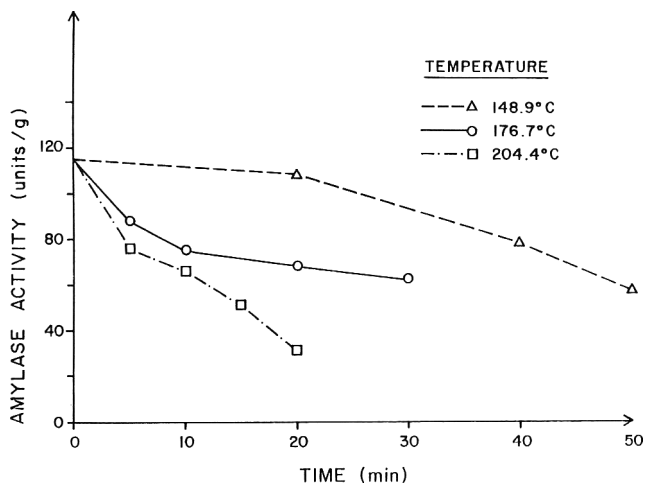

Fig. 2—Effect of heat treatment on α-amylase activity of wheat flour.

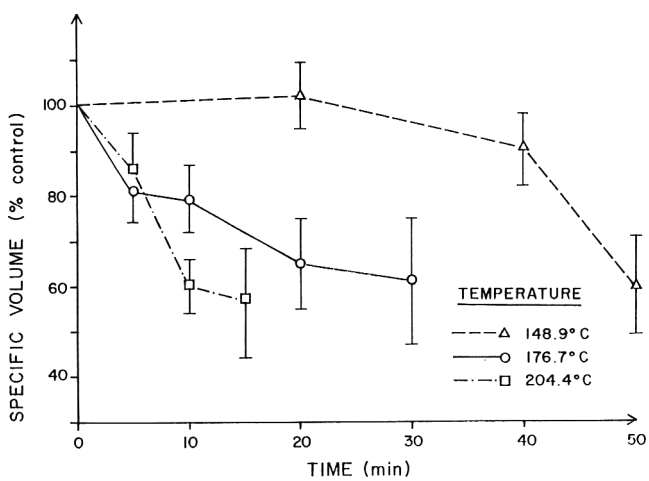

Fig. 3—Effect of heating wheat flour on specific volume of puff pastry. The error bars indicate standard deviations.

Table 2—Effect of sorbitol concentration on water activity of puff pastry dough and quality of puff pastry

Sorbitol (%)[a]	Water activity of dough				Pastry	
	V.P.M.[c]	Hygrometer	Interpolation	Mean	Sp. vol.[b]	Height[b]
0	0.97	0.97	0.99	0.98	100a[d]	100a
10	0.91	0.92	0.93	0.92	88b	88b
12	0.90	0.91	0.90	0.90	85b	77c
14	0.90	0.91	0.89	0.90	71c	62d
16	0.90	0.90	0.88	0.89	–	–
18	0.88	0.89	0.87	0.88	–	–

[a] Flour basis (14% moisture basis)
[b] % Control
[c] Vapor Pressure Manonmeter
[d] Means in the same column followed by different letters are significantly different ($P < 0.05$).

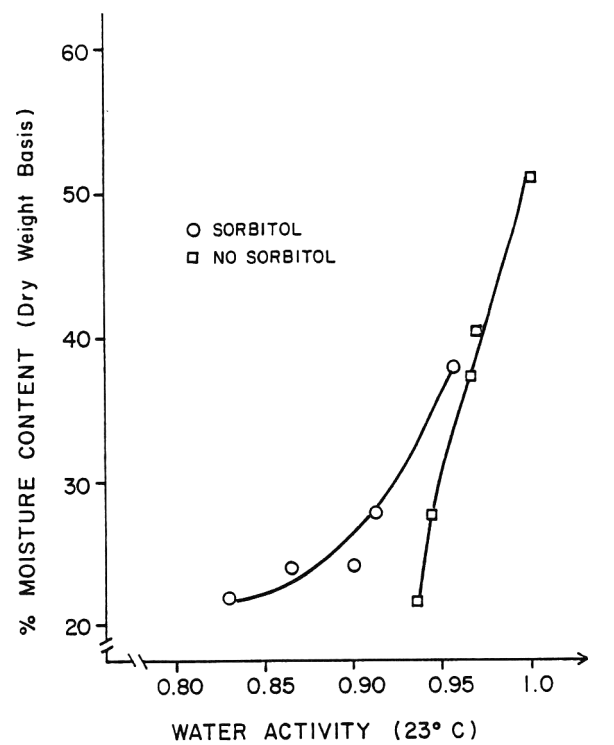

Fig. 4—Effect of 12% sorbitol on water adsorption isotherm of puff pastry dough at 23° C.

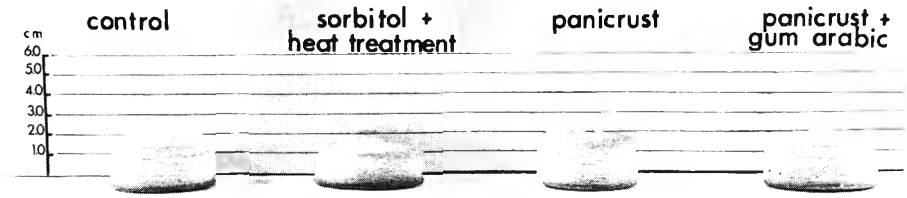

Fig. 5—Puff pastries baked from doughs containing heat treated flour, 12% sorbitol, 1% Panicrust and/or 0.5% gum arabic.

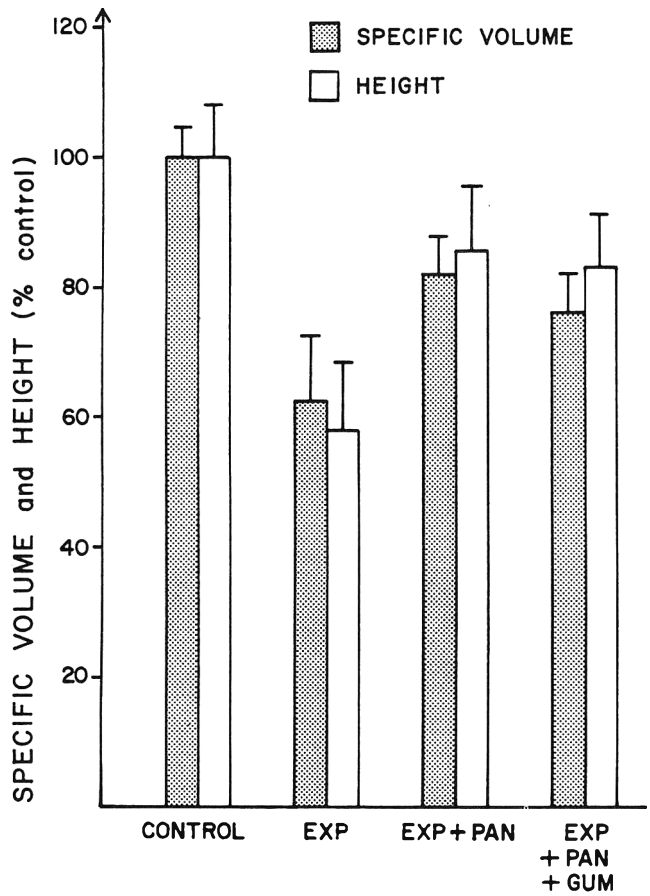

Fig. 6—Effect of sorbitol, Panicrust and gum arabic on specific volume and height of puff pastry. EXP = Experimental Formula, PAN = Panicrust, GUM = Gum Arabic

sorbitol concentration from 12 to 14% resulted in a significant decrease in specific volume from 85 to 71% of the control.

Effect of Panicrust and gum arabic

To alleviate the deleterious effects of sorbitol on puff pastry, various additives were evaluated. Preliminary studies indicated that a commercial dough conditioner, "Panicrust," and gum arabic were effective in improving dough properties and pastry quality. Panicrust contains 50% sodium stearoyl lactylate (SSL) which is approved for use in baked products for up to 0.5% on flour basis. Thus the use of Panicrust (50% SSL) in puff pastry is limited to 1% on flour basis. Addition of 1% Panicrust facilitated incorporation of fat in dough during mixing. As a result, the dough was not greasy and was easier to handle. Also it was observed that "oiling out" of puff pastry was greatly reduced during baking after addition of Panicrust. The improvement of dough handling and baking properties of puff pastry was probably due to the emulsifying properits of SSL (Tenney and Schmidt, 1968). Addition of 0.5% gum arabic also improved the handling properties of the dough and flaky structure of the baked product. Gum arabic functions as a hydrocolloid and increases the viscosity of the aqueous phase to help obtain optimum emulsion stability (Powrie and Tung, 1976).

Fig. 5 and 6 demonstrate the effects of Panicrust and gum arabic on the baking properties of puff pastry dough. The combined effect of 12g sorbitol and heat treated flour (EXP) decreased the specific volume of the baked product to about 62±10%, and the pastry height to about 58±11% of the control. Addition of 1% Panicrust to the experimental formula (EXP) significantly increased the specific volume (82±6%) and the height (85±10%) of the pastry. At the 5% level of probability, there was no significant difference in specific volume of pastry height among the control, the sample containing Panicrust, and the sample containing Panicrust and gum arabic. Although gum arabic had no effect on specific volume of height of the pastry, it improved handling properties of the dough and layering structure of the pastry.

Based on the above observations, 12% sorbitol, 1% Panicrust and 0.5% gum arabic (flour basis) were included in the final experimental dough formula. Potassium sorbate was added as an antimicrobial and butylated hydroxyanisole (BHA) was added as an antioxidant. The formula for puff pastry dough with reduced water activity is shown in Table 1.

Storage stability studies

Specific volume of the products remained relatively stable for doughs stored at 5°C in air and nitrogen (Fig. 7). Doughs stored at 15°C in nitrogen exhibited consistently lower specific volume than those stored at 5°C throughout the storage period. The results of pastry height (data not shown) were similar.

The deterioration of the baking quality of the dough stored at 15°C is probably related to the crystal structure of the puff pastry margarine. Dough samples from the 15°C storage were weaker and less firm than samples held at 5°C. Slight oiling or weeping of some melted puff pastry margarine into the pouches was also observed. The problem of oiling was evident during baking for the samples from 15°C storage, and appeared to become more severe during the final bakes of the storage study. The intact fat layer structure of puff pastry dough is critical in retaining water vapor formed during baking (Lagendijk and Van Dalfsen, 1965). Migration of fat into dough layers of the laminate structure or out of the dough during storage at 15°C may affect its ability to retain vapor during baking. Consequently, the product failed to rise properly during baking. The problem might be remedied by using a puff pastry margarine with a higher melting point.

Total carbonyl content of the doughs stored at 5 and 15°C showed a gradual increase from 2.2 μmoles/g at day

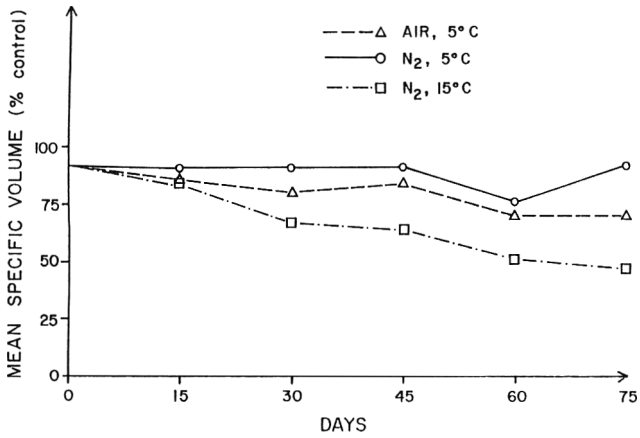

Fig. 7—Effect of storing puff pastry doughs on specific volume of the baked products.

zero to 2.8-3.7 μmoles/g at day 56. Henick et al. (1954) showed that corn and soybean oil do not become rancid until their total carbonyl contents reach 20-30 μmoles/g. The low carbonyl content in puff pastry dough indicated that rancidity was not a problem.

Standard microbial plate counts for dough samples showed some downward fluctuation during storage at 5 and 15°C (data not shown). Variations in standard plate counts were probably due to differences in initial microbial population of dough samples. Standard plate counts showed a decreasing trend from $3x10^3$ CFU/g at day zero to about $3x10^2$CFU/g at day 63. This range is well within the acceptable aerobic plate counts of 10^2-10^3CFU/g for refrigerated and frozen doughs (Hobbs and Greene, 1976). Anaerobic plate count estimates of dough samples varied from 1,400 to less than 100 CFU/g throughout the second half of the storage period. There appear to be no differences in microbial counts among samples stored at 5°C and 15°C in air or nitrogen.

CONCLUSIONS

WATER ACTIVITY of puff pastry dough could be lowered from 0.98 to 0.90 by reducing the normal moisture content by half and by addition of 12% sorbitol (flour basis). The adverse effects of sorbitol on dough handling properties and puff pastry quality were partly alleviated by addition of 1% Panicrust, a dough conditioner containing 50% SSL, and 0.5% gum arabic. Specific volume and height of puff pastries prepared from doughs stored at 5°C in air or nitrogen remained relatively stable throughout the 75-day storage period. No microbial spoilage or oxidative rancidity of the products was observed. However, puff pastry dough stored at 15°C showed significant loss in baking quality after 30 days of storage. The loss of baking quality was attributed to partial melting of puff pastry margarine.

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Effects of Harvesting, Transportation, and Cryogenic Processing on the Microflora of Southern Peas

S. D. SENTER, N. A. COX, J. S. BAILEY, and F. I. MEREDITH

ABSTRACT

Samples of southern peas [*Vigna unguiculata* (L.) Walp., cv. Purple Hull Pinkeye] were obtained at critical stages in a normal harvesting, transportation, and cryogenic processing operation and microbiologically analyzed for total plate count (TPC), Enterobacteriaceae count (ENT), and yeast and mold counts. Molds were identified to genera while ENT were identified to species. Operations involving extended holding times tended to increase all counts significantly, although subsequent processing reduced these counts to an acceptable level. Predominant genera of ENT and molds appeared to be soil borne organisms such as *Enterobacter agglomerans, Enterobacter cloacae, Klebsella pneumoniae,* and *Serratia liquefaciens,* and *Fusarium, Cladisporium, Alternaria, Phoma,* and *Aspergillus,* respectively.

INTRODUCTION

SOUTHERN PEAS are subjected to many harvest and postharvest conditions that favor microbial contamination and proliferation. Large quantities of soil are deposited on the shelled peas by rotary drum combines, and the peas are then held in collecting bins for extended periods prior to dumping. Fields are commonly located long distances from processing facilities, and large open trucks are used for transportation. Consequently, large quantities of the combined peas are exposed to high temperatures and mechanical damage before processing. The holding times and handling conditions necessary for preparation of the peas for processing are conducive to microbial growth.

Smittle and Kays (1976), Smittle and Hayes (1979), and Smittle et al. (1981) studied the effects of harvesting and handling on the chemical and sensory properties of southern peas and found that combining and transportation operations caused physical damage to the immature pea seeds and accelerated quality deterioration. Decreases in chlorophyll, sugars, starch, ascorbic acid, and sensory quality were related to the stress conditions of these operations.

Determination of the microflora prevalent on southern peas in relation to modern methods of harvesting, transporting, and cryogenic processing has not been made. Since this commodity is a frozen food that is quickly prepared for consumption in the home, comprehensive information on the predominant microflora as affected by the various harvesting and processing procedures would assist in pinpointing problems. This study was conducted to enumerate total aerobic bacteria, *Enterobacteriaceae,* yeasts, and molds and to identify the predominant microorganisms encountered at different stages in a typical, well-managed harvesting and processing operation for frozen southern peas.

MATERIALS & METHODS

Sampling

Shelled southern pea (cow pea) [*Vigna unguiculata* (L.) Walp cv. Purple Hull Pinkeye] samples were taken in a typical commercial harvesting, transporting, and cryogenic processing operation in southern Georgia. Samples, consisting of peas that were approximately 50% immature (green) and 50% vine dry, were taken in September, 1983 during the harvesting of a single field (ca 200 ha) and were aliquots of a single truck load during loading and holding, after transporting, and during processing. Combining operations began at about 10 AM with a rotary-drum, air cleaned combine, and the first bin load was dumped into the truck at 12 noon. The truck loading (ca 18,000 kg) was completed at 6 PM and the load was delivered to the processing plant at 9 PM. The load was immediately dumped into receiving pits and conveyed through air cleaning and washing operations for trash and dirt removal. After cleaning, the peas were conveyed to holding tanks with water, covered with ambient temperature potable water and held for approximately 10 hr for rehydration of the dried peas. After rehydration, the peas were conveyed through a 10 sec steam blanch for partial enzyme inactivation, then pneumatically transported without further washing to inspection lines for residual trash and defect removal. The peas were then conveyed to chambers for blast-freezing with liquid N_2, and packaged in polyethylene-lined cardboard containers for bulk storage.

Sampling points (Table 1) were significant events and operations within harvesting and processing, and were chosen because they might be expected to affect the microbial population of the peas. The initial sample was obtained during the first unloading of the combine with subsequent samples obtained from the same approximate location in the load two hr later and at the time of unloading at the processing plant. Samples thereafter were representative composites of the entire load.

Samples (300g) were taken aseptically in duplicate at the sampling points, placed in Whirl-Pac bags, and stored in crushed ice until analyzed. Time from harvest until analysis was approximately 24 hr.

Microbial analysis

Twenty-five grams of each sample were weighed, placed in a Waring Blendor jar with 225 mL sterile physiological saline and blended for 1 min. Appropriate serial dilutions were made using sterile physiological saline.

Table 1—Log-count/g of total aerobic bacteria (TPC), Enterobacteriaceae *(ENT), yeasts, and molds found in commercial harvesting and processing of Southern peas*

Sampling point	TPC	ENT	Yeast	Mold
Harvesting and transporting				
1. Freshly combined	7.4^{c}	4.6^{bc}	4.5^{ab}	4.4^{a}
2. Two hr postharvest	8.3^{ab}	5.6^{a}	5.1^{a}	4.2^{ab}
3. Nine hr postharvest	8.4^{a}	5.3^{a}	4.3^{b}	4.4^{a}
Processing				
4. After 1st wash	7.6^{bc}	4.7^{bc}	3.2^{c}	2.9^{bc}
5. After 2nd wash	7.3^{c}	4.2^{c}	2.7^{cd}	2.6^{c}
6. Soak tank, time = 0 hr	7.5^{c}	4.6^{bc}	2.6^{cde}	2.6^{c}
7. Soak tank, time = 10 hr	8.3^{ab}	5.0^{ab}	3.2^{c}	3.0^{abc}
8. After blanching	3.9^{f}	2.6^{e}	$<1^{9*}$	$<1^{e*}$
9. After inspection	5.4^{d}	3.3^{d}	1.4^{f}	0.9^{de}
10. Pre-freezing	4.4^{ef}	2.8^{de}	2.0^{e}	0.9^{de}
11. Post-freezing	4.8^{de}	2.5^{e}	2.3^{de}	1.7^{cd}

$^{a-g}$ Means separation by the Duncan's Multiple Range and Multiple F test (Duncan, 1955); numbers within columns followed by a common letter are not significantly different at the 5% level.
* <1 means no visible colonies appeared on the 10^{-1} dilutions.

The authors are affiliated with the USDA–ARS, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30613.

Table 2—Enterobacteriaceae *and molds identified in a commercial harvesting and frozen processing operation for Southern peas*

Sampling point	*Enterobacteriaceae*	Molds
Harvesting and transporting		
1. Freshly combined	*E. cloacae, E. agglomerans, S. marcescens*	*Fusarium, Phoma*
2. Two hr postharvest	*E. cloacae, K. pneumoniae, S. liquefacienes*	*Fusarium*
3. Nine hr postharvest	*E. agglomerans, K. pneumoniae, E. cloacae*	*Cladosporium, Blakeslea, Fusarium, Epicoccum*
Processing		
4. After 1st wash	*E. cloacae, S. rubidaea, K. pneumoniae*	*Fusarium, Colletotrichum*
5. After 2nd wash	*E. cloacae, K. pneumoniae*	*Phomopsis, Blakeslea, Cladosporium, Alternaria, Fusarium*
6. Soak tank, time = 0 hr	*E. gergoviae, K, pneumoniae, E. cloacae, K. oxytoca*	*Fusarium*
7. Soak tank, time = 10 hr	*E. cloacae, K. pneumoniae, E. agglomerans*	*Aspergillus, Fusarium, Alternaria, Fusarium*
8. After blanching	*K. pneumoniae, E. cloacae, E. gergoviae, H. alvei, S. liquefaciene*	---
9. After inspection	*E. cloacae, S. liquefaciens, H. alvei, K. pneumoniae*	---
10. Pre-freezing	*E. cloacae, E. agglomerans, K. pneumoniae*	---
11. Post-freezing	*E. agglomerans, E. cloacae, K. pneumoniae, P. mirabilis, C. freundii*	*Phoma, Penicillium*

For the total aerobic plate count (TPC), Plate Count Agar was the medium of choice and the plates were incubated in an inverted position for 72 hr at 20°C. The counts were reported as logarithmic averages and expressed as TPC/g product. Violet Red Bile agar with 1% glucose (VRBG) was used to estimate the *Enterobacteriaceae* (ENT) count (Mossel et al., 1962). Double-poured plates were incubated at 35°C for 18-24 hr. Counts were reported as logarithmic averages and expressed as ENT/g.

Potato Dextrose Agar (PDA) with sterile 10% tartaric acid added (pH 3.5) was used for enumeration of yeast and mold. Plates were incubated at 25°C for 72 hr then counted and examined microscopically to determine the actual number of yeast and molds. Counts were reported as logarithmic averages and expressed as yeast or mold counts per g.

Colony forming units appearing on the VRBG plates were randomly selected using a numerical grid (Cox and Mercuri, 1978) and a table of random numbers (Snedecor, 1957) and transferred to plates of Brain Heart Infusion agar to achieve purity. Following this, each isolate was identified to genus and species with Micro-ID and API. These two systems had been previously shown to be very accurate for identification of *Enterobacteriaceae* isolates from raw foods (Cox et al., 1983).

Mold colonies were also randomly selected and identified to genus by microscopic examination of morphological characteristics after growth on one-half and one-fourth strength PDA and V-8 agar.

One-way analysis of variance was performed on the data sets to determine if significant differences occurred in counts during harvesting and processing operations. Differences in mean counts at the 5% probability level were then evaluated by the Multiple Range and Multiple F Tests (Duncan, 1955). When significant differences are hereafter indicated, significance at the 5% level may be inferred.

RESULTS & DISCUSSION

TOTAL PLATE COUNTS were elevated during harvesting and transporting operations (Table 1). The time incurred betwen harvest and delivery to the processing facilities was 9 hr, and the peas were subjected to elevated temperatures, mechanical agitations, and impact; conditions which increased the TPC significantly by one log count/g. The first stages of processing, i.e., air clarification and washing, reduced the TPC back to that of the freshly combined peas.

Significant differences were not found in TPC during washing and delivery to the soak tank; however, TPC increased significantly during the 10 hr soak in potable water to that of samples 9 hr postharvest. Blanching significantly reduced TPC approximately 5 log/g. Recontamination of the peas during subsequent inspection and conveying significantly increased TPC; the count after inspection was higher than on samples prior to freezing but this may be attributable to variability within the load. The counts on the frozen peas were not significantly different from those obtained on samples taken after inspection or before freezing, and were within the limits specified by the International Commission on Microbiological Specifications for Foods (ICMSF) (1974) and the levels recommended by Splittstoesser and Corlett (1980).

ENT counts during harvesting, transporting, and processing followed essentially the same trends as TPC. Counts increased significantly during the 9 hr interim from the time of harvest utnil delivery to the processing plant, again attributable to the elevated temperature and mechanical damage in the conveying vehicle. Washing significantly reduced ENT load by approximately one log per g. After 10 hr of soaking for rehydration, the ENT count had increased, but was not significantly different from the ENT count of the unwashed peas 9 hr postharvest. Blanching significantly reduced the ENT count but operations within the plant recontaminated the peas, and a significant increase was observed after inspection. Freezing was somewhat effective in reducing the ENT counts; however, differences were not significant between this and the preceeding sample point. The post-freezing ENT count was not significantly different from the ENT count after blanching.

Enterobacteriaceae prevalent during harvesting and processing were *Enterobacter agglomerans, Enterobacter cloacae, Klebsiella pneumoniae,* and *Serratia liquefaciens*; of the elven sampling points, they appeared at 5, 11, 10, and 3 respectively. These organisms are found frequently in soil and have been found previously on raw fruits and vegetables (Cox et al., 1979).

Yeast and mold counts did not increase significantly during harvesting and transporting. The significantly higher yeast count two hr post harvest is probably an abberation since this count is higher than 9 hr postharvest. Washing of the peas at the processing facility reduced yeast and mold counts significantly, but unlike TPC and ENT counts, yeast and mold counts did not increase significantly during the 11 hr rehydration. Blanching eliminated yeast and molds, but recontamination during inspection and freezing increased both counts significantly.

Yeasts were not identified to genus and species. Identification of molds showed genera commonly associated with soil borne contamination; *Fusarium, Cladisporium, Alternaria, Phoma,* and *Aspergillus* appear to be the more common genera associated with the harvesting and processing of field peas (Table 2). *—Continued on page 1437*

Time and Temperature Parameters of Corn Popping

T. H. ROSHDY, K. HAYAKAWA, and H. DAUN

ABSTRACT

High quality commercial popcorn containing 14.0% moisture was processed in a continuous hot air domestic corn popper. The time required to pop individual kernels ranged from 35 - 120 sec, with 43% of the corn being cooked between 60 and 75 sec. The temperature of the popping chamber ranged from 196 - 277°C. The average temperature at the center of the kernels when they burst was 187°C. The empirical parameters, f and j values for heat transfer were determined.

INTRODUCTION

HEATING TEMPERATURE and thermal responses during popping greatly influence the quality of popped corn. However, there is no information on these physical parameters available in the literature.

The present study was conducted to determine the mean values and statistical distribution of the empirical parameters of heat transfer, such as j and f values; the popping temperature at the center of the popcorn kernel at the moment it pops; and the time required for the kernel to pop.

MATERIALS & METHODS

Popping procedure

The hot air popper used was a Presto "PopCornNow" which is a continuous corn popper, model No. 0481001 (Fig. 1). Popcorn grains (Orville Redenbacher's Gourmet) were used for these experiments.

The popper was preheated for 10 min. Then 100g units of corn kernels, each unit containing an average of 781 kernels, were popped by feeding the popper through its corn bin. At the end of the popping an average of 42 kernels remained unpopped (5.4% of the total kernels) which had to be replaced. The total popping operation took between 20 and 25 min.

Popping time

To determine the number of popped kernels versus time, three groups of popcorn kernels were prepared. Six runs were made with each group using 20g samples containing an average of 171.6 kernels of popcorn.

The first run (20g) of each group were popped for 45 sec. The number of popped kernels were counted and recorded. The second run (20g) of each group was popped for 60 sec, and the number of popped kernels counted and recorded. Then, the third, fourth, fifth, and sixth runs were popped for 75, 90, 105, and 120 sec, respectively, and the popped kernels counted and recorded.

To determine the popping time of the first kernel, eighteen 20g samples of popcorn were prepared and popped. The time that the first kernel popped was observed and recorded. The average time for the first kernel to pop in each sample was calculated.

Temperature in the empty popping chamber

The popper was modified to allow temperature measurements. Four 1.6 mm diameter holes 25.4 mm apart were drilled through a round wooden stick. A (T type) teflon coated gauged copper constantan thermocouple wire (0.254 mm diameter) was fixed in each hole (Fig. 1). The thermocouple wire from each zone was connected to a channel in a digital recorder computer, specially equipped for T type copper constantan thermocouples (Ester Line Angus Co.).

The recorder was turned on and the temperatures at the four zones were measured for 60 sec in the empty popping chamber. The average temperature of each zone was calculated.

Temperature inside and outside of the corn kernel

In order to determine the temperature inside the kernels, a thermocouple wire was inserted into a small hole drilled into a kernel and sealed with high temperature silicon glue (General Electric Co.). Thirteen kernels were prepared, each with a thermocouple wire inserted just before the test to assure no change in moisture content.

The experiment was carried out under two conditions: the first using ten 20g samples of popcorn kernels, and the second using three 100g samples of popcorn kernels. In each run the kernel with the thermocouple was located and fixed in the second zone 50.8 mm from the bottom of the popping chamber. This thermocouple was connected to the computer channel in addition to the other four channels for measuring the temperatures at the four zones at the same time.

In every run under both conditions, the temperature inside the kernel with thermocouple was recorded as well as the tempera-

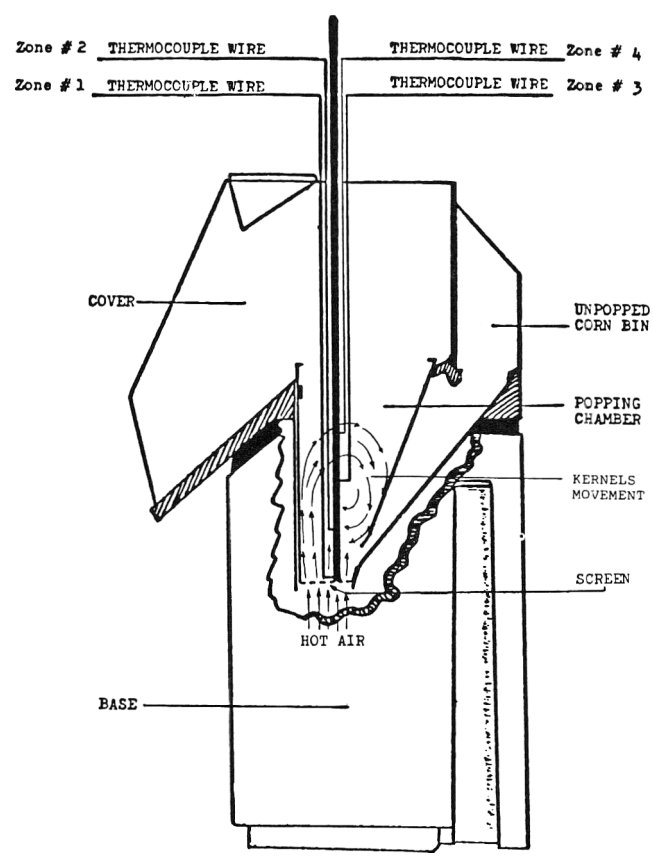

Fig. 1—Longitudinal section of the modified hot air popper.

Authors Roshdy, Hayakawa, and Daun are with the Dept. of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers Univ., New Brunswick, NJ 08903.

tures outside the kernels at the four zones. The time when the kernel with thermocouple popped was observed and recorded.

The mean temperature of each zone was calculated for each set of conditions.

The mean temperature at zone #2 (where the kernels with thermocouples were located) and the mean temperature inside the kernels at the time the kernel with the thermocouple was popped, were calculated.

The mean popping time of the kernels with thermocouples was calculated.

In the 20g experiments, the empirical parameters, f and j values for heat transfer, were determined according to Hayakawa (1972) using the data from popping temperatures.

Since it is rather difficult to determine accurately t_{br} and T_{br}, this determination was accomplished as described below.

In the 20g experimetns the collected data were used to obtain a semilogarithmic temperature history curve of sample kernels, which shows a relationship between $\log_{10}(T_1 - T)$ and t.

The temperature history curve showed a break point and the major part of this curve is represented by:

$$\log_{10}(T_1 - T) = m_1 t + b_1 \text{ when } t \leq t_{br} \quad (1)$$

$$\log_{10}(T_1 - T) = m_2 t + b_2 \text{ when } t \geq t_{br} \quad (2)$$

The values of b_1, b_2, m_1, and m_2 in Eq. (1) and (2) were estimated by applying a linear regression analysis to straight line portions of each temperature history curve. These values were correlated to empirical constants for heat transfer as shown below:

$$b_1 = \log_{10}[j_1 (T_1 - T_o)] \text{ and } b_2 = \log_{10}[j_2 (T_1 - T_o)]$$

$$\frac{1}{m_1} = f_1 \text{ and } \frac{1}{m_2} = f_2$$

The two lines intersected at the break point t_{br}, $\log_{10}(T_1 - T_{br})$. Therefore, from Eq. (1) and (2) we had:

$$m_1 t_{br} + b_1 = m_2 t_{br} + b_2 \quad (3)$$

By solving Eq. (3), we obtained:

$$t_{br} = \frac{b_2 - b_1}{m_1 - m_2} \quad (4)$$

Then the kernel temperature at the break point T_{br} was calculated using the following equiation:

$$\log_{10}(T_1 - T_p) = -(t_p - t_{br})/f_2 + \log_{10}(T_1 - T_{br})$$

The mean and standard deviations were calculated for j, f_1, f_2, T_{br}, T_p and t_p values.

RESULTS & DISCUSSION

Popping time

The time required to pop individual kernels in the 20g experiments ranged from 35 - 120 sec. Forty-three percent of the kernels popped between 60 and 75 sec. The relationship between the number of popped kernels and the popping time in the 20g experiments is shown in Table 1.

The mean popping time was 74.5 sec with a standard deviation (SD) of 17.8 sec in the 20g experiments.

The mean value for the first kernel to pop was 39.2 sec with a SD of 2.9 sec.

Popping temperature

The temperature in the four zones of the popping chamber became higher when 20g samples were processed. The zone temperatures were even higher when 100g of corn samples were used.

When the chamber contained 20g of kernels, the mean temperature at the center of the kernel was 187.0°C with a standard deviation of 2.2°C. When 100g of kernels were used this temperature was 191.2°C with a standard deviation of 19.2°C. The bursting time of the individual kernels with the thermocouples, immobilized in the second zone in the 100g experiments, occurred on the average after 45 sec and in the 20g experiments after 61.8 sec, with standard deviations of 5.2 and 9.4 sec, respectively (Fig. 2).

The temperatures of the popping chamber determined in our experiments were significantly lower than the stack reading found in commercial operations (Matz, 1976).

The mean values of the empirical parameters for heat transfer, j, f, and f_2, the mean temperature at the break point (T_{br}) of the temperature history curve, the mean temperature at the center of the kernels when they burst (T_p), as well as the mean popping time (t_p) required for kernels to burst, are shown in Table 2.

Experimental temperature history curve (Fig. 3) consists of two parts, when $t \leq t_{br}$ and $t \geq t_{br}$, respectively. A break point occurs where $t = t_{br}$ and the curve shows sudden change in the slope.

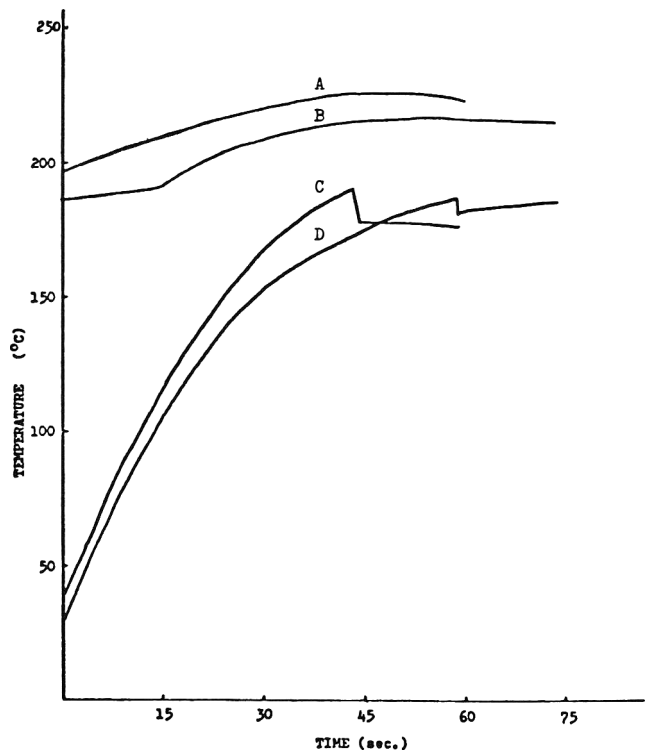

Fig. 2—Ambient temperatures A and B, temperatures at the center of the kernels C and D, in 100g and 20g of corn popping experiments.

Table 1—Number of popped kernels at various time intervals

Sample no.	Popping time (sec)						
	30	45	60	75	90	105	120
1	0	2	14	48	80	90	97
2	0	4	16	53	82	89	96
3	0	6	19	76	85	98	108
$\bar{X}$	0	4 ± 2.0	16 ± 2.5	59 ± 14.9	82 ± 2.5	92 ± 4.9	100 ± 6.7

The difference between ambient temperature and the temperature at the center of the kernel ($T_1 - T$) decreased with processing time. The experimental and predicted points on both parts of the temperature history curve correlate very well, with a correlation coefficient of 0.986.

If we assume that the following are known:

$T_o = 24.6°C$, $T_1 = 233.3°C$, $T_{br} = 148.2°C$
$T_p = 187°C$, $f_1 = 58.8$ sec, $f_2 = 93.5$ sec

we can calculate the time at the break point of the broken heating curve (t_{br}) using the following equation:

$$\log_{10}(T_1 - T_{br}) = -t_{br}/f_1 + \log_{10}[j(T_1 - T_o)]$$
$$\log_{10}(233.3-148.2) = -t_{br}/58.8 + \log_{10}[1.2(233.3-24.6)]$$
$$1.92992956 = -t_{br}/58.8 + 2.398703695$$

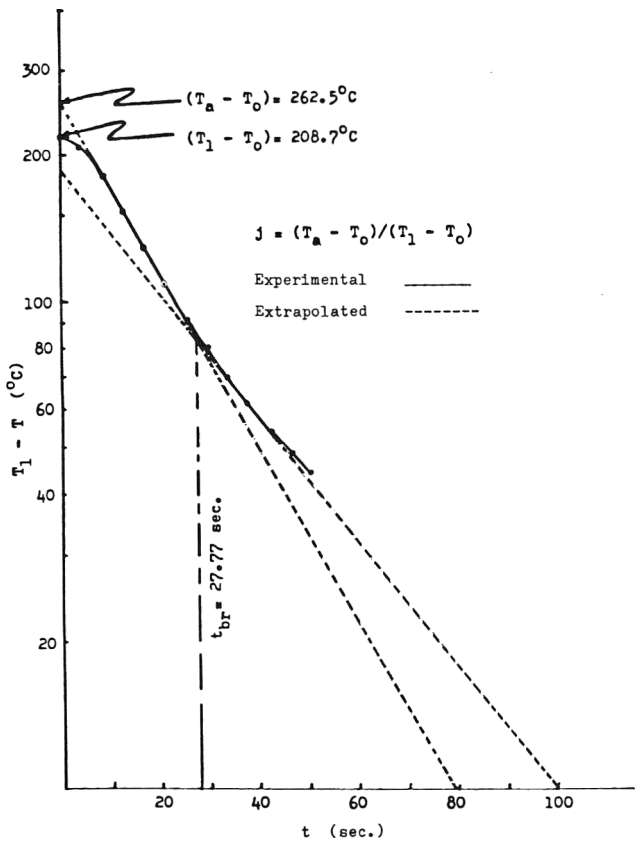

Fig. 3—Temperature history curve at the center of the kernel until it bursts.

$$t_{br} = (58.8)(0.468774135)$$
$$= 27.5691914 \text{ sec} = 27.6 \text{ sec}$$

The observed time from the graph = 27.8 sec.

Then we can use the t_{br} to estimate the time required for popping (t_p) using the following equation:

$$\log_{10}(T_1 - T_p) = -(bt_p - t_{br})/f_2 + \log_{10}(T_1 - T_{br})$$
$$\log_{10}(233.3-187.0) = -t_p - 27.6)/93.5 + \log_{10}(233.3-148.2)$$
$$1.665580991 = -(t_p - 27.6)/93.5 + 1.92992956$$
$$t_p = (93.5)(0.2643485691) + 27.6$$
$$= 52.28051035 \text{ sec} = 52.3 \text{ sec}$$

The observed popping time = 51 sec.

The calculated empirical parameters for heat transfer, j, f_1, f_2 and T_p can be used to determine the popping time for any initial temperature of corn and at any ambient temperature level assuming that the ambient temperature increased similarly to that observed during the experiment.

CONCLUSIONS

THE TEMPERATURE PROFILE of the popcorn kernels during popping showed a broken heating curve. The empirical parameters for heat transfer, j, f_1 and f_2 can be used to determine the popping time and the temperature required for popping. They can also be used to determine the time of the break point.

A similar approach can be established using any other popping operation. The accumulated data constitute a basis which can be used to optimize the equipment and evaluate both physical and chemical changes during the popping of corn and some other roasting operations.

NOMENCLATURE

b_1 = Intercept of the extrapolated line when $t \leqslant t_{br}$
b_2 = Intercept of the extrapolated line when $t \geqslant t_{br}$
f_1 = Slope index (sec.) for $t \leqslant t_{br}$
f_2 = Slope index (sec.) for $t \geqslant t_{br}$
j_1 = Intercept coefficient of temperature history curve when $t \leqslant t_{br}$
j_2 = Intercept coefficient of temperature history curve when $t \geqslant t_{br}$
m_1 = The slope of straight line when $t \leqslant t_{br}$
m_2 = The slope of straight line when $t \geqslant t_{br}$
T = Temperature at the center of the kernel at any time (°C)
T_o = Initial temperature at the center of the kernel (°C)
T_1 = Constant ambient temperature (°C)
T_a = Phantom initial temperature at the center of the

—Continued on page 1418

Table 2—Heat transfer parameters[a]

Exp. no.	j Value	f_1 (sec)	f_2 (sec)	T_{br} (C°)	T_p (C°)	t_p (sec)
1	1.38	62.10	98.97	160.9	186.5	77
2	1.24	54.98	86.52	144.1	186.3	64
3	1.08	62.29	100.11	158.1	188.6	64
4	1.19	69.70	106.00	140.8	183.4	77
5	1.23	57.05	82.47	144.2	188.9	51
6	1.14	51.00	74.36	145.2	189.1	60
7	1.16	60.90	107.10	151.7	189.2	59
8	1.21	62.10	106.24	143.6	188.2	59
9	1.14	52.55	97.24	151.2	186.4	56
10	1.24	55.68	75.60	142.4	183.2	51
$\bar{X}$	1.20 ± 0.08	58.83 ± 5.62	93.50 ± 12.70	148.2 ± 6.9	187.0 ± 2.2	61.8 ± 9.2

[a] j = Intercept coefficient of temperature history curve; f_1 = Slope index for $t \leqslant t_{br}$; f_2 = Slope index for $t \geqslant t_{br}$; T_{br} = Temperature at the break point; T_p = Temperature at the moment the kernel burst; t_p = Time required for popping.

Evaluation of Lye and Steam Peeling Using Four Processing Tomato Cultivars

D. V. SCHLIMME, K. A. COREY, and B. C. FREY

ABSTRACT

Fruit of four eastern tomato cultivars (cvs) were peeled using either high pressure steam or a lye bath under commercial conditions. Percent yield of whole fruit and finished product quality were determined. Yield of 5 sec steam-peeled fruit was 4.1% greater than lye-peeled fruit; no cvs differences were detected. Quantity of blossom-end peel was not affected by peel treatment, but differences among cvs were shown. Steam-peeled tomatoes averaged 6.2 cm^2 and lye-peeled < 1 cm^2 total peel/can. There were no differences among treatments or cvs in sensory color scores. Differences in surface bL/a values among cvs at the equator region of fruit were found. Although recovery was greater for steam-peeled than for lye-peeled fruit, canned product quality was better for lye-peeled fruit.

INTRODUCTION

ON THE DELMARVA PENINSULA virtually all canned whole tomatoes are peeled using hot, sodium hydroxide solutions. Currently, most whole tomatoes canned in the eastern United States are lye-peeled (Weaver et al., 1980). The use of pressurized steam to peel tomatoes has been evaluated as an alternative to lye peeling in both in-plant and laboratory studies (Leatherman and Tanklage, 1984; Weaver et al., 1980; Thomas et al., 1976). Steam peeling has been used in Europe to peel tomatoes for several years. Various pressurized steam peeling machines are available in the United States for peeling fruits and vegetables and some are in commercial use in California (Leatherman and Tanklage, 1984).

The tomato cultivars used by tomato processors in the eastern United States are largely machine-harvested, determinate types which tend to set and mature a large proportion of their fruit at one time. Nevertheless, a large number of fruit showing incomplete ripening and yellow shoulders are often delivered to tomato canneries. The duration of exposure to hot caustic necessary to adequately remove epidermis from unripened tomato tissue and from fully ripe tomatoes developed to withstand the rigors of mechanical harvesting results in substantial losses of subepiermal tissue.

Reeve (1976) reported that the skin of the tomato is composed of a cutinized epidermal layer and most of the cuticular wax is found in the cellulosic matrices of the outer and radial cell walls. He indicated that steaming or scalding increases the ease of cell separation in the subepidermal tissue and noted that cultivars lacking uniform ripening are not suitable for whole canning because of their inferior peeling qualities. Chih-Yu and Thompson (1972) found several layers of small, flattened, thick-walled cells below the cutinized layer of tomato epidermal cells. These have been identified as collenchyma cells and comprise the hypodermis. Beneath the hypodermis are several to many layers of mesocarp which are composed of parenchyma cells. Vascular bundles of different sizes are dispersed throughout the mesocarp. The innermost cells of the mesocarp are adjacent to the single-layered endocarp.

Chih-Yu and Thompson (1972) found that tomato mutants with the easy peeling gene (ep) demonstrate ready breakdown of the delicate mesocarp parenchyma cells subtending the hypodermis. When mechanically harvested tomatoes are lye peeled, the epidermis and hypodermis are removed to expose the mesocarp parenchyma with its numerous dispersed vascular bundles often referred to as "veins."

There are several disadvantages associated with the use of hot caustic to remove tomato epidermis. Among these are: the steadily increasing cost of NaOH, waste disposal problems and associated cost, the 25 - 30% peeling losses, the complexity of methods to recover peel loss tissue for subsequent by-product utilization, and the alleged reduction in the color quality of lye-peeled fruit as a consequence of the loss of mesocarp parenchyma and exposure of vascular tissue. Color evaluations of whole canned tomatoes have traditionally been presented as Hunter-Gardner L, a, b values or as a/b values obtained from blended can contents. Yeatman (1969) indicated that the value bL/a provided a high linear correlation (r = 0.902) with visual color scores of processed tomato juice.

The objectives of this study were to: (1) investigate the percent recovery of whole fruit, and (2) evaluate finished product quality of tomatoes peeled by both caustic and high pressure steam methods under commercial conditions.

MATERIALS & METHODS

Tomatoes

Fruit of four eastern-grown tomato cultivars viz. 'FM 6203', 'Peto 76', 'Peto 95', and 'VF 134' were obtained from local growers within 1 day of harvest at four separate times. Each lot was graded to remove fruit that were undersize, immature, damaged and diseased. Graded fruit were then washed and 25 - 30 kg of raw product were weighed for each treatment combination.

Peeling treatments

Peeling treatments on the four cultivars were replicated in time. Fruit were subjected to one lye peel or two steam peel treatments. The concentration of NaOH in the commercial lye peeler (Chisholm Ryder Co., Inc. – Fox Caustic Peeling Unit, Anonymous, 1971) was measured on an ambient temperature sample by titration with 0.1629N H_2SO_4 and then adjusted to achieve an initial concentration of 13.0 ± 0.5% (v/v) NaOH. Faspeel peeling aid (a surfactant obtained from The Maryland Chemical Co. (Baltimore, MD) which is a proprietary mixture of medium to long fatty acids in an aqueous solvent) was added to the lye bath at a rate of 31 g/MT of raw product throughput. Dwell time of raw product in the lye bath was 30 ± 1 sec at a temperature of 99 ± 1°C. Preliminary studies conducted during the 1982 tomato canning season demonstrated that these peeling parameters provided adequate peel removal and tended to hold peel loss to a minimum. After emerging from the CRCO-Fox Caustic Peeling Unit, the peeled tomatoes were discharged into collecting baskets. The fruit was rinsed with cold tap water and allowed to drain before weighing. Any minor peel not removed by lye treatment was included in the recovery weight.

Steam peeling treatments were conducted with a P.K.C. Steam Peeling System (Model 400 3 B, Zimmerman & Jansen, Inc; Denshaw, PA); a 425 liter capacity vessel at an inlet steam pressure of

Authors Schlimme and Corey are affiliated with the Dept. of Horticulture, and Author Frey is affiliated with the Dept. of Agricultural Engineering, Univ. of Maryland, College Park, MD 20742.

7.1 atm was used. Prior to loading the tomatoes into the vessel, it received two complete cycles of preheating at the selected steam pressure to temper it to treatment temperature. The two steam peel treatments used were obtained as a recommendation from a representative of the manufacturer, and consisted of 5 sec and 7 sec durations in the pressurized vessel. Following the steam treatments the tomatoes were unloaded into an ambient water bath and removed for immediate peel removal. Peel was removed from each fruit by hand in a manner comparable to that achieved by mechanical peel eliminators. Peel was found to adhere tenaciously to the fruit surface on regions near the stem scar and where normal or complete ripening had not occurred. Care was taken not to remove peel aggressively from these areas, based on the assumption that a mechanical peel eliminator would also not achieve removal.

Following peel removal, the rinsed and drained fruit were weighed and the percent recovery of initial raw product was calculated for the three peeling treatments.

Processing

Ambient temperature juice was prepared using a CRCO Model B Juice Extractor-Finisher (Anonymous, 1971) from a portion of the peeled fruit for each separate treatment lot. A 0.1 cm screen was used. One 2.9g calcium, acidulant A, salt tablet containing 17.0% (w/w) calcium sulfate dihydrate, 15.5% (w/w) citric acid and 62.9% (w/w) NaCl was placed in the bottom of each 303 x 406 can prior to addition of 60 - 90 mL juice. The can bodies and ends were #0.25 electrotin plated with GLD enameled interiors; the cans had welded side seams. Tomatoes were handpacked and the remaining can volume filled with the appropriate juice. The fill was adjusted with a paddle packer to provide an average 0.6 cm net headspace. Cans were sealed on a CCC 304-CR-1, steam-vac, closing machine, processed in a FMC 1.4m, round bottom, continuous cooker for 11 min at 100°C, cased and air-cooled. A total of 24 cans were processed for each treatment combination and replication.

Grading procedure

The average lot size for a typical eastern tomato processor is approximately in the range 12,001 - 39,000 303 x 406 cans. According to USDA quality grade standards – general requirements, a lot size in this range requires 13 sample units to establish lot grade (Anonymous, 1983). Therefore, a 13 unit sample from each 24-can batch was evaluated for surface color and defects according to USDA-AMS methodology and grade scales. Cans were drained, tomatoes rinsed, and the peel recovered. The quantity of peel adhering to the blossom-end (often referred to as tag-end peel) and the remainder of the fruit was measured graphically according to USDA, AMS methods. In addition, the total areas of discolored and blemished regions were measured by the same method.

Hunter color measurements

Samples of three cans from each treatment were taken for determination of Hunter L, a, b measurements using the Pacific Scientific Spectrogard Color System with spectral reflectance included. The four largest fruit from each can were selected and measurements of L, a, and b were taken on two different surface portions of the equatorial and shoulder regions after the fruit were rinsed in tap water. The set-up parameters for these measurements were: 2° observer angle, Illuminant C, and a 2.2 cm diam viewing port. The instrument was calibrated with the white and black standards provided.

Mold counting procedure

A sample of three cans was taken from each 24-can batch of replicates I and IV for each peeling treatment for the cultivars Peto 95 and Peto 6203. Packing media tomato juice was removed from each can and 50 fields were counted according to established procedures (Anonymous, 1978). The number of positive fields was converted to a percentage.

Data analysis

Analysis of variance and mean separation by Duncan's Multiple Range Test ($p \leq 0.05$) were applied to the data to obtain statistical comparison of treatments.

RESULTS

Percent recovery

Steam peeling for 5 sec resulted in significantly higher % recovery than lye peeling for all cultivars (Fig. 1). Percent recovery for 7 sec steam peel treatments were significantly higher than lye-peeled fruit for Peto 95 and VF 134. Steam peeling for 7 sec resulted in intermediate % recovery for all cultivars and was not significantly different from 5 sec steam-peel or lye-peel for FM 6203 and Peto 76. There was no significant main effect of cultivar on % recovery.

Defects

Quantity of blossom-end peel was not affected by peel treatment (Table 1). There were significant differences among cultivars with VF 134 having the least and FM 6203 having the most adhering blossom-end peel. The quantity of nonblossom-end peel over all peeling treatments averaged 330% greater than blossom-end peel. Steam-peeled (5 sec and 7 sec) fruit averaged seven times greater nonblossom-end peel than lye-peeled fruit. Thus, total peel from steam-peeled fruit was four times greater than lye-peeled fruit even though there was no significant effect of peel treatment on blossom-end peel. However, there was a trend toward a lower quantity of blossom-end peel for lye-treated fruit.

In addition to peel defects, the total quantity of blemish and discoloration defects was also found to be greater in steam-peeled than in lye-peeled product (Table 2). Blemished areas are defined as "abnormal areas that contrast strongly in color and/or texture with normal tomato tissue" and discolored portions refer to "imperfections which may or may not contrast strongly in color with normal

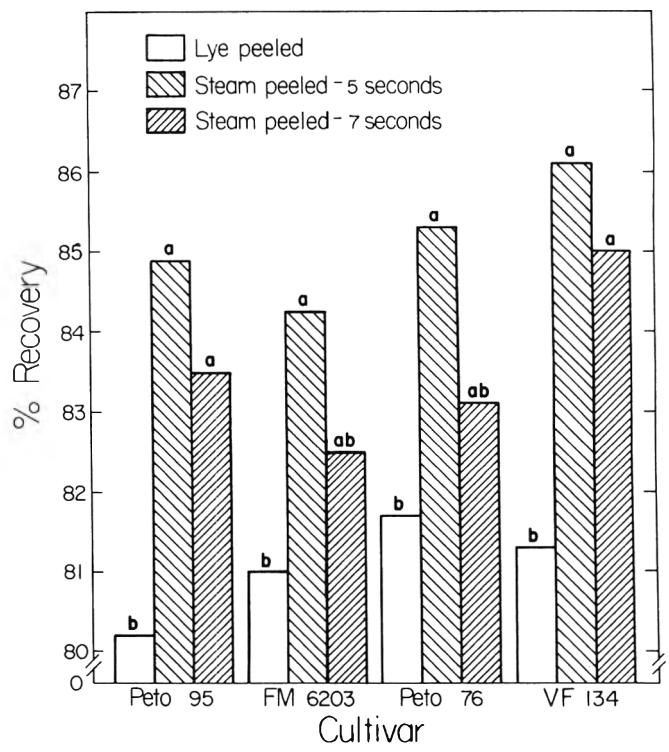

Fig. 1—Percent recovery of four tomato cultivars following lye and steam peeling treatments. Each bar represents the mean of three replications. Comparison of peel treatment means within a cultivar is by Duncan's Multiple Range Test ($P \leq 0.05$).

tomato tissue, but which detract slightly from the appearance" (Anonymous, 1964). Peto 76 and VF 134 contained the least amount of blemish and discoloration.

The average quantity of various defects in a lot sample were converted to U.S. Grades (Table 3). Comparison of the results in Tables 1 and 2 to the grade limits presented in Table 3 reveal that lye-peeled fruit averaged U.S. Grade B for defects and steam-peeled fruit averaged substandard for defects.

Color evaluations

There were no significant effects of peel treatment or cultivar on average USDA color scores (Table 4). All treatment combinations averaged USDA Grade B for color. It is noteworthy that steam-peeled VF 134 fruit had USDA color scores of Grade A.

Analytical color measurements of the equatorial and shoulder regions of all treatments are presented in Table 5. There was a significant ($P \leq 0.01$) effect of surface region on Hunter bL/a values. The overall mean bL/a value for the equator was 7.64 compared to 10.23 for the shoulder. The poorer color value of the shoulder surface tissue is presumably a consequence of the tendency for fruit to demonstrate slower ripening and for a higher concentration of vascular tissue in that region. Peel treatments had no effect on color quality at either region. There were differences among cultivars in equatorial region surface color. VF 134 exhibited superior color having a bL/a value 1.26 units lower than Peto 95. Although there were no differences among cultivars in bL/a values measured on the shoulder regions, VF 134 showed the best color of all cultivars tested.

Mold counts

There was no significant difference in mold counts between cultivars or among the three peeling treatments. The mean percent positive fields for 5 sec steam, 7 sec steam and lye-peeled treatments were 1.0, 1.8 and 0.7, respectively. Peto 95 averaged 1.2% and Peto 6203 averaged 1.1% positive fields. Lye peeling did not significantly reduce mold counts as compared to steam peeling.

Table 1—Quantity of blossom-end peel, nonblossom-end peel and total peel of cultivars of whole canned tomatoes after lye and steam peeling

	Cultivar				Peel treatment
Peel treatment	Peto 76	Peto 95	VF 134	FM 6203	means
	Blossom-end Peel (cm^2/can)[ab]				
Lye	0.50	1.46	0.61	1.89	1.04
Steam 5 s	1.37	2.91	0.15	2.06	1.58
Steam 7 s	0.73	1.22	0.12	4.62	1.40
					NS
Cultivar means	0.87 bc	1.86 ab	0.29 c	2.85 a	
	Nonblossom-end Peel (cm^2/can)[ab]				
Lye	0.79	0.63	1.19	0.99	0.89 b
Steam 5 s	5.29	6.44	6.57	6.45	6.16 a
Steam 7 s	7.23	6.07	4.40	8.07	6.29 a
Cultivar means	4.44	4.38	4.05	5.17 NS	
	Total peel (cm^2/can)[ab]				
Lye	1.29	2.08	1.80	2.88	1.94 b
Steam 5 s	6.67	9.35	6.72	8.51	7.75 a
Steam 7 s	7.96	7.30	4.52	12.68	7.70 a
Cultivar means	5.30	6.24	4.35	8.02 NS	

[a] Values represent means of three replications. Each observation was calculated as the mean of 13 cans. Row means provide for peel treatment means that differ from arithmetic means, because FM 6203 data is based on two replications.
[b] Means within each row or column followed by the same letter are not significantly different ($P > 0.05$).

DISCUSSION

THE TREND IN PEELING METHODS for whole pack processing tomatoes is toward the use of high pressure steam and scalding treatments. These methods are being commercially used in California. However, commercial scale research on the use of steam peeling methods for cultivars adapted to eastern conditions has not been reported. Results of this study indicate that recovery of high (7 atm) pressure, short exposure time, steam-peeled tomatoes is greater than for lye-peeled fruit. However, the efficacy of peel and defect removal by steam peeling was inferior to that attained with lye peeling for the peeling variables employed. This is of major practical importance with regard to industry objectives.

The quantity of defects measured for the steam-peel treatments provided substantial potential for lots to have received USDA substandard grade. It is most important that canned whole tomatoes receive grades not less than U.S. Grade C. The quantity of defects found in the steam-peeled test products would have resulted in substantial substandard grade product in a USDA lot sampling program. In order to achieve greater efficacy of defect removal, and thus attainment of higher U.S. Grade, longer dwell times and/or different steam pressures must be utilized to steam peel these tomatoes. A probable consequence of increased intensity of steam peeling is a decline in percent recovery. However, the potential for recovery of peel and pulp material from steam peeling for subsequent use in sauce and puree manufacture exists. Recovery of such material for use in puree and sauce from lye-peeled fruit is impractical, especially for small eastern canneries. Thus, greater economic gain may be realized with steam peeling even if recovery of whole tomatoes is equivalent to that experienced with lye peeling.

The alleged improvement in the surface color of steam-peeled tomatoes due to a greater quantity of remaining mesocarp tissue subtending the peel than for lye-peeled tomatoes was not confirmed in this work. Although a minor trend toward improved color for steam-peeled tomatoes was noted in the nalytical color data, there was no statistically significant difference. Overall, VF 134 exhibited the best color and least quantity of defects.

Table 2—Quantity of blemish and discoloration defects of four cultivars of whole canned tomatoes after lye and steam peeling

	Blemish and discoloration defects (cm^2/can)[ab] Cultivar				Peel treatment
Peel treatment	Peto 76	Peto 95	VF 134	FM 6203	means
Lye	1.04	0.88	0.74	1.52	1.05 b
Steam 5 s	1.19	2.99	1.85	3.06	2.27 a
Steam 7 s	2.03	2.85	1.60	3.16	2.41 a
Cultivar means	1.42 b	2.24 ab	1.40 b	2.58 a	

[a] Values represent means of three replications. Each observation was calculated as the mean of 13 cans.
[b] Means within each row or column followed by the same letter are not significantly different ($P > 0.05$).

Table 3—Maximum permitted quantities of defect categories for USDA grades of canned tomatoes

	Defects (Mean cm^2/can)		
Grade[a]	Peel	Blemish	Discoloration
A	1.27	0.16	0.64
B	2.54	0.32	1.27
C	2.54	0.64	1.90
Substandard	>2.54	>0.64	>1.90

[a] U.S. Standards for grades of canned tomatoes — effective July 24, 1964.

Table 4—USDA color score points of 4 cultivars of whole canned tomatoes after lye and steam peeling

	USDA color score points[ab]				
	Cultivar				Peel treatment
Peel treatment	Peto 76	Peto 95	VF 134	FM 6203	means
Lye	25.9	25.3	24.9	25.6	25.4
Steam 5 s	26.2	25.5	27.4	25.8	26.2
Steam 7 s	25.5	25.5	27.0	25.9	26.0
					NS
Cultivar means	25.9	25.4	26.4	25.8 NS	

[a] Color score points in the range of 27—30 are classified as USDA Grade A, 24—26 is USDA Grade B, 21—23 is USDA Grade C, and 0—20 is USDA Substandard. Individual sample units falling into the 24—26 range limit the sample grade to USDA Grade B regardless of total score.
[b] Values represent means of three replications. Each observation was calculated as the mean of 13 cans.

Cultivar selection is, therefore, an important consideration for both grower and processor. Additional testing of cultivar response to several steam-peeling variables will be needed.

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Table 5—Hunter bL/a values for different surface regions of four cultivars of whole canned tomatoes after lye and steam peeling

	Cultivar				Peel
Peel treatment	Peto 76	Peto 95	VF 134	FM 6203	treatment means
	Hunter bL/a — Equatorial region[abc]				
Lye	8.13	8.87	7.50	7.43	7.98
Steam 5 sec	7.77	7.90	6.57	7.17	7.35
Steam 7 sec	7.77	7.67	6.57	8.37	7.59
					NS
Cultivar means	7.89 a	8.14 a	6.88 b	7.66 ab	
	Hunter bL/a — Shoulder region[abc]				
Lye	10.50	11.20	9.87	11.27	10.71
Steam 5 sec	10.43	10.73	8.93	10.30	10.10
Steam 7 sec	10.87	9.67	8.20	10.77	9.88
					NS
Cultivar means	10.60	10.53	9.00	10.78 NS	

[a] Values represent means of three replications. Each observation was calculated as the mean of two readings on four fruit from each of three cans.
[b] Hunter bL/a values are inversely proportional to sensory perception of tomato color quality.
[c] Means within each row or column followed by the same letter are not significantly different ($P > 0.05$).

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TTP OF CORN POPPING . . . From page 1414

nel, obtained by linearly extrapolating the straight line portion of temperature history curve which is plotted on semi-log paper (°C)
T_{br} = Temperature at the break point (°C)
T_p = Temperature at the moment the kernel pops (°C)
t = Time variable (sec)
t_{br} = Time at the break point
t_p = Time required for popping (sec)
W_1 = Initial weight in grams of the corn kernels sample
W_d = Weight in grams of the corn kernels sample at the end of the drying time
W_b = Weight in grams of the corn kernels before popping
W_a = Weight in grams of the corn kernels after popping

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A Laboratory and Field Study of the Relationship Between Calcium Sources and Browning in Apple Juice

R. B. SMITH and R. A. CLINE

ABSTRACT

Chloride ions added to freshly pressed apple juice at the rate of 0.2 mg/100 mL significantly slowed browning. Levels of chloride considerably higher than this were detected in juice produced from apples of the cultivar Northern Spy which had been sprayed with $CaCl_2$ (44.8 Kg/ha). Juice containing these chloride spray residues browned at a significantly slower rate than juice from apples off control trees. Chelated $CaCl_2$ sprays applied at considerably lower rates of chloride ion (13.6 and 27.2 Kg/ha of formulation containing 6.0% calcium and 10.1% chloride) and chelated $Ca(NO_3)_2$ (13.6 and 27.2 Kg/ha of formulation containing 6.0% calcium and 18.6% nitrate) did not significantly change the level of browning from that of the control.

INTRODUCTION

IN THE COMMERCIAL PRODUCTION of apple juice, limited levels of oxidative enzymatic browning are desired by many processors and consumers (Luthi, 1953). Polyphenol oxidase catalyzes this oxidative browning process in apple juice (Mathew and Parpia, 1971). Its action is inhibited by ions such as Br^- and Cl^- and by compounds such as SO_2 and ascorbic acid (Joslyn and Ponting, 1951; Taufel and Voigt, 1964). Complete elimination of the oxidative process causes an undesirable greenish color in the juice (Luthi, 1953). Apple juice which had a greenish color and would not brown was a problem for one processor in the Georgian Bay area of Ontario in 1980 (Wilson, K., personal communication). Sprays of $CaCl_2$ applied to apple trees at the appropriate time are effective in the prevention of bitter pit in the fruit (Cline, 1975). The possibility existed that the absence or reduction of browning was due to the use of $CaCl_2$. The objectives of this study were to determine the minimum concentration of chloride ion which would affect the browning process in apple juice relative to chloride ion concentrations present in apple juice produced from apples receiving $CaCl_2$ sprays. The effect of chelated $CaCl_2$ and $Ca(NO_3)_2$ compared to $CaCl_2$ on juice browning was also studied.

MATERIALS & METHODS

Apparatus

Apples used in these investigations were examined after storage at 0°C for intervals of 2-3 wk or 6 months. Upon removal from storage, the apples were spray washed in tap water and rinsed in distilled water. The fruit was milled while cold using the apple mill of a Bucher-Guyer TPZ 7 fruit press. The pulp from 8 kg apples was collected in 30 mesh heavy duty cheesecloth and the juice squeezed by hand to minimize the interval from milling to juicing. Samples of juice from the various treatment combinations were placed in a 20°C water bath. The time between milling and placing in the water bath was standardized to 1.5 min. The color of the juice was measured with a HunterLab D25-3A Color Difference Meter. Duplicate 50 mL samples were placed in sample cups (Agtron sample cup 11595), and the color read through the bottom of the cup using reflected light. The final standardization of the Hunter Color meter was made on the yellow tile, (L = 77.4, a = −2.1, and b = 23.0), the focus was 10.3 mm and the port diameter was 54.0 mm.

Chloride ion determinations were made using the potentiometric method (AOAC, 1975) using 50.0 mL juice and titrating with 0.01N $AgNO_3$. A Fisher model 420 pH/ion meter equipped with Fisher universal glass (16-639-3) and silver billet (16-639-122) electrodes was used for making the determinations. Total titratable acids and pH of the samples were measured using a Metrohm Dosimat titrator (model E415) and pH meter (model E512). The percent soluble solids was determined using an American Optical Model 10450 Abbe type refractometer.

Laboratory tests

Stock solutions of $CaCl_2$, $BaCl_2$, $Ca(NO_3)_2$, C_6H_5OH, CH_3Cl, chelated $CaCl_2$ and chelated $Ca(NO_3)_2$ were prepared. Sufficient quantities of each of these stock solutions were pipeted into flasks to give final concentrations of 5.0, 50.0 and 500.0 mg/L for each source of chloride, nitrate or phenylic acid when diluted with apple juice. A stock solution of HCl was prepared so as to give a chloride concentration of 2.0, 20.0 and 200.0 mg/L. Immediately after pressing, juice from Northern Spy apples, which had not received pre or postharvest treatments of $CaCl_2$, was added to the premeasured stock solutions. The color of these samples was measured after 30 min and, for some selected samples, after 24 hr. The control consisted of equivalent volumes of water. After the same time intervals, six samples of juice from selected treatments were ranked for browning by eight panelists. The panelists were selected from research and technical staff trained in sensory evaluation techniques (Larmond, 1977). The samples of juice for sensory evaluation were placed in Agtron sample cups. The samples were ranked in a neutral, light gray color room with controlled illumination (Fuleki, 1971). The samples, identified numerically and presented in a random display, were ranked from lightest to darkest by the panelists. These rankings were correlated with Hunter L, a, b, a/L, a/b and hue to determine which Hunter Color reading best measured the browning in apple juice. Samples of juice for chloride ion, pH and titratable acidity determinations were frozen at −30°C until analyzed. The effects of selected anions on enzymatic browning were further evaluated using juice from the cultivars Delicious and McIntosh. The data were subjected to a 3-way analysis of variance followed by regression analysis (Steel and Torrie, 1960).

Field tests

Calcium chloride, chelated $CaCl_2$ and chelated $Ca(NO_3)_2$ were applied to the cultivar Northern Spy in an orchard in the Georgian Bay area for two seasons, and for one season to an orchard near Simcoe, Ontario, as well as to several cultivars grown at the Horticultural Research Institute of Ontario, Vineland Station. The $CaCl_2$ was applied at 44.8 kg/ha as recommended for the control of bitter pit (Cline, 1975). The chelated $CaCl_2$ (6% calcium and 10.1% chloride) and $Ca(NO_3)_2$ (6% Calcium and 18.6% nitrate) were applied at 13.6 kg/ha, the rate currently being evaluated for the control of bitter pit (Stoller Chemical Co., Houston, TX) and at 27.2 kg/ha. Since calcium is thought to be more effective in the chelated form in controlling physiological disorders than $CaCl_2$ salt, less calcium was applied when the chelated form was used (Cline, 1975). Samples of apples were randomly selected during the commercial harvest when the firmness of the fruit was at 144 ± 7 KPa (21 ± 1 psi) to determine the amount of chloride ion present and its effect on browning. The color of this juice was determined objectively 30 min and 24 hr after pressing. Samples of juice were frozen for chloride ion, pH, titratable acidity and soluble solids determinations. Randomly selected samples of fruit to which the six field sprays had been applied were ranked for blush. The blush was ranked by panelists under the same conditions of background

Authors Smith and Cline are affiliated with the Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada L0R 2E0.

color and lighting as was used to rank juice browning. Samples from these same trials were subjected to firmness measurements using a Magness-Taylor pressure tester with an 11 mm diameter probe.

RESULTS & DISCUSSIONS

IN A VISUAL EVALUATION of selected juice samples, each panelist ranked the six samples in the same order. Correlations between these visual rankings for color and Hunter color index values L, a, b, a/L, a/b and hue, revealed that there was high correlation between L values and visual rankings for lightness of juice samples. The L values from the instrumental measurements were in the same order for lightness as the visual ranking resulting in a correlation coefficient of 1.0. The correlations between the other readings and visual rankings were not statistically significant. Thus, Hunter L values were used as a measure of browning in apple juice in all experimental evaluations; the higher the L value, the lighter the juice.

The addition of known quantities of chloride ion to fresh pressed apple juice resulted in significantly ($P = 0.01$) less browning in the juice. Since the analysis of variance comparing the experimental means indicated that there were significant interactions, an analysis of variance for regression was performed for each source of ion. This analysis showed that when chloride ion was present, the juice became significantly lighter in color as concentration of chloride ion increased. Samples containing CH_3Cl were discarded since this compound was immiscible and nonionizing and did not retard the browning process. Data obtained from juice to which chelated compounds had been added were not used in statistical analyses because the dark brown color of the formulation imparted an interfering brown tinge to the juice. Data from all other combinations were combined and a regression analysis conducted. There was a significant linear correlation (Fig. 1) between the log of chloride ion concentration and color readings, similar to that found for specific chloride ion sources (Fig. 1). This indicates that each source of chloride ion had an effect on browning; the magnitude was proportional to chloride ion concentration.

The potentiometric titration method used gave good recovery of added chloride ion up to 20 mg/L (Table 1). The discrepancy between added and detected chloride ion increased with levels of chloride when additions exceeded 20 mg/L. Levels of chloride ion found in apples from trees sprayed with $CaCl_2$ were less than 20 mg/L, thus the method used should accurately reflect the quantity of chloride ion present in these fruits. The potentiometric titration method was reviewed by Cantliffe et al. (1970) and was found to be accurate and sensitive. Chloride ion determinations were made on unfiltered samples, a method reported by Cantliffe et al. (1970) to give the highest chloride ion recovery. It is possible that at high concentrations, some chloride ion became fixed by chemical combination with constituents in the juice and thus was not titrated with $AgNO_3$.

Analysis of variance of the data for color of juice from Northern Spy apples showed that there were significant differences between the treatment means for juice from apples receiving various field sprays. Comparison of the means (Table 2) showed that this effect was due to $CaCl_2$ sprays. Similar trends were observed for the cultivars McIntosh and Delicious receiving similar field sprays. The effect was most pronounced at 20 and 30 min after pressing. However, there was still a significant difference in color readings and visual rankings 24 hr after pressing. In several trials, the juice from apples sprayed with chelated $CaCl_2$ was lighter than that from apples from control and chelated $Ca(NO_3)_2$ sprayed trees, but the differences were not statistically significant. Analysis of variance and comparison of the treatment means for chloride content showed trends similar to that for color (Table 2).

When chloride ion concentration was correlated with color readings (Fig. 2) there was a highly significant ($P = 0.01$) relationship between chloride ion levels and color of juice. In contrast to the correlation between levels of added chloride ion (Fig. 1) and color readings, the relationship was linear. After 6 months in storage, the effect of the chloride ion on browning was still present. There appeared to be no alteration of the effect of $CaCl_2$ on browning whether the apples were washed or unwashed before the juice was extracted.

In many of the laboratory experiments, there was a statistically significant replicate effect. Each replicate was a random selection of apples from one tree. Variations in stages of maturation of the fruit as a result of tree and fruit response to small in-orchard and storage factors probably account for the replicate effect (Harel et al., 1966). There were only small differences in the chloride ion content of fruit receiving similar treatments, thus within fruit, chloride ion should not have been a factor contributing to the significance. In some instances, with Delicious, there were significant differences in detected chloride ion among the

Table 1—Effects of adding various quantities of salts on levels of chloride ion and on browning of Northern Spy apple juice

Treatment	Anion added mg/L	Chloride detected mg/L	Browning after 30 min (Hunter L[a])
Control	–	2.4	16.5
Control	–	2.7	15.8
Control	–	3.5	16.7
HCl	2.0	5.2	17.3
HCl	20.0	22.6	17.1
HCl	200.0	179.8	19.9
$BaCl_2$	5.0	8.1	18.1
$BaCl_2$	50.0	47.5	18.6
$BaCl_2$	500.0	438.6	20.4
$CaCl_2$	5.0	8.5	17.0
$CaCl_2$	50.0	50.1	18.5
$CaCl_2$	500.0	445.3	20.6
$Ca(NO_3)_2$	5.0	3.1	16.6
$Ca(NO_3)_2$	50.0	2.8	16.3
$Ca(NO_3)_2$	500.0	2.7	16.9[b]
$CaCl_2$ Chelate	5.0	6.3	17.3
$CaCl_2$ Chelate	50.0	41.5	17.9
$CaCl_2$ Chelate	500.0	449.2	15.0
$Ca(NO_3)_2$ Chelate	5.0	3.8	16.7
$Ca(NO_3)_2$ Chelate	50.0	2.8	15.4
$Ca(NO_3)_2$ Chelate	500.0	3.8	11.4

[a] Lower Hunter L values represent more browning.
[b] Chelates added color.

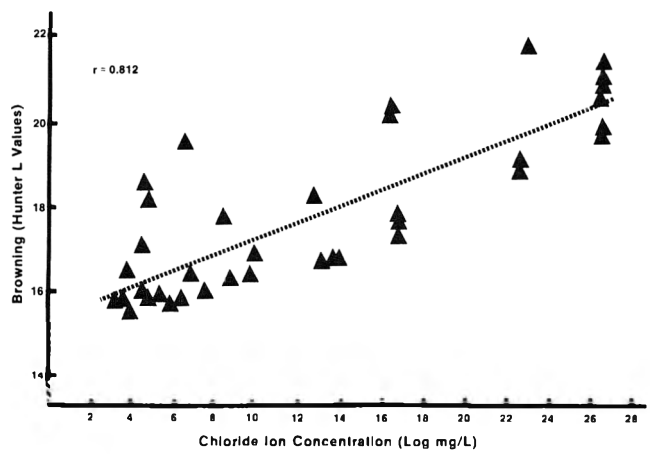

Fig. 1—Effect of added chloride ion on browning of apple juice.

Table 2—Levels of chloride ion and browning in Northern Spy apple juice produced from trees which had received field sprays of $CaCl_2$ and chelated $CaCl_2$ and $Ca(NO_3)_2$

Treatment	Rates applied Kg/ha	Chloride ion applied Kg/ha	Chloride ion detected mg/L	Browning (Hunter L[a]) 30 min	Browning (Hunter L[a]) 24 hr
$CaCl_2$	44.8[b]	28.7	11.8a[c]	29.10a[c]	21.07a[c]
$CaCl_2$ chelated	13.6	1.4	7.0b	23.90b	17.71b
$CaCl_2$ chelated	27.2	2.7	6.7b	23.32b	18.27b
$Ca(NO_3)_2$ chelated	13.6	0.0	6.5b	23.91b	19.04b
$Ca(NO_3)_2$ chelated	27.2	0.0	5.4b	22.90b	18.33b
Control	0.0	0.0	6.1b	22.72b	18.20b

[a] Lowest Hunter L values represent most browning.
[b] $CaCl_2$ — 36% calcium, 64% chloride; chelated $CaCl_2$ — 6% calcium, 10.1% chloride; chelated $Ca(NO_3)_2$ — 6% calcium, 18.6% nitrate.
[c] Nonorthogonal single degree of freedom comparisons: Means followed by the same letter are not significantly different at 0.01 probability level.

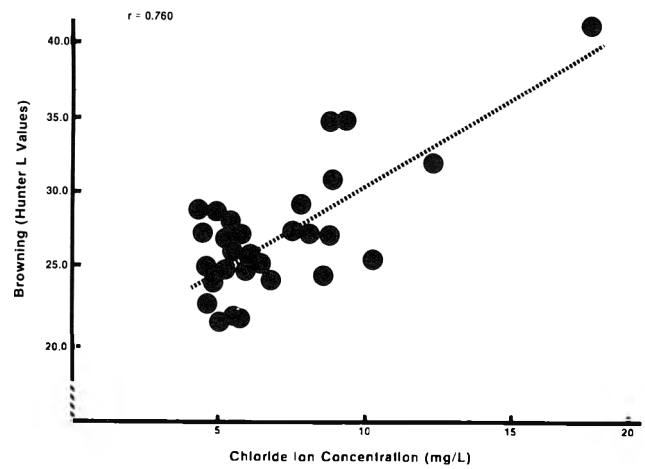

Fig. 2—Concentration of chloride ion detected and level of browning in juice from apples which had received field sprays of $CaCl_2$, chelated $CaCl_2$ and chelated $Ca(NO_3)_2$.

replicates when chloride was applied to trees in the orchard. Where there were significant differences among replicates, the concentration of chloride ion between replicates in the control varied considerably. In some instances, the natural chloride content of the fruit was at a level found to be inhibitory to browning in laboratory experiments.

This study confirmed that if chloride ion is added to fresh pressed apple juice, enzymatic browning will be suppressed. The application of $CaCl_2$ as a field spray for the control of bitter pit had essentially the same effect on chloride ion levels in the juice and on browning as adding chloride ion after pressing. The chloride ion was present in the juice from the application of $CaCl_2$ in the field. The average amount of chloride ion found in the Northern Spy juice samples which had received $CaCl_2$ sprays was 11.8 mg/L compared to 6.1 mg/L in the control. This level of chloride ion is within the concentration range found to inhibit browning when chloride ion was added to juice. The level of chloride ion found in juice from some nonsprayed Northern Spy apples was in a range of concentration found to have an effect on browning when chloride was added to juice. It is possible that levels of chloride ion naturally occurring in soils could vary and may effect the range of concentration found in fruit from different orchards and thus, rates of oxidation.

The form of the response curve to chloride ion in the juice was not similar when chloride ion was added as compared to that absorbed by apples from field sprays (Fig. 1 and 2). It is possible that this change in response was caused by differences in maturity since the rate of browning of apple slices decreases as the fruit ripens (Harel et al., 1966). Also, levels of browning in apple juice can be altered by factors such as temperature and pH (Joslyn and Ponting, 1951). Throughout this investigation, the holding temperature for all juice samples was 20°C. Except for the addition of HCl which changed the pH and acidity slightly, none of the added salts of field sprays caused a change in these variables. A more likely explanation for the different response is the range of concentration of chloride ion found in the juice when chloride ion was added compared to that absorbed through field sprays. A linear relationship can exist between two variables when compared over a limited range and a nonlinear response can be obtained when these same variables are compared over a broader range (Steel and Torrie, 1960). These situations existed in this investigation.

The use of chelated $CaCl_2$ sprays does reduce the amount of chloride ion applied and found in the juice compared to $CaCl_2$ salt. With both levels of applied chelated calcium chloride there was a slight increase in the levels of chloride ion in the juice when compared to the control (Table 2). The increase was not proportional to the amount of chelated $CaCl_2$ applied. The juice was slightly lighter in color than the control in some instances, but the differences were not significant. Higher rates of application would probably have caused a significant increase in chloride ion in the juice but higher levels of chelated calcium were not needed for the control of bitter pit. Ranking apples from the six different field treatments for blush development showed that those samples receiving chelated $Ca(NO_3)_2$ were always ranked in the four lowest places, never in the top two. This could be an effect of the increased nitrogen levels. Cline and Hutchinson (1970) have reported increased storage breakdown in apples receiving $Ca(NO_3)_2$ spray applications. Sprays of chelated $Ca(NO_3)_2$ could be used in place of chelated $CaCl_2$. However, because of the potential for loss of quality, chelated $Ca(NO_3)_2$ should be used with caution. In this investigation, the applied sprays had no effect on firmness or soluble solids level in the fruit at the time of harvest. It has been shown (Bramlage et al., 1979) that apples which have received sprays of $CaCl_2$ are firmer after several months of storage than apples which have not received these sprays.

Where $CaCl_2$ salt is used for the prevention of physiological disorders in apples, there would likely be only slight effects on browning of the juice as long as the sprayed fruit constitutes no more than 20% of the total apple volume. The concentration of chloride ion in the juice from apples sprayed with $CaCl_2$, which ranged as high as 19 mg/L (Fig. 2) should be diluted to 6.2-9.9 mg/L range. At this level of chloride ion, processors may have to hold their juice for several hours before processing to give at least a slightly oxidized color. Alternatively, processors desiring a light colored juice could achieve this by using a high percentage of $CaCl_2$ sprayed apples.

Calcium is used for the prevention of physiological disorders in a number of apple cultivars (Cline, 1975; Mason et al., 1974; Shear, 1975; Drake et al., 1979). If chloride ion residue is a problem for processors, $CaCl_2$ and $Ca(NO_3)_2$ chelates offer alternate sources of calcium.

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Control Studies in an Industrial Apple Juice Evaporator

J. E. LOZANO, M. P. ELUSTONDO, and J. A. ROMAGNOLI

ABSTRACT

Real plant data were processed by using two available system identification techniques and the applicability and limitations of such techniques in an industrial scale unit were discussed. Empirical dynamic models obtained were used to compare alternative control configurations. Predictive strategies to compensate for delays are recommended and the use of secondary measurements with an adaptive set point configuration is proposed for future implementation.

INTRODUCTION

PROCESS CONTROL is being increasingly adopted in the food industry to produce better products at a lower cost, particularly on operations and equipment involving high energy consumption.

Many food industries rely heavily on evaporation as the concentration operation to facilitate product storage, preservation and transportation. Steam economies vary significantly among industries as different types of evaporators with various numbers of effects are utilized. Upgrading of these evaporators with minor investments such as additional insulation, major capital outlays for effects or development of alternative control strategies could have an effect on energy consumption (Frost, 1977; Carter and Chen, 1980; Carter and Chen, 1982). Using a triple-effect evaporator in a simulated commercial-scale application (Chen et al., 1982) it was found that computer control reduced energy consumption by 17%.

The purpose of this study was to obtain empirical dynamic models for an existing industrial juice evaporator and to implement alternative control configurations, comparing conventional control loops with more sophisticated structures based on predictive strategies.

The authors are affiliated with Planta Piloto de Ingenieria Quimica (UNS-CONICET), 8000 Bahia Blanca, Argentina.

MATERIAL & METHODS

Description of the industrial unit

A schematic diagram of the industrial scale triple effect evaporator is shown in Fig. 1. The process variables are also shown in the figure and their nominal steady-state values (during the tests) are given in Table 1. The evaporator is normally operated with a feed stream of approximately 9360 (L/hr) with a 16.3°Brix stripped juice. The feed is preheated by passing through the heater shells before going into the first effect. Each effect has the configuration of a shell and tube arrangement with two passes in the tube and one pass in the shell, the juice being the tube fluid while steam (or vapor) goes through the shell. Product cooling is accomplished in three plate heat exchangers. The existing control system consists of a single loop feedback of the steam pressure (first body) using a PI controller.

System monitoring and data collection

Temperatures and pressures were measured by the existing indicators located in the evaporator as shown in Fig. 1. Final concentration indication and transducing were carried out by means of an on-line refractometer mounted between flanges in the product line just

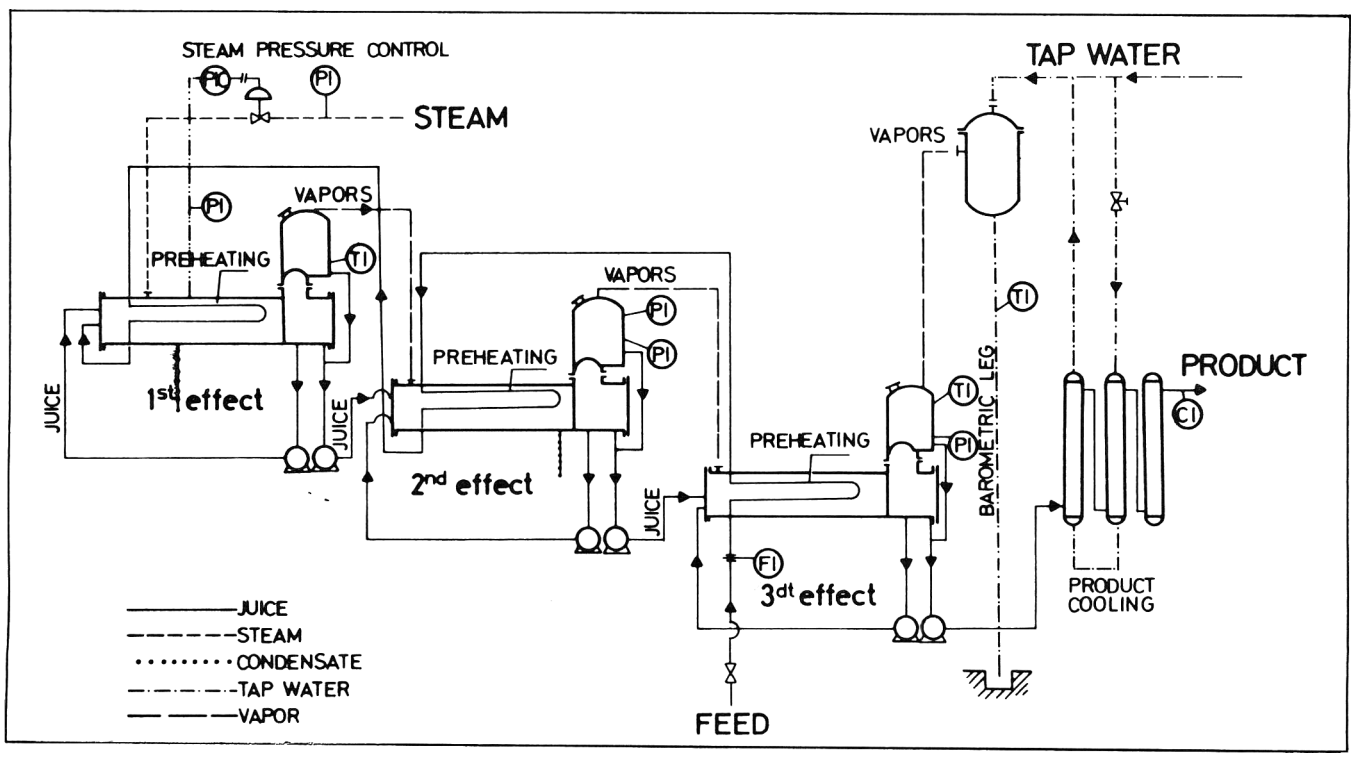

Fig. 1—Schematic diagram of the industrial scale triple effect evaporator.

after the cooling operation. Concentration changes were continuously recorded as °Brix (±0.1°Brix) in a strip-chart recorder.

Steps and pulse signals were introduced through the steam pressure controller set point and the main feed valve. More than 20 steps and pulses, different in magnitude and duration, were run. Test were carried out with a width of the pulses of less than 50-60% of the smallest time constant of the process. Steps were up to 0.3 kg/cm^2 for the steam pressure and 10% for the feed flow rate. Estimated errors were 1% and 5%, respectively.

System identification

Two experimental techniques were applied: (1) a graphical approach on a step response of the industrial unit; (2) pulse testing, a frequency domain method which yields the frequency diagram of the open loop process.

Step-test data method

This technique consists of submitting the process, while it is operating under steady-state conditions with the feedback controller in manual, to a step change in input and recording the resulting transient response. The plot of the step response of an open-loop process versus time is called a process reaction curve. The plot of the typical process response corresponding to the industrial evaporator (for a step in the steam pressure) under analysis is shown in Fig. 2. The curve is the classical over-damped second order plus dead-time system whose transfer function is given by (Stephanopoulos, 1983):

$$G_p(s) = \frac{\exp(-\tau_d s)\, K_p}{(\tau_1 s + 1)(\tau_2 s + 1)} \tag{1}$$

where $G_p(s)$ is the soluble solids content (response) to the steam pressure (perturbation) ratio.

On the other hand the response for a step in the feed flow rate was under-damped and followed an equation of the type (Stephanopoulos, 1983),

$$G_p(s) = \frac{\exp(-\tau_d d)\, K_p}{(1/\omega_n^2)\, s^2 + (2\xi/\omega_n)\, s + 1} \tag{2}$$

The objective is to determine the three parameters τ_D, τ_1 and τ_2 or τ_D, ω_n and ξ from the reaction curve.

Most of the available methods for parameter estimation of second order models depend on the accurate location of the inflection point. Here a methodology will be considered to overcome this difficulty. As pointed out by Sundaresan et al. (1978) the first moment of the response function is:

$$m_1 = -\left.\frac{d\, G_p(s)}{ds}\right|_{s=0} = \tau_d + \tau_1 + \tau_2 \tag{3}$$

In Fig. 2, m_1 is the shaded area. Differentiating the time domain solution of Eq. (1)and (2) twice, and setting the resulting second derivative to zero, the following expressions are obtained:

$$(t_m - m_1)\, M_i = \begin{cases} \dfrac{\eta^{1/(1-\eta)}}{(\eta-1)} \ln \eta & \text{if } \xi \geqslant 1 \\ \dfrac{\cos^{-1}\xi}{(1-\xi^2)^{1/2}} \exp\left[-\dfrac{\xi}{(1-\xi^2)^{1/2}} \cos^{-1}\xi\right] & \text{if } \xi < 1 \end{cases} \tag{4}$$

where

$$M_i = \eta^{1/(1-\eta)}/(\eta-1)\alpha \tag{5}$$

$$\alpha = \tau_1\tau_2/(\tau_1 - \tau_2) \tag{6}$$

Table 1—Normal steady state values during experiment

	Conc c (°Brix)	Feed flow rate Q (1/h)	Temp T (°C)	Pressure P (K/cm^2)
Feed	16.3	9,360	45	–
First body	23.2	–	103	0.45
Second body	36.8	–	82	–0.20
Third body	72.0	1,179	44	–0.80

$$\eta = \tau_1/\tau_2 \tag{7}$$

As Fig. 2 shows, M_i is the slope of the tangent that passes through the point of inflection and intersects the fmal value of the response at time t_m. Parameter η (or ξ) can be evaluated by trial and error on Eq. (4).

The equations resulting from the application of Eq. (3) to (7) for the parameters under consideration are:

$$\tau_1 = \eta^{[\eta/(1-\eta)]}/M_i \tag{8}$$

$$\tau_2 = \eta^{[1/(1-\eta)]}/M_i \tag{9}$$

$$\tau_D = m_1 - \eta^{1/(1-\eta)}\, \frac{(\eta + 1/\eta)}{M} \tag{10}$$

$$\omega_n = \frac{\cos^{-1}\xi}{1 - \xi^2 (t_m - m_1)} \tag{11}$$

$$\tau_D = m_1 - \frac{2\xi}{\omega_n} \tag{12}$$

Pulse testing method

Unlike the reaction curve method, this approach is a frequency domain method yielding a frequency response diagram of the open-loop process. In the pulse-testing method, a pulse of arbitrary shape is applied at the input to the process [F(s)], while it is operating under steady state conditions, and the transient of the process is recorded. The Laplace transform of the input F(s) is related to the Laplace transform of the output C(s) via the model transfer function G(s); thus,

$$G(s) = \frac{C(s)}{F(s)} = \frac{\int_0^\infty C(t)\, e^{-st}\, dt}{\int_0^\infty F(t)\, e^{-st}\, dt} \tag{13}$$

If "ω" is substituted for "s" this relationship can also be expressed as

$$G(j\omega) = \frac{\int_0^{t_c} C(t) \cos\omega\, dt - j \int_0^{t_c} C(t) \sin\omega t\, dt}{\int_0^{t_c} F(t) \cos\omega\, dt - j \int_0^{if} F(t) \sin\omega t\, dt} = \frac{A - j\,B}{C - j\,D} \tag{14}$$

Where numerator and denominator are the Fourier transformation of C(t) and F(t) respectively, or equivalently

$$G(j\omega) = \mathrm{Re}\, G(j\omega) + j\, \mathrm{Im}\, G(j\omega) \tag{15}$$

From this equation the amplitude ratio AR and the phase angle ϕ are readily obtained as,

$$AR = G(j\omega) = \left\{\left(\frac{AC + BD}{C^2 + D^2}\right) + \left(\frac{AD - BC}{C^2 + D^2}\right)^2\right\}^{1/2} \tag{16}$$

and

$$\phi = \arctan\left(\frac{AD - BC}{AC + BD}\right) \tag{17}$$

A computer program based on these equations was developed (Tonelli and Romagnoli, 1982) for analyzing a pulse-test data. The

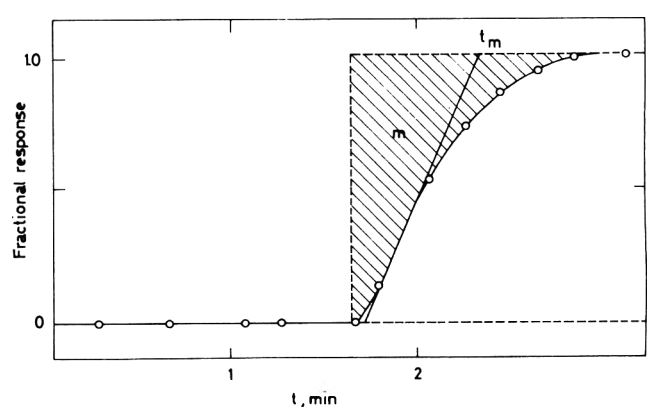

Fig. 2—Process reaction curve for a step in the feed flow rate.

program performs the numerical integration of the integrals appearing in Eq. (14) and yields different values of A, B, C and D for several frequencies (ω). The complete frequency response diagram can be prepared from these. The program also fits the points to a curve using a nonlinear regression routine.

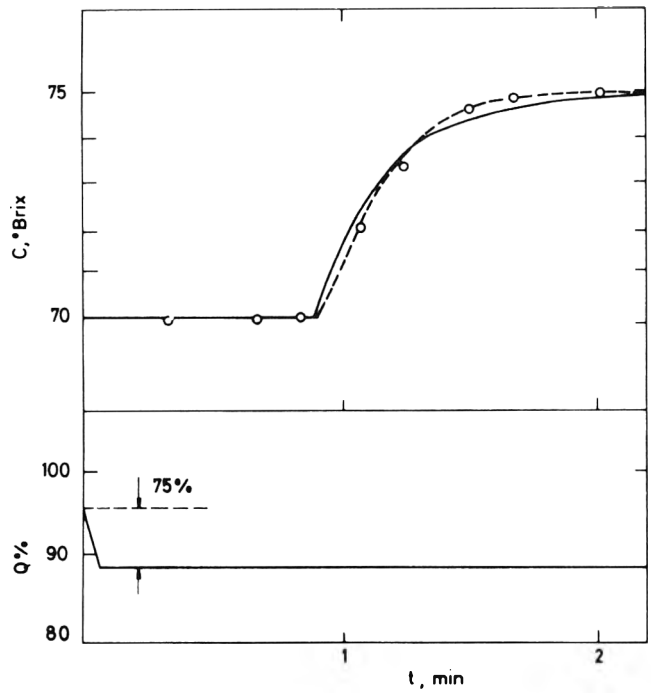

Fig. 3—Comparison between predicted response and the actual plant data for a step in the feed flow rate Q(7.5% negative step). ○, Experimental data; ---, Pulse test method; ——, Reaction curve method.

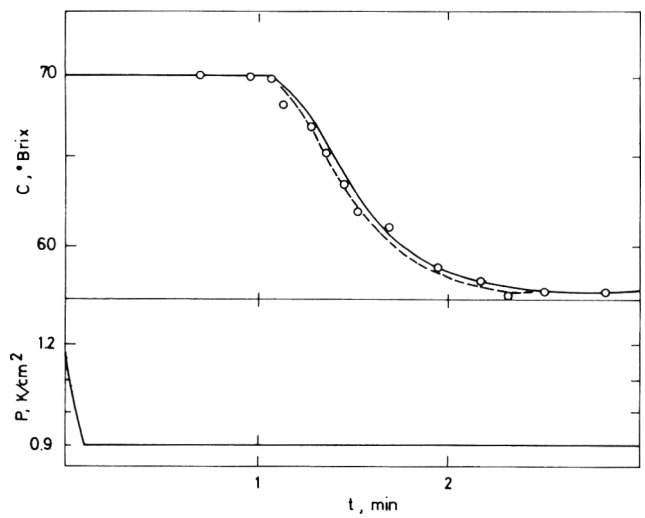

Fig. 4—Comparison between predicted response and the actual plant data for a step in the steam pressure P(0.3 kg/cm^2). ○, Experimental data; ---, Pulse test method; ——, Reaction curve method.

RESULTS & DISCUSSION

EQUATIONS representing the temporal response of the evaporator can be expressed as:

For a step change in the steam pressure:

$$C(t) = C_o + K_p P\left[1 + \frac{\tau_1}{(\tau_2 - \tau_1)} e^{-(t-\tau_D/\tau_1)} - \frac{\tau_2}{(\tau_2 - \tau_1)} e^{-(t-\tau_D/\tau_2)}\right] \quad (18)$$

where P = steam pressure.

For a change in the feed flow rate:

$$C(t) = C_o + K_p Q\left[1 - \text{Exp}\left(\frac{t-\tau_D}{\omega_n}\right) \xi \cdot \{*\}\right]$$

where

$$\{*\} \frac{\xi}{(1-\xi^2)^{1/2}} \sin[(1-\xi^2)^{1/2}\omega_n (t-\tau)] + \cos[(1-\xi^2)^{1/2}\omega_n (t-\tau_D)] \quad (19)$$

and Q = Feed flow rate.

Eq. (18) and (19) were obtained by anti-transforming Eq. (1) and (2). Fig. 3 and 4 illustrate the comparison between the predicted responses given by Eq. (18) and (19) and the actual plant data obtained by experiments. In these figures only a few experimental data obtained from a single step in the feed flow rate and in the steam pressure are shown. In Table 2 additional values obtained through the computation of several runs, not plotted here, are also given. As can be readily seen, the model represented by Eq. (18) and (19) with the conditions given in Table 1 follows very closely the behavior of the real system for the given operating conditions.

Fig. 5 illustrates the comparison between the predicted response for a change in the vapor pressure obtained with

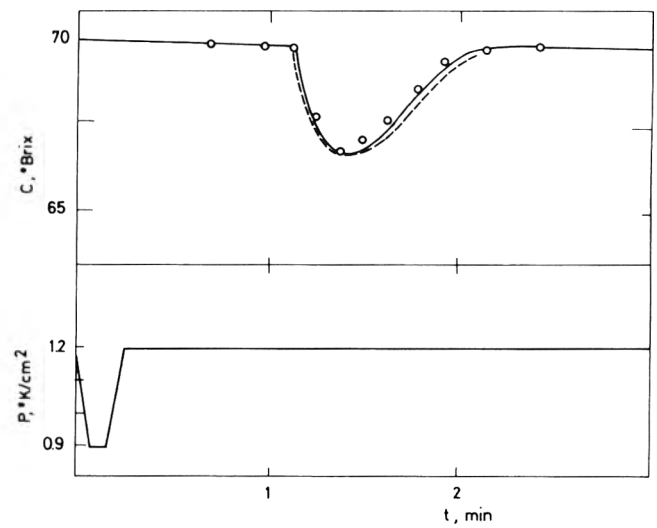

Fig. 5—Comparison between predicted and actual response for a pulse in the vapor pressure. ○, Experimental data; ---, Pulse test method; ——, Reaction curve method.

Table 2—Estimated model parameters resulting from the application of the identification techniques

Inlet	Method	K_p	τ_1 (sec)	τ_2 (sec)	τ_d (sec)	ξ	ω_n (sec^{-1})
Steam pressure	Reaction curve	34.7	9.56	12.60	64.3	–	–
Feed rate	Reaction curve	1.2	–	–	54.36	0.74	0.084
Steam pressure	Pulse test	34.7	12.0	17.60	64.2	–	–
Feed rate	Pulse test	1.2	–	–	55.02	0.79	0.080

the pulse analysis program and the actual experimental data.

In general, the test with the step function is easier to perform than the pulse test. In both cases, however, it is necessary to find the appropriate magnitude of the change since a model was used which was linearized around the steady state operating level from a process which is inherently nonlinear. If the change is too large, the process may be driven out of the linear range. Therefore, several changes, different in height and sign, were tried to detect nonlinearities in the response.

In the case of the pulse method, the shape of the pulse should be such that its frequency content, i.e. amplitude of the FIT (Fourier Integral Transform), should be finite over the frequency range of interest. For our system, it was only possible to perform rectangular pulses. The FIT of a rectangular pulse of width D is:

$$FIT = (h \sin \omega D)/\omega - i\frac{h}{\omega}(1 - \cos \omega D)$$

when the frequency $\omega = 2\pi/D$, FIT goes to zero and the calculation of the transfer function is meaningless. Therefore, the smaller D can be made, the higher is the frequency to which $G(j\omega)$ can be found.

Another practical consideration is that in general, it is easier to operate on the vapor pressure rather than on the feed flow rate. The latter, for this kind of process, is usually set to the maximum value to work at full production. On the other hand, the response of the vapor pressure (steam) closed loop is dynamically more favorable (faster response) making it possible to obtain an input signal closer to the ideal.

Control studies

Alternative schemes will be presented for the automatic control of the industrial unit. The results of these comparisons provide new elements for further improvements and in selecting the correct configuration.

Control configuration using conventional feedback refractrometric loop

The model transfer function developed between the inlet steam and product concentration, according to the previous sections, was used to develop the conventional control system shown in Fig. 6. The empirical model was programmed in a PDP 11/70 digital computer and an attempt was made to determine suitable P + I controller constants by simulating the closed loop system. For the purpose of this study, we simulated the frequency response, with a Ziegler and Nichols setting of the controllers, from which it was assimilated to an overdamped second order system.

There is an important measure for the attainable control quality when time delays are present, which is called the controllability ratio (ν) defined according to the equation.

$$\nu = \frac{\tau_D}{\tau_p} \qquad (20)$$

where $\tau_p = (\tau_1 + \tau_2)$

for the second order model.

For properly tuned loop gain ($K_c\ K_p$) can be plotted as a function of ν. This loop gain decreases as ν increases and for large values of ν approaches 0.45 for loops tuned by the Ziegler-Nichols method. In our case, since $\nu = 2.89$, the loop gain should be very low ($K_c\ K_p = 7.23$) which, for a constant process gain ($K_p = 34.7$), allows a very low admissible control gain. For this value of ν the settling time approaches $9\tau_d$. These characteristics show "a priori" that poor control can be expected if conventional strategies are to be used.

The scheme shown in Fig. 6 was implemented and tuned, and Fig. 7 gives typical responses of this simple arrangement under a load and set point changes. It can be readily seen that the system is controllable but oscillatory when the disturbance is important even for very low values of the gains. This is mainly due to the inherent time delay associated with the process.

Since the juice goes to an accumulator tank, the error introduced can be compensated in the final mixture. However, this results in a waste of energy and decreases production. Consequently, alternative schemes have to be considered.

Control configuration using predictive strategies

Since in this case the process exhibits apparent dead time characteristics and since dead time has been shown to be detrimental to control, there is an incentive to analyze the behavior of the system with control algorithms that can compensate for such delays.

The best known of the time delay compensation techniques currently in use is the Smith (1957) predictor technique. Few experimental investigations have demonstrated that the SP can result in significant improvements over conventional PID controllers. A block diagram of the SP is shown in Fig. 8 where $G_c(s)$ is a conventional PI or PID controller. In this case they can be tuned much more

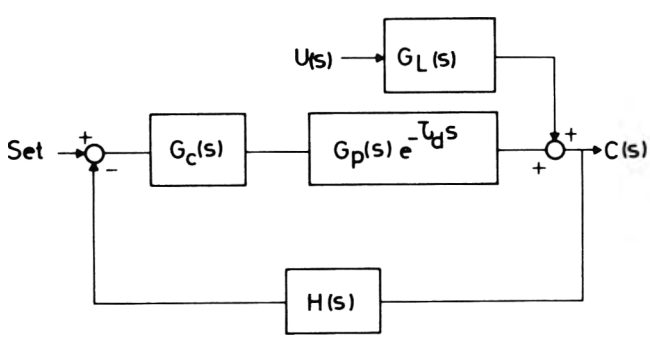

Fig. 6—Conventional feedback refractrometric loop.

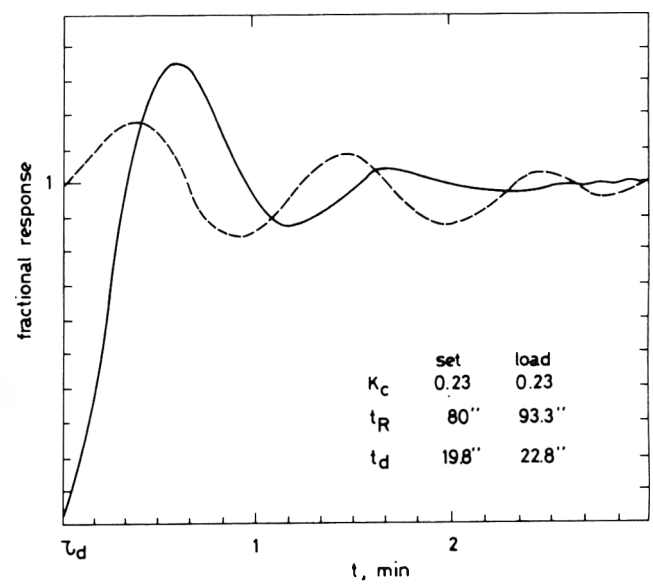

Fig. 7—Response for a change in the set point (——) and load (-----) for the conventional control loop of Fig. (6).

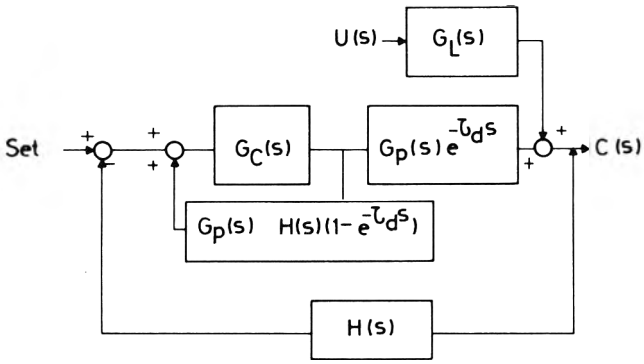

Fig. 8—Block diagram for a "SP" control configuration.

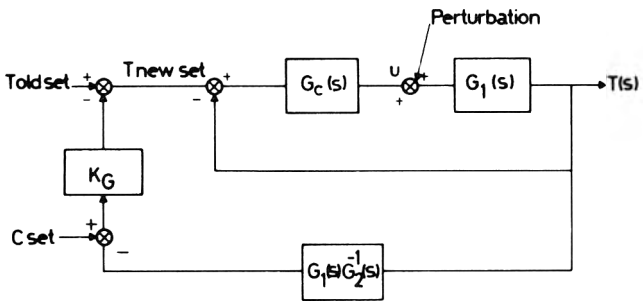

Fig. 10—Block diagram of the proposed control alternative.

tightly because of the elimination of dead time from the loop.

For the feedback control system shown in Fig. 8 the closed loop transfer function for load disturbances is

$$\frac{C(s)}{U(s)} = G(s) = \frac{G_c\, G_p\, G_L\, e^{-\tau D}}{1 + G_c(s)\, G_p(s)\, H(s)} \qquad (21)$$

The characteristic equation for this system is now

$$1 + G_c(s)\, G_p(s)\, H(s) = 0 \qquad (22)$$

which is also the characteristic equation for the system in Fig. 6 when the time delay is zero. Thus by using the SP the time delay has been eliminated from the characteristic equation and consequently larger controller gains may be used.

Simulations were conducted for a step change in set point and a typical result is shown in Fig. 9. As it can be readily seen the SP gives substantial improvement over the classical PID controller when used for controlling the process.

Alternative control configuration

An alternative control configuration can be implemented using the easily available temperature or pressure (at any of the stages) in an inner loop and concentration measurements in an outer loop (done in a different time scale) to check periodically the set points of the control variables. If it is assumed that the temperature or pressure and composition measurements are possible on a different time scale, two functions will be available, i.e.

$$T(s) = G_1(s)\, U(s) \qquad (23)$$

$$C(s) = G_2(s)\, U(s) \qquad (24)$$

A control scheme can now be developed where temperatures or pressures will be controlled in an inner loop and concentration (implicitly) in an outer loop by adjusting the set points of the temperature or pressure controller. The

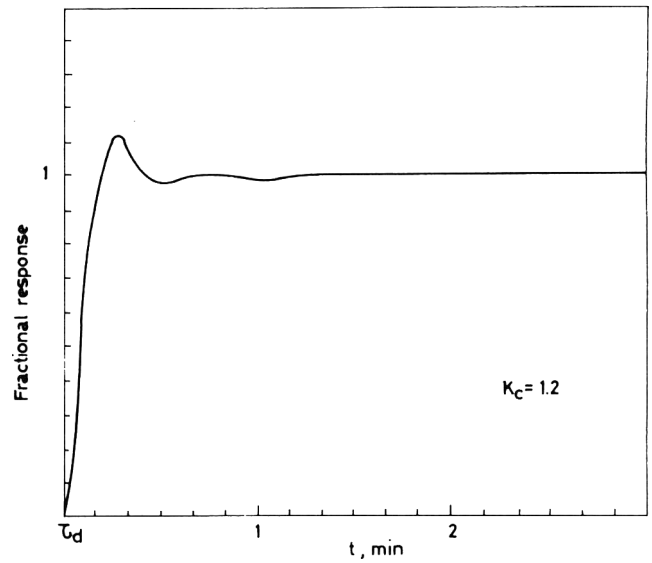

Fig. 9—Response obtained using predictive structures (change in the set point).

block diagram of this scheme is illustrated in Fig. 10.

Furthermore, from Eq. (23) and (24) a direct relation between temperature and concentration can be obtained as follows:

$$T(s) = G_1(s)\, G_2^{-1}(s)\, C(s) \qquad (25)$$

Thus, when composition measurements are not available, the above relation can be used to predict the composition which can then update the set point for the temperature controller. Currently, work is underway to implement this adaptive setpoint configuration in the context of a direct digital control of the unit.

NOMENCLATURE

A	Integral defined in Eq. (14)
AR	Amplitude ratio
B	Integral defined in Eq. (14)
c	Concentration, °Brix
C	Integral defined in Eq. (14)
CI	Soluble solids content indicator
C(s)	Output of the process.
D	Integral defined in Eq. (14)
FI	Flow rate indicator
F(s)	Input to the process
G(s)	Transfer function (closed loop)
H(s)	Transfer function for the beed back transmitter
Im	Imaginary part
j	$\sqrt{(-1)}$
K	Steady state gain
L(s)	Transfer function for load disturbance
m_1	First moment of the response function, defined in Eq. (3)
M_i	Slope, defined in Eq. (5)
P	Pressure, kg/cm^2
PI	Pressure indicators
PIC	Proportional integral controller
PID	Proportional integral derivative controller
Q	Feed Flow rate, % maximum
Re	Real part
s	Laplace operator
SP	Smith predictor
T	Temperature
TI	Temperature indicator
T(s)	Laplace transformation of T
t	Time, sec.

t_m Time to reach final value at maximum slope, sec
U(s) Load to the process

Greek letters

α Defined in Equation (6)
η Time constant ratio, defined in Equation (7)
ξ Damping coefficient
ν Controllability ratio, defined in Equation (20)
τ_d Time constant, sec
τ Dead time, sec.
ϕ Phase angle, defined in Equation (18)
ω Frequency rad/sec.

Subscripts

c Controller
d Derivative
G Inlet to outlet loop gain ratio
o Steady state
p Process
R Reset (integral action)

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Kinetics of Hydrolyses of Sorghum Molasses with Dilute Mineral Acids and Oxalic Acid and Melibiose with Oxalic Acid

SUI-SHENG ZHONG, L. T. FAN, and R. W. WISECUP

ABSTRACT

Investigations were carried out on the kinetics of hydrolyses of sorghum molasses with dilute mineral acids and oxalic acid and melibiose with oxalic acid at relatively high temperatures. Kinetic equations for hydrolysis of sorghum molasses and melibiose have been derived from the experimentally determined hydrolysis rate constants as functions of the acid concentration and temperature. It has been shown that the hydrolysis activity of oxalic acid is weaker than those of hydrochloric and sulfuric acid, and that the second hydrogen atom in oxalic acid does not significantly participate in hydrolysis.

INTRODUCTION

SORGHUM MOLASSES is a mixture consisting of sucrose, glucose, and fructose in ratios of roughly 2:1:1. Unfortunately, kinetic studies of direct hydrolysis of sorghum molasses with both mineral and organic acids have not been reported. If the hydrolysis of sorghum molasses is controlled in such a way that glucose and fructose do not decompose appreciably, the rate of hydrolysis of sorghum molasses can be considered essentially as that of sucrose. It has been reported that the hydrolysis rate of sucrose possibly changes at a concentration of hydrochloric acid greater than 5.68M (Gorokhov et al., 1976, 1977). A hydrolysis isocatalytic point where the effects of H^+ and OH^- are equal was also observed when the pH value of the hydrolysis medium was nearly 8. Decomposition of the inverted sugars at this point increased about two to four times with each 10°C increase in temperature (Sapronov and Koltscheva, 1975). Actually, the decomposition (Seaman, 1945; Shaw et al., 1967; Velasco and Dowling, 1975; Kuster and Temmink, 1977) and condensation (Boguslaw, 1978) occurred under almost any hydrolysis condition; and thus, flavored and colored compounds are produced. In syrup production, therefore, the temperature and acid concentration should be selected so that the decomposition of invert sugars be minimized (Fleming and GrootWassink, 1979).

Both sucrose and melibiose are disaccharides. Sucrose, consisting of glucose and fructose linked with a glucosyl-fructosyl bond is sensitive to acids; sucrose is inverted as illustrated in Fig. 1 (Junk and Pancoast, 1973). Melibiose, consisting of galactose and glucose linked with a galactosyl-glucosyl bond, is hydrolyzed by acids to galactose and glucose as illustrated in Fig. 2; however, this bond is highly resistant to hydrolysis action (Moelwyn-Hughes, 1929). It has been reported that the rate of G-F bond destruction was approximately 955 times greater than that of Ga-G bond destruction, when hydrolysis was carried out in a solution of hydrochloric acid (Moelwyn-Hughes, 1934; Szejtli et al., 1970).

Mineral acids, such as hydrochloric and sulfuric acids, are most frequently used for the hydrolysis of biomass because of their effectiveness and low cost. According to Conti (1953), the ash contents in the final product were higher with hydrochloric acid than with other acids. Organic acids, such as citric, tartaric, and oxalic acids, have also been used for syrup production (Conti, 1953; Sandkuhl and Halbach, 1957; Grandel and Neumann, 1958).

According to Szejtli et al. (1970), the study of acid-catalyzed hydrolysis of sucrose was first carried out in the middle of the 19th century. The rates of hydrolysis of sucrose with different acids were measured by many investigators (Armstrong and Wheeler, 1911; Worley, 1911; Hantzsch and Weissberger, 1927; Kautz and Robinson,

The authors are affiliated with the Dept. of Chemical Engineering, Kansas State Univ., Manhattan, 66506. Author Zhong is on leave from the Dept. of Chemical Engineering, Taiyuan Inst. of Technology, China.

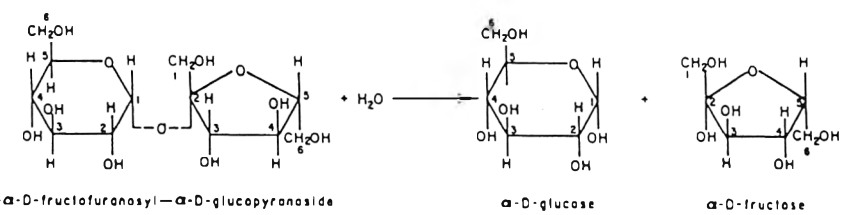
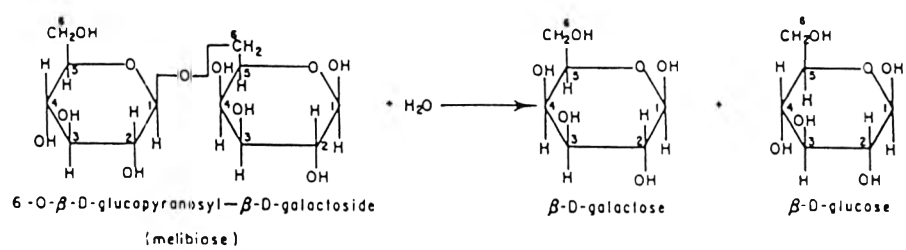


Fig. 1—Products of sucrose hydrolysis (Junk and Pancoast, 1973).

Fig. 2—Products of melibiose hydrolysis (Moelwyn-Hughes, 1929).



1928; Timell, 1964; Narasaraju and Rao, 1972; Yamaguchi and Kawabuchi, 1974; Moiseev et al., 1976). A staircase nonisothermal method was used to measure the rates of hydrolysis of sucrose (Pang et al., 1981). To correlate the hydrolysis rate with acid concentration in the medium, a hypothesis has been proposed (Hammett and Deyrup, 1932; Hammett and Paul, 1934), postulating that the rate of acid-catalyzed hydrolysis of sucrose in a strong mineral acid is closely dependent on the so-called Hammett acidity function, H_o,$\{[H_o = -\log (a_{H^+})(f_B)/(f_{BH^+})\}$ where a_{H^+} is the hydrogen-ion activity and f_B is the molar-concentration activity coefficient], that the logarithmic plot of the first order hydrolysis rate constant, k, against H_o is linear, and that the ratio of the increments of these quantities is equal to unity. Later studies have shown that the relationship between ln k and H_o for the hydrolysis of sucrose is influenced by the temperature (Krieble, 1935; Krieble and Reinhart, 1935; Leininger and Kilpatrick, 1938a, b; Narasaraju and Rao, 1972). Although the logarithmic plots of k against H_o for many other glucosides were also linear, the ratios of Δln k to ΔH_o for these substances were not necessarily equal to unity (Timell, 1964; Bunnett, 1961; DeBruyne and Van Wijnendaele, 1968). The Hammitt hypothesis is not satisfied by extremely weak organic acids because the ratio of Δln k to ΔH_o is considerably greater than 1 (Long and Paul, 1957).

Extensive studies have shown that a plot of Δln k against pH of the medium of sucrose hydrolysis, instead of H_o, is also linear; however, its slope departs from unity (Thies et al., 1953; Bunton et al., 1957; Szejtli, 1965a, b, c, 1968; Szejtli et al., 1970; O'Connor and Barnett, 1971; Kharin, 1973). So far no attempt has been made to correlate the rate of hydrolysis of either sorghum molasses or sucrose with acid concentration in terms of volume concentration.

The major objectives of this work were to establish the relationship between the rate of hydrolysis of sorghum molasses and mineral acid concentration in terms of volume concentration; to determine the kinetic equations of the hydrolysis of sorghum molasses with mineral acids; to determine whether the second hydrogen atom in sulfuric acid participates in the hydrolysis as in the case of acid-hydrolysis of cellulose (Wenzl, 1970); to study the kinetic characteristics of the hydrolyzing action of oxalic acid; to establish the relationship between the rate of hydrolysis of sorghum molasses and melibiose and oxalic acid concentration in terms of volume concentration [%(w/v)]; and to derive their kinetic equations based on the information generated from experimental determination of the kinetic parameters.

MATERIALS & METHODS

Sorghum molasses

Sorghum molasses (d = 1.370) was obtained from a farm cooperative located in Alma, KS. It contained sucrose, glucose, and fructose in ratios of roughly 2:1:1. A 2.8% molasses medium was used for acid-catalyzed hydrolysis. The total sugar content of the medium was about 2%.

Melibiose

A 2.0% (w/v) melibiose solution (specific rotation n_d^{20} = +142 degrees) prepared from white crystalline melibiose (M.P. 184–185°C, Sigma Chemical Company), was employed in the experiments.

Sulfuric acid

Sulfuric acid solutions were prepared by mixing 98% H_2SO_4 (d = 1.84, Fisher Scientific Company, U.S.A.) with distilled water into concentrations of 2.015, 0.2015, and 0.02015 g/100 mL. After mixing with the same volume of molasses solution, the concentrations became 1.0075, 0.10075, and 0.010075 g/100 mL, respectively.

Hydrochloric acid

Hydrochloric acid concentrations of 2.0, 0.2, and 0.02 g/100 mL were used for hydrolysis. After mixing with the same volume of molasses solution, the concentrations became 1.0, 0.1, and 0.01 g/100 mL, respectively.

Oxalic acid

Oxalic acid solutions of 0.4, 0.8, 1.6, 3.2, and 6.4% (w/v) were prepared from crystalline oxalic acid, $HOOCCOOH \cdot 2H_2O$ (Fisher Scientific Company). After mixing with the same volume of either sorghum molasses or melibiose solution, the acid concentrations in hydrolysis mixtures became 0.2, 0.4, 0.8, 1.6 and 3.2% (w/v), respectively.

Hydrolysis

Hydrolysis was carried out in a 200 mL three-neck flask reactor with a mixer and a thermometer. An acid solution (25 mL), prepared in an appropriate concentration, was placed into the reactor and warmed to the desired temperature of 60, 70, 80, 90, or 100°C. Twenty-five milliliters of molasses solution (C = 2.8%), which had been warmed to the same temperature, were then poured into the reactor. Samples (2 mL) were withdrawn at regular intervals, neutralized, and then placed immediately into an ice-bath.

Analysis

Sucrose, glucose, and fructose were measured by a Varian Model 5020 high pressure liquid chromatography (HPLC) equipped with an Aminex ion exchange resin column (HPX-87H, Bio-Rad Laboratories). For protection, the column was preceded by a Micro-guard column which was packed with ion exchange resin (Aminex HPX-85H, Bio-Rad Laboratories). The Microguard column was replaced routinely after every 100 injections. The sugar was analyzed by a refractive index detector (Waters Model 401). The temperature of the column was kept at 45°C during analysis. A 0.01N sulfuric acid solution was used as the mobile phase at a flow rate of 0.9 mL/min. n-Propanol was used as the internal standard.

RESULTS & DISCUSSION

THIS INVESTIGATION was divided into two phases. The first involved hydrolysis using dilute mineral acids and the second using oxalic acid.

Mineral acids

The hydrolysis curves of sorghum molasses with dilute sulfuric and hydrochloric acids are illustrated in Fig. 3 and 4, respectively. The first-order hydrolysis rate constants obtained through linear regression analysis of these data are listed in Table 1.

Effects of acid concentration and temperature. The rate and extent of the hydrolysis of sorghum molasses are dependent on the acid concentration and temperature (Fig. 3 and 4).

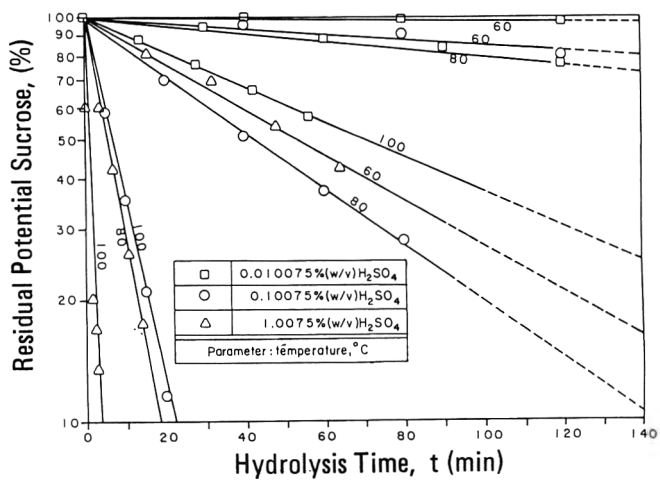


Fig. 3—Hydrolysis curves of sorghum molasses with sulfuric acid.

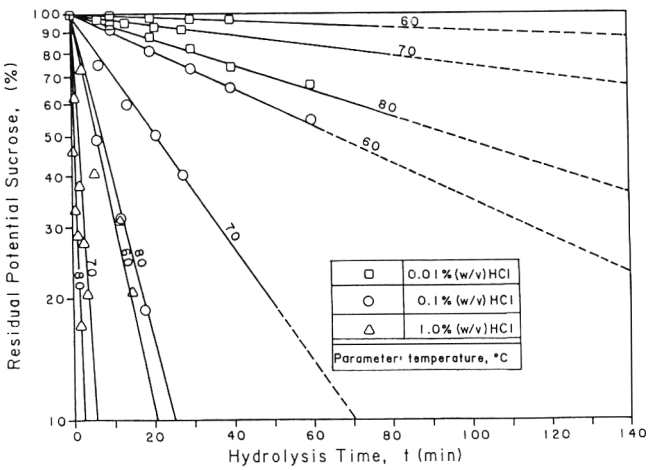


Fig. 4—Hydrolysis curves of sorghum molasses with hydrochloric acid.

A logarithmic plot of the first-order hydrolysis rate constant, k, in Table 1 against the acid concentration, C, at three temperature levels yielded three approximately parallel, linear correlations (Fig. 5) indicating that:

$$k = k'C^m \quad (1)$$

for each acid, where k' and m are empirical constants. The magnitude of the slope of each plot, or m, indicates the extent of the influence of the acid concentration on the rate of hydrolysis. Various investigators have found ln k to be a linear function of H_0 and pH (Hammett and Deyrup, 1932; Hammett and Paul, 1934).

Plotting the logarithms of hydrolysis rate constants in Table 1 against the reciprocal of absolute temperature for three different acid concentrations has yielded three approximately parallel, linear correlations (Fig. 6), indicating that the dependence of the hydrolysis rate constant on the temperature obeys the classical Arrhenius equation:

$$k = A \exp(-E/RT) \quad (2)$$

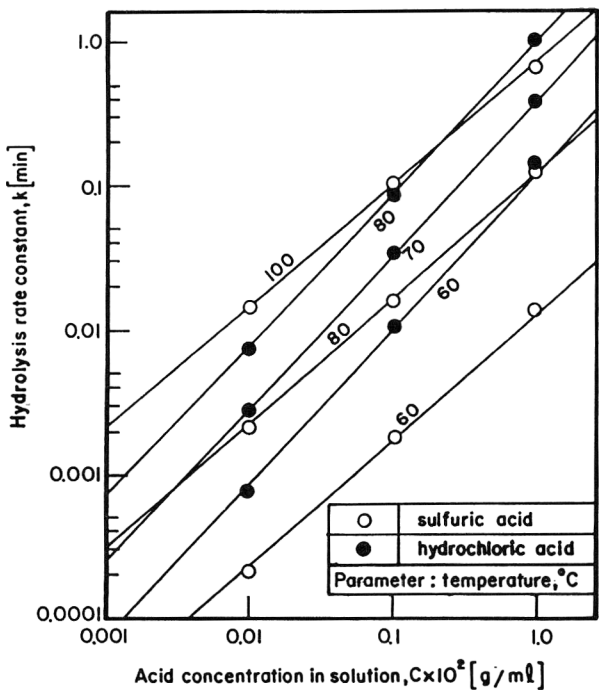


Fig. 5—Dependence of the hydrolysis rate constant on the acid concentration.

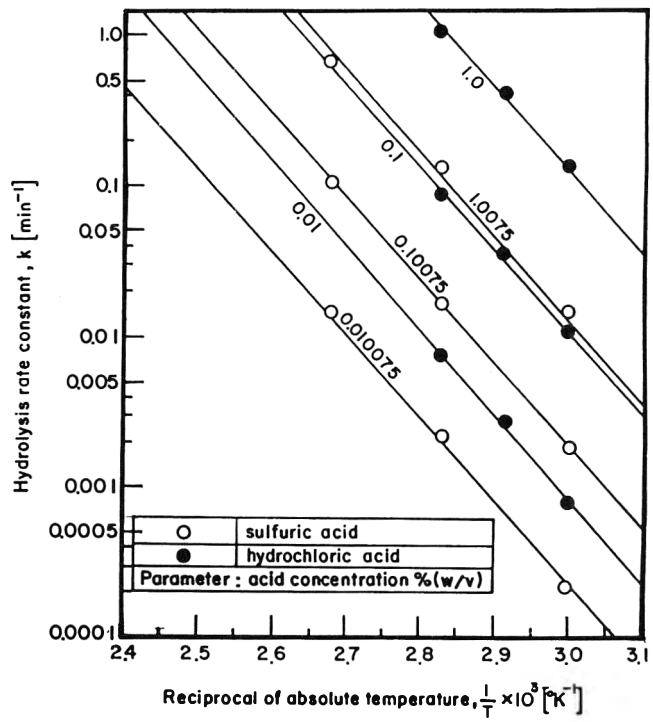


Fig. 6—Dependence of the hydrolysis rate constant on the temperature.

Table 1—First-order hydrolysis rate constants for the hydrolysis of molasses with mineral acids in different concentrations at various temperatures

Temp °C (K)	Sulfuric acid Concentration % (w/v)	(pH)	k, min⁻¹ Observed	Calculated[a]	Calculated[b]	Temp °C (K)	Hydrochloric acid Concentration % (w/v)	(pH)	k, min⁻¹ Observed	Calculated[c]	Calculated[d]
60	0.01008	2.80	0.000209	0.000239	0.000236	60	0.01	2.80	0.000762	0.000862	0.000863
(333.15)	0.10075	2.34	0.001801	0.001805	0.001860	(333.15)	0.10	2.00	0.010828	0.010593	0.010601
	1.0075	1.90	0.014131	0.013617	0.013414		1.00	1.20	0.133470	0.130236	0.130271
80	0.01008	2.80	0.002157	0.002051	0.001986	70	0.01	2.80	0.002712	0.002575	0.002577
(353.15)	0.10075	2.34	0.015944	0.015209	0.015669	(343.15)	0.10	2.00	0.034913	0.031653	0.031664
	1.0075	1.90	0.126351	0.114742	0.113026		1.00	1.20	0.394230	0.389172	0.389100
100	0.01008	2.80	0.014122	0.013511	0.013313	80	0.01	2.80	0.007488	0.007231	0.007234
(373.15)	0.10075	2.34	0.101947	0.101977	0.105061	(353.15)	0.10	2.00	0.085215	0.088900	0.088891
	1.0075	1.90	0.658380	0.769360	0.757858		1.00	1.20	1.012730	0.914895	0.915458

[a] Calculated from: $k = 1.70 \times 10^{16} C_{H_2SO_4}^{0.878} \exp(-24{,}913/RT)$.

[b] Calculated from: $k = 1.5 \times 10^{18} \exp[-(4.49pH + \frac{24{,}913}{RT})]$.

[c] Calculated from: $k = 3.988 \times 10^{17} C_{HCl}^{1.09} \exp(-24{,}861/RT)$.

[d] Calculated from $k = 1.14 \times 10^{17} \exp[-(3.14pH + \frac{24{,}861}{RT})]$.

where A is the pre-exponential factor, E the activation energy (cal/g-mole), T the temperature (°K), and R the gas constant (cal/g-mole·°K).

Kinetic equations. By combining Eq. (1) and (2), the kinetic equations describing the hydrolysis of sorghum molasses with mineral acids, which take into account the effects of the acid concentration and temperature, can be determined. This yields:

$$k = k_o C^m \exp(-E/RT) \text{ or } \ln k = \ln k_o + m \ln C - E/RT \quad (3)$$

where k_o is an empirical constant, C the acid concentration (g/mL), and m an empirical constant. A linear least-square fitting of Eq. (3) to the experimental data has yielded:

$$k = 1.70 \times 10^{16} C_{H_2SO_4}^{0.878} \exp(-24{,}913/RT) \quad (4)$$

for sulfuric acid, and:

$$k = 3.998 \times 10^{17} C_{HCl}^{1.09} \exp(-24{,}861/RT) \quad (5)$$

for hydrochloric acid. When the acid concentration is expressed in terms of pH, the expression of dependence of the hydrolysis rate constant on the acid concentration and temperature becomes:

$$k = k'_o \exp\left\{-\left(npH + \frac{E}{RT}\right)\right\} \text{ or } \ln k = \ln k'_o - npH - \frac{E}{RT} \quad (6)$$

where k'_o and n are empirical constants, and a_{H^+} the activity of hydrogen ion in the hydrolysis medium. Linear least-square fitting of Eq. (6) to the data has resulted in:

$$k = 1.50 \times 10^{18} \exp\left\{-\left(4.94pH + \frac{24{,}913}{RT}\right)\right\} \quad (7)$$

for sulfuric acid, and:

$$k = 1.14 \times 10^{17} \exp\left\{-\left(3.14pH + \frac{24{,}861}{RT}\right)\right\} \quad (8)$$

for hydrochloric acid. The values of k calculated from Eq. (4), (5), (7), and (8) are compared with the experimentally determined values in Table 1.

The magnitudes of activation energy obtained in this work are essentially in agreement with those of other investigators (Table 2). Statistical analysis of the available data has yielded E = 25,550 ± 260 cal/g-mole (Vukov, 1965; Szejtli et al., 1970). Note that the R^2, measuring the extent of variation in the dependent variable attributable to the model, is 0.9987 with sulfuric acid and 0.9991 with hydrochloric acid.

Oxalic acid

The sucrose content in the sorghum molasses medium during hydrolysis by oxalic acid and the fraction of glucose produced in the melibiose medium during hydrolysis are shown in Fig. 7 and Fig. 8, respectively. It can be seen that these semi-logarithmic plots are essentially linear, giving rise to the first order rate expressions. Upon integration, they yield:

$$\ln\left(\frac{C_{At}}{C_{Ao}}\right) = -kt \quad (9)$$

Table 2—Activation energy for the acid-catalyzed hydrolysis of sucrose

Investigator	Acid (conc)	Temp °C	Activation energy Cal/mole
Jones and Lewis (1920)	H_2SO_4 (0.1N)	20- 50	26,400
Moelwyn-Hughes (1929)	HCl (a_{H^+} = 1.0)	60	25,830
Heidt and Purves (1938)	HCl (0.00965N)	30- 60	25,700
Leininger and Kilpatrick (1938a)	HCl (1.1235M)	0- 10	26,200
Leininger and Kilpatrick (1938a)	HCl (4.822M)	0- 10	24,200
Leininger and Kilpatrick (1938b)	HCl (1.0M)	30- 40	25,160
Bodamer and Kunin (1951)	0.001 N[a]	25- 50	28,200
Bodamer and Kunin (1951)	0.18 N[a]	25- 50	30,000
Timell (1964)	H_2SO_4 (0.01M, 0.5M)	30- 60	24,400
Lund et al. (1969)	HCl (1.48N)	12- –7	26,000
Szejtli et al. (1970)	HCl (0.001-1.0N)	30- 60	25,541
Present work	HCl (0.01-1.0 g/100 mL)	60- 80	24.756
Present work	H_2SO_4 (0.01-1.0 g/100 mL)	60-100	25,054

[a] By using cation exchange resin.

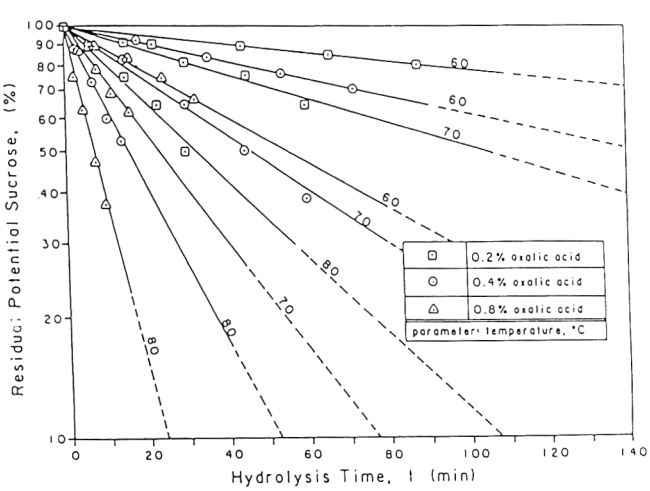


Fig. 7—Hydrolysis curves of sorghum molasses with oxalic acid.

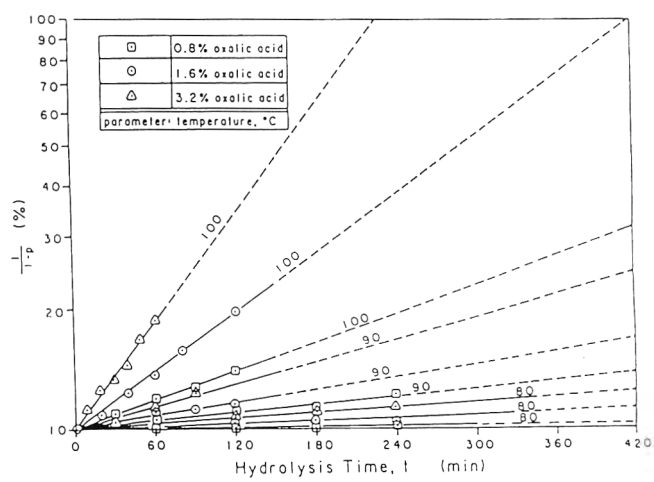


Fig. 8—Hydrolysis curves of melibiose with oxalic acid.

for sorghum molasses, and:

$$\ln\left(\frac{1}{1-p}\right) = kt \qquad (10)$$

for melibiose where k is the first-order hydrolysis rate constant (min^{-1}), C_{Ao} and C_{At} the sucrose concentrations [% (w/v)] at the beginning and at time t, respectively, t the hydrolysis time (min), and p the fraction of glucose produced up to time t. The first-order hydrolysis rate constants, k, were determined from the data by means of regression analysis. The resultant values of k are listed in Table 3.

A comparison between oxalic acid and the mineral acids shows that the three acids were different in their hydrolyzing action (Table 4). At the same concentration, in terms of molarity, the specific rates of hydrolysis with the three acids were of the same order of magnitude, indicating that the second hydrogen atom in oxalic acid as well as that in sulfuric acid play an insignificant role in hydrolysis. This is probably due to the low dissociation constants of their second hydrogen atoms. Sulfurous and phosphoric acids also have low dissociation constants and, thus, are only slightly catalytically active in hydrolysis of cellulose (Wenzl, 1970).

Effects of acid concentration and temperature. The effect of oxalic acid concentration on the hydrolysis rates of sorghum molasses at three temperature levels is shown in Fig. 9. At a given temperature, the hydrolysis rate constant, k, is related to the acid concentration, C_{oxalic}, as:

$$k = k_c C_{oxalic}^m \text{ or } \ln k = m \ln C_{oxalic} + \ln k_c \qquad (11)$$

where m is the slope and $\ln k_c$ the intercept of a logarithmic plot of k against C_{oxalic}. This result is very similar to those obtained by earlier investigators (Reva et al., 1975; Muro et al., 1976) when hydrolysis of sucrose was carried out in a system buffered with inverted sugars. The logarithm of the hydrolysis rate of sucrose was found to be linear with respect to the pH of the reaction mixture.

Arrhenius plots illustrating the effect of temperature on the hydrolysis rate of sorghum molasses and melibiose at three oxalic acid concentrations is shown in Fig. 10. The hydrolysis rates of sorghum molasses and melibiose increase with increasing temperature and acid concentration. Increasing the temperature 10°C was approximately equivalent to doubling the acid concentration for sorghum molasses and increasing the temperature 10°C was approximately equivalent to quadrupling the acid concentration for melibiose. The hydrolysis rate constant, k, is exponentially dependent on the reciprocal of absolute temperature; (Fig. 10):

$$k = A\, e^{-E/RT} \text{ or } \ln k = \ln A - \frac{E}{R}\left(\frac{1}{T}\right) \qquad (12)$$

where E is the activation energy (cal/g-mole), R the gas constant (cal/g-mole°K), T the absolute temperature (°K), and A the Arrhenius constant.

Kinetic equations. Combining Eq. (11) and (12) gives rise to the kinetic equations for hydrolysis of sorghum molasses and melibiose with dilute oxalic acid, which takes into account the acid concentration and temperature. The resultant expression is

$$\left.\begin{aligned} k &= k_o C_{oxalic}^m \exp(-E/RT) \\ \text{or} \quad \ln k &= \ln k_o + m \ln C_{oxalic} - \frac{E}{R}\left(\frac{1}{T}\right) \end{aligned}\right\} \qquad (13)$$

A linear regression of Eq. (13) to the experimental data has yielded:

$$k = 1.306 \times 10^{17} C_{oxalic}^{1.136} \exp(-25{,}407/RT) \qquad (14)$$

for sorghum molasses, and:

$$k = 1.796 \times 10^{24} C_{oxalic}^{0.951} \exp(-42{,}368/RT) \qquad (15)$$

for melibiose. For comparison, the values of k calculated from Eq. (14) and (15) are also listed in Table 3.

Table 3—Observed and calculated first-order hydrolysis rate constants for hydrolysis of sorghum molasses and melibiose by oxalic acid

Temp °C	Oxalic acid concentration %(w/v)	Molasses $k \times 10^3$ (min^{-1}) Observed	Molasses $k \times 10^3$ (min^{-1}) Calculated[a]	Melibiose $k \times 10^4$ (min^{-1}) Observed	Melibiose $k \times 10^4$ (min^{-1}) Calculated[b]
60	0.2	2.401	2.365	–	–
	0.4	5.167	5.197	–	–
	0.8	12.26	11.42	–	–
70	0.2	6.987	7.244	–	–
	0.4	16.13	15.92	–	–
	0.8	36.14	34.99	–	–
80	0.2	22.87	20.83	–	–
	0.4	46.48	45.77	–	–
	0.8	97.35	100.6	0.98	1.064
	1.6	–	–	1.96	2.057
	3.2	–	–	4.44	3.977
90	0.8	–	–	6.81	5.620
	1.6	–	–	10.92	10.86
	3.2	–	–	23.42	21.00
100	0.8	–	–	27.10	27.14
	1.6	–	–	55.78	52.47
	3.2	–	–	90.83	101.4

[a] Calculated from: $k = 1.306 \times 10^{17} C_{oxalic}^{1.136} \exp(-25{,}407/RT)$.

[b] Calculated from: $k = 1.796 \times 10^{24} C_{oxalic}^{0.951} \exp(-42{,}368/RT)$.

Table 4—Comparison of the rates of the hydrolysis of sorghum molasses in various acids (experimental conditions: acid conc 0.1 molar, temp 80°C)

Item	Oxalic acid	Sulfuric acid	Hydrochloric acid
Rate constant, min^{-1}	0.112210	0.126351	0.359073
Activity of the acids	0.31	0.35	1.00[a]

[a] The activity of hydrochloric acid to invert sucrose is taken as 1.00.

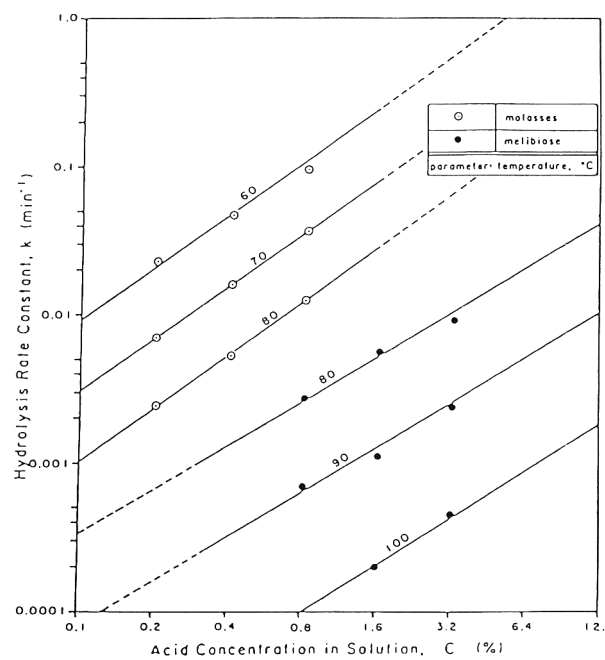


Fig. 9—Dependence of the hydrolysis rate constant on the oxalic acid concentration.

The activation energy of 25,407 cal/g-mole for sorghum molasses is close to those reported by earlier investigators for hydrolysis of sucrose mostly by hydrochloric acid. For example, from the results of numerous investigators, Vukov (1965) and Szejtli et al. (1970) obtained average values of the activation energy of 25,920 cal/g-mole, and 25,550 ± 260 cal/g-mole, respectively. The activation energy of 42,368 cal/g-mole for melibiose is substantially greater than the values of 38,590 and 33,800 cal/g-mole obtained by Moelwyn-Hughes (1929) and Timell (1964), respectively (Table 2). Since hydrochloric acid was employed by the earlier investigators, this difference is not surprising. Statistical analysis of the data from this phase of investigation has shown that the R^2 was nearly 1.0, indicating that the kinetic equations derived are accurate.

CONCLUSIONS

THE PLOTS of ln k obtained from hydrolysis of sorghum molasses by dilute sulfuric and hydrochloric acids against ln C are linear as in the cases of plots of ln k verses H_o or ln k versus pH as reported by earlier investigators. The kinetic equations for hydrolysis of sorghum molasses by dilute mineral acids have been determined. A close agreement between the observed rates and those calculated from the present kinetic equations indicates that the slight differences among the values of activation energy at various acid concentrations are within the experimental error.

Sulfuric and hydrochloric acids appear to be different in their hydrolyzing action. At the same molarity, the specific rates of hydrolysis with both acids are of the same order of magnitude. The value of k is 0.126351 for sulfuric acid and 0.359073 min^{-1} for hydrochloric acid in a 0.1M acid medium at 80°C. This implies that the second hydrogen atom in sulfuric acid plays an insignificant role in the hydrolysis. At 80°C, sorghum molasses can be hydrolyzed essentially completely in a 1.0 g/100 mL sulrufic acid or 0.1 g/100 mL hydrochloric acid solution within 12 or 16 min, respectively, with only slight decomposition of both glucose and fructose.

Hydrolysis of sorghum molasses and that of melibiose in dilute oxalic acid obey the law of first-order reaction. The hydrolysis rate constant depends on the oxalic acid concentration in terms of % (w/v); this dependency can be expressed as $k = k_o C^m$. The kinetic equations for hydrolysis of sorghum molasses and melibiose with oxalic acid have been derived. These equations take into account the effect of temperature and oxalic acid concentration on hydrolysis. The hydrolyzing action of oxalic acid is different from that of hydrochloric acid. The second hydrogen atom in oxalic acid does not appear to participate in hydrolysis. The destruction of G-F bond of sucrose in sorghum molasses is approximately 993 times faster than that of Ga-G bond in melibiose under the same condition when hydrolysis is carried out in the solution of oxalic acid.

The activation energies of destruction of G-F bond in molasses are 25,407 cal/g-mole with oxalic acid, 24,861 cal/g-mole with hydrochloric acid, and 24,913 cal/g-mole with sulfuric acid. These values are approximately equal, indicating that the rupture of G-F bond in molasses is independent of the kind of acids. The hydrolysis rates are different in various acid media at the same set of conditions, indicating that their activities are distinct. The activity of oxalic acid is weaker than those of hydrochloric acid and sulfuric acid.

NOMENCLATURE

A	pre-exponential factor in the Arrhenius equation (min^{-1})
a_{H^+}	hydrogen-ion acitivity (g/mL)

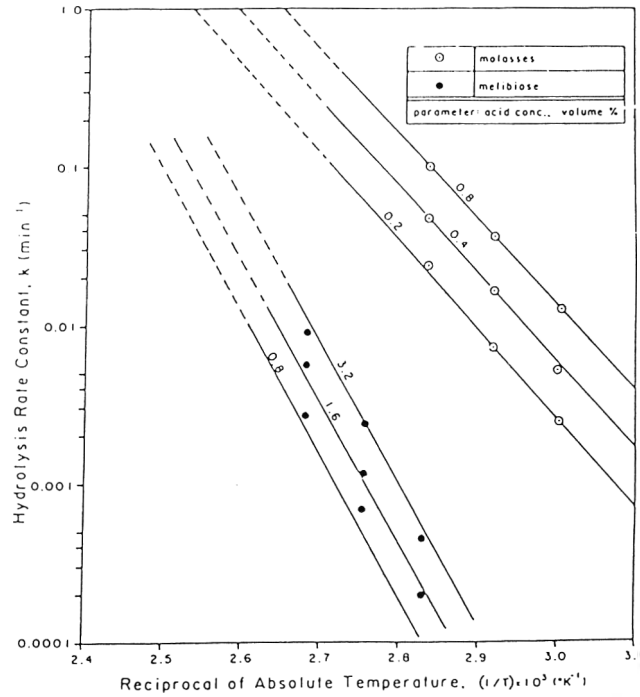


Fig. 10—Dependence of the hydrolysis rate constant on the temperature in hydrolysis with oxalic acid.

C	acid concentration (g/mL)
C_{Ao}	sucrose concentration in hydrolysis solution at the beginning [% w/v)]
C_{At}	sucrose concentration in hydrolysis solution at time t [% (w/v)]
C_{oxalic}	oxalic acid concentration in hydrolysis solution (g/mL)
E	activation energy (cal/g-mole)
f_B	molar-concentration activity coefficients (dimensionless)
H_o	acidity function (g/mL)
k	first-order hydrolysis rate constant (min^{-1})
k′	empirical constant in Eq. (1)
k_o	empirical constant in Eq. (3) and (13)
k'_o	empirical constant in Eq. (6)
k_c	empirical constant in Eq. (11)
M	molar concentration (moles of solute per liter solution)
m	empirical constant (dimensionless)
n	empirical constant in Eq. (6) (dimensionless)
p	fraction of produced glucose at time t (dimensionless)
R	gas constant (cal/g-mole·°K)
t	reaction time (min)
T	absolute temperature (°K)

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Lemon Juice Color Evaluation: Sensory and Instrumental Studies

KURT ANTHONEY, DANIEL ENNIS, and PETER COOK

ABSTRACT

The ability to use the Hunter Citrus Colorimeter D45 for the objective measurement of lemon juice color was studied. The correlation coefficient between 25 panelists' visual ranking of lemon juice lightness to darkness and the colorimeter D45 color scores was 0.98. This paper presents the methods used in preparing the lemon juice sample for color measurement and the implication of the sensory results for the potential use of the Hunter Citrus Colorimeter D45 for lemon juice color measurement by the citrus industry.

INTRODUCTION

THE COLOR OF FOODS and beverages is an important component of total product acceptability and value. Over the years, objective color measurements have been developed for a number of foods and beverages. The need for a simple, objective instrument to measure juice color was reported by Hunter in 1967. At the request of the Florida Citrus Commission, Hunter Associates Laboratory developed a Citrus Colorimeter prototype and calibration procedure for the objective color measurement of frozen concentrated orange juice. Subsequent improvements of the initial prototype colorimeter led to the development of the Model D45 Citrus Colorimeter (Wenzel and Huggart, 1969). The instrument color scores correlated well with the average grade color scores of skilled human observers. The instrument has been primarily used for the objective color measurement of orange juice and with the additional capability of measuring other citrus juices including lemon juice (Hunter, 1967).

Two color scales were developed by Hunter: Citrus Redness (CR), and Citrus Yellowness (CY). The citrus colorimeter color score value, N is derived from a linear equation for the best fit between color scores Citrus Red (CR) and Citrus Yellow (CY) and the USDA color scores for reconstituted frozen orange juice. The color score value, N (N = 22.51 + 0.165 CR + 0.111 CY) represents a color score which fits into the yellow-orange region of the Hunter Rd., a, b color space (Hunter, 1975). Wenzel and Huggart (1969) reported a squared correlation coefficient between visual panel scores and the citrus colorimeter D45 color scores values, N of 0.98, for reconstituted orange juice. Additional studies by Hunter gave correlation coefficients between visual panel scores and the citrus colorimeter color scores of 0.80-0.94 for reconstituted orange juice (Hunter, 1967).

Currently, the citrus industry does not have an industry accepted standard for an objective method of measuring lemon juice color. During lemon juice pasteurization and concentrating procedures, the resulting lemon juice concentrate can be darkened to an undesirable and unacceptable brown-yellow color. Typical commercial pasteurization time and temperature prior to evaporation is 30 sec at 77°C. Although lemon juice varies in its enzyme activity, heat treatment of this temperature and duration is generally required to achieve denaturation of lemon juice enzymes and stabilization of lemon juice cloud (Swisher and Swisher, 1971).

The objective of this study was to examine and verify the ability of using the Hunter Citrus Colorimeter D45 to objectively measure the color of reconstituted lemon juice from lemon concentrate.

Author Anthoney is with the R&D Dept., Seven-Up Foods, 2325 Vista Del Mar Drive, Ventura CA 93002. Author Ennis is with the Product Evaluation Division, R&D Dept., Philip Morris, Commerce Rd., Richmond VA 23261. Author Cook is affiliated with Consulting Statisticians, Inc., 20 William Str., Wellesley Hills, MA 02181.

MATERIALS & METHODS

Preparation of lemon juice for color measurement

Thirty-two samples of 9.0 ± 0.2°Brix lemon juice were prepared from a single original production lot of California lemon concentrate. Twenty-nine varying shades of lemon juice color were prepared by browning a portion of the original lemon concentrate by direct heat and adding volumetric proportions of the darkened lemon concentrate back to the original lot of lemon concentrate. An additional three color sample of lemon juice concentrate of varying shades of color were prepared by the oxidation browning of the original lemon concentrate for 3, 5, and 10 days at 24°C.

The 32 lemon concentrate samples were diluted to 9.0 ± 0.2° Brix with water. Fifty milliliters of each sample were centrifuged at 1310 rpm using an International SBV centrifuge with a 15 inch arm diameter. The supernatant was decanted into the colorimeter sample tube, gently inverted three times and immediately placed into the Hunter Citrus Colorimeter D45 sample port for the color score value, N measurement. The Citrus Colorimeter had previously been calibrated with the Hunter white standard calibration tube immediately prior to the 32 lemon juice sample color measurements. The lemon juice samples were allowed exactly 1 min equilibration within the colorimeter sample port prior to the recording of the color score values, N.

Preparation of samples for sensory studies

Each sample was contained in a 1-oz screw top glass vial, 3.75 inches in length and 0.75 inches in diameter. All thirty-two samples were presented to the panelists horizontally against a white backing under white lights. The 32 samples were prepared and the color scores were measured immediately prior to presentation to each of the 26 panelists. The vials were filled completely to eliminate any air bubbles within the vial. Each panelist was isolated from the other panelists during the sensory evaluation. The sensory testing was conducted in two sets under identical conditions. The twenty-six panelists were not pre-screened or trained. Those panelists with corrective lenses were requested to wear them during the experiment. The panel consisted of 13 males and 13 females, all under the age of 30 years. The lemon juice samples were presented in random order and the panelists' task was to rank the vials of lemon juice from lightest to darkest.

RESULTS & DISCUSSION

Panel homogeneity

For each vial, the average rank across subjects and the average normal score was computed. The normal scores for each subject are the expected values of the rank-order statistics from a standard normal distribution. The normal

scores are the standardized values expected if the assigned rankings are to be maintained. In all of the analyses conducted, both the average ranks and average normal scores were used.

For each vial, there was an average rank computed across subjects and each subject was compared to this average ranking by computing the Euclidean distance across vials. A subject whose ranking agreed with the average ranking would have a small distance and a subject whose ranking differed greatly from the average would have a large distance. Table 1 gives the Euclidean distances from the average rankings for each subject. Subject 9 was the furthest from the mean and subjects 3 and 26 showed more than average deviation. In order to further explore these differences, plots for each subject of the assigned rank and the average rank were generated and correlations were computed. Fig. 1 shows that subject 9 appears to be using quite different criteria to establish the rank-order for the vials than the average panelist. The correlation coefficient for most subjects between average between average rank and assigned rank was 0.90-0.96, but subject 9 ranks were correlated only at 0.78. Subject 9 was omitted from further analyses.

Table 1—Euclidean distance of vial scores from the mean for each subject

Subject	Rank score distance	Normal score distance
1	8.26	1.04
2	10.41	1.38
3	23.10	3.00
4	8.96	1.35
5	11.12	1.43
6	17.74	2.41
7	17.90	2.14
8	8.53	1.18
9	33.88	3.79
10	11.76	1.75
11	9.01	1.36
12	16.29	2.48
13	12.96	1.91
14	9.26	1.27
15	9.43	1.17
16	10.71	1.44
17	13.98	1.66
18	9.15	1.40
19	8.36	1.21
20	12.06	1.70
21	10.65	1.46
22	14.23	1.82
23	9.08	1.19
24	13.62	1.56
25	9.45	1.22
26	21.16	2.78
Mean	13.12	1.73
Standard deviation	5.86	0.67

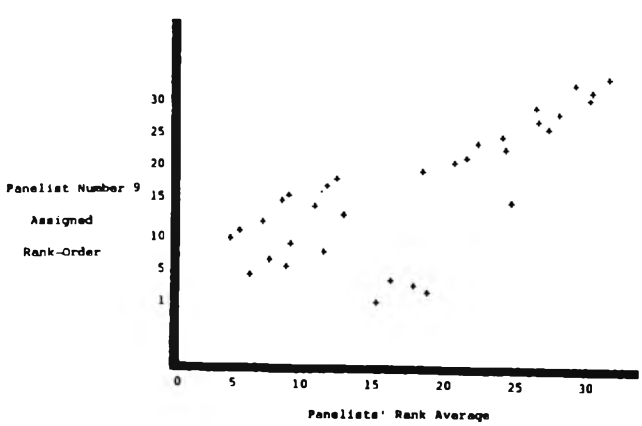

Fig. 1—Sensory rank-order comparison of panelist number 9 vs the panel average.

Differences among the vials

An analysis of variance on the normal scores for the remaining 25 panelists indicated a highly significant difference between the vials ($p < 0.001$). There was no effect on the scores evident due to the test set, the sex of the subject or whether corrective lenses were used. Since the vials exhibited substantially varying standard deviations in their assigned ranks, a weighted analysis of variance was considered appropriate. The weights were computed as the reciprocal of the standard deviation so that vials with high standard deviations received a smaller weight in the analysis. Table 2 shows the colorimeter value, N average normal scores and average ranks for each vial along with the Duncan groupings ($p < 0.01$).

Table 2—Duncan multiple range test groupings for panel testing results (using P < 0.01) for 32 lemon juice samples of varying color score N values

N	Average ranks Duncan grouping	Mean	Normal scores Duncan grouping	Mean
29.6	A	31.440	A	1.8500
30.0	B	30.280	B A	1.5555
29.6	C B	30.000	B A	1.4866
29.1	C	29.000	B	1.2395
28.9	D	27.760	C	1.0549
28.4	D	27.120	D C	0.9704
28.2	D F	26.200	D C	0.8526
28.3	F	25.920	D C E	0.8228
27.9	G	23.720	D F E	0.5957
27.8	G	23.480	D F E	0.5739
27.3	H	21.720	G F E	0.4182
27.1	H I	20.920	G F H	0.3513
27.5	I	20.120	G F H	0.2860
26.6	I J	18.600	G I H	0.1644
27.0	J	17.720	G I H	0.0954
26.2	J	17.600	G I H	0.0859
25.9	K	15.720	I J H	−0.0612
25.8	K	14.680	K I J	−0.1765
25.9	K L	11.800	K J L	−0.4085
25.8	M L	11.080	K M J L	−0.4692
25.5	M N	10.440	K M N L	−0.5461
25.7	M N	10.360	K M N L	−0.5673
25.8	N	9.640	K M N L	−0.5823
25.5	O	7.600	O M N L	−0.7962
25.5	O	7.560	O M N L	−0.8123
25.1	O	7.400	O M N L	−0.8130
25.5	P O	6.960	O M N	−0.8865
25.3	P Q	6.240	O P	−0.9591
25.1	R Q	5.400	O P	−1.0606
25.1	R S	4.760	Q P	−1.3193
24.5	T S	3.840	Q P	−1.3377
24.4	T	2.920	Q	−1.6075

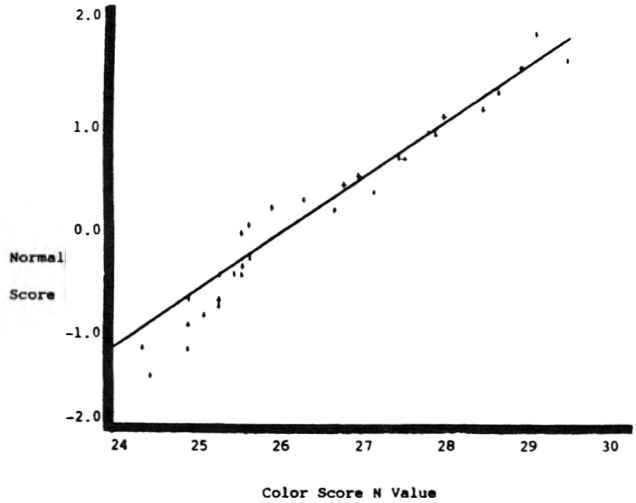

Fig. 2—Relationship between panel average normal scores and instrumental color score, N values.

Sensory/colorimeter correlations

The correlation between N value and the sensory measures was 0.98 for the average rank and 0.97 for the normal score. These are both significant correlations at $p < 0.001$. Fig. 2 is a plot of the normal scores versus the N values with the calculated regression equation, Normal Score = −14.15 + 0.53 (N value).

Detectable difference in N value

From Table 2 it can be seen that there was considerable overlap in the Duncan groups for the average normal score and the average rank measures. Apparently, the panel is not able to distinguish between the vials when the colorimeter scores were within about 0.3 or 0.4N value units of each other. When they were further apart than this, 0.5 units or more, discriminiation appeared to be possible.

CONCLUSIONS

RANK-ORDERING of lemon juice samples in glass vials on the basis of lightness to darkness appears to be extremely well related to the Citrus Colorimeter value, N. Based on the means of 25 panelists' rankings, the panel appears to be able to discriminate between varying shades of lemon juice color which differ by 0.5 units of N or more, but confusion between the varying shades of lemon juice color begins to occur when the samples differ by 0.3–0.4 units of N or less. There was good general agreement between panelists on the ranking of the vials with the exception of one panelist, who was not included in the analysis. The relevance of lemon juice color to consumer perception of lemon juice "quality" needs to be researched further.

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MICROFLORA OF SOUTHERN PEAS . . . From page 1411

CONCLUSION

DATA on microbial populations normally encountered during the harvesting and processing of southern peas are provided in the present study and can serve as a baseline to evaluate and compare sanitary conditions in similar plants and operations. While microorganisms of public health significance were not encountered among the isolates in this study, this does not preclude the possibility of encountering organisms under adverse conditions that would be significant to public safety. However, isolates of *Enterobacteriaceae* from southern peas would normally be those associated with soil and not considered pathogenic to humans.

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Reference to a company or product name does not imply approval or recommendation by the U.S. Dept. of Agriculture.

Orange Juice Adulteration: Detection and Quality Effects of Dilution, Added Orange Pulpwash, Turmeric and Sorbate

D. R. PETRUS, P. J. FELLERS, and H. E. ANDERSON

ABSTRACT

Adulteration of frozen concentrated and single-strength orange juices with orange pulpwash (PW) have been detected regularly in samples obtained from the North American retail market. This practice is a direct economic fraud on the consumer and to the legitimate grower, processor and distributor. The addition of PW to frozen concentrated or single-strength orange juices may have a profound detrimental effect on the juice flavor and other quality factors. Turmeric, as colorant, alone and in combination with PW has been found in concentrated orange juice for manufacturing imported by some Florida processors. Sorbate preservative has been detected in single-strength orange juices from concentrate obtained from the retail market and packed out of Florida.

INTRODUCTION

THE QUALITY of reconstituted frozen concentrated orange juice (FCOJ) and single-strength orange juice (SSOJ) is directly related to quality of fruit used and to the processing variables involved during juice extraction.

There is an attempt to assure quality of FCOJ and SSOJ by many Florida Rules (Official Rules Affecting the Florida Citrus Industry, 1975) and Federal regulations (Code of Federal Regulations, 1980; United States Standards for Grades, 1976). To further assure product quality, United States Standards for Grades (1983) of various orange juice products have been established. The quality standards cover flavor, color, and defects. The analytical standards cover minimum degrees Brix, minimum and maximum degrees Brix to percent acid ratio and maximum recoverable oil.

The rules and regulations presented are only a part of those established to help assure quality of orange juice products. However, in spite of all the rules and regulations present, Petrus and Attaway (1980) reported gross adulteration of U.S. retail FCOJ and SSOJ (packed outside Florida) with orange pulpwash (PW) and/or by dilution (water or sugar solutions) of these products.

PW is a byproduct of the citrus processing industry. The product may also be denoted as WESOS (water-extracted soluble orange solids), OWP (orange washed pulp) or WEOS (water extraction of orange solids). After extraction of juice from oranges the juice is separated from the rag, excess pulp, seeds and other components. These products contain adsorbed orange juice solids, and are further processed by washing with water (to extract the adhered juice) using one or a number of washing stages and concentrating the washings to 45-65° Brix. The resulting product is concentrated PW. Department of Citrus Rule 20-64.07 (Official Rules Affecting the Florida Citrus Industry, 1975, amended 1982) prohibits the addition of PW to FCOJ and concentrated orange juice for manufacturing (COJFM) packed in Florida. Rule 20-69.02(1) (d) states that an imported product to be used in the production of FCOJ in Florida cannot contain PW. However, Code of Federal Regulations, section 146.146, does permit the use of PW in FCOJ or COJFM (packed outside of Florida) as long as the PW and orange juice, prior to concentrating, are obtained from the same batch of oranges. The final product would contain about 6% and at most 10% PW (Petrus and Attaway, 1980). There are no established standards for PW production.

Fellers (1984) determined the flavor characteristics of 242 samples of PW product commercially produced in Florida during late season 1979 and throughout the 1979-80 citrus season. He concluded, flavor scores of late-season (Valencia variety) PW samples were significantly better than for early-mid season (Hamlin, Pineapple varieties) PW samples. Seventy-three percent of the samples possessed some bitterness characteristics from extremely bitter to slightly bitter. However, some samples (27%) possessed flavor characteristics of orange juice either slightly, moderately, or very much. In many instances there were significant plant-to-plant flavor differences due to the processing or fruit variations. Mean hedonic flavor score for all samples was 2.5 or between "dislike very much" and "dislike moderately."

The purpose of this investigation was to evaluate the effects of orange juice adulteration on flavor, color, spectral characteristics, degrees Brix, percent acid, degrees Brix to percent acid ratio and limonin.

MATERIALS & METHODS

Model system

A model system was developed to investigate the effects on orange juice quality and other analyses by the addition of PW and/or by dilution of the product. A U.S. Grade A (United States Standards, 1976) Florida-produced FCOJ and a commercial Florida-produced late-season PW sample were obtained and reconstituted to approximately 12.0° Brix (corrected for temperature and acid). Various amounts of orange juice and PW were mixed. Some samples were also diluted with a sugar solution (approximately 12.0° Brix cane sugar solutions). The Grade A orange juice used had a color number of 37.3 (better than required minimum color score of 36) and a mean flavor score of 6.2, or the upper end of the "like slightly" flavor category. The late-season PW sample had a color number of 35.1 and a flavor score of 3.7 (low end of the "dislike slightly" category), or the sample was somewhat above average flavor-wise for a 100% PW product as determined by Fellers (1984).

Sample preparation

Sample preparation for instrumental analysis was as described by Petrus and Attaway (1980).

Analyses

Visible and ultraviolet absorption were determined with a Perkin Elmer Model 124 recording spectrophotometer, and fluorescence with a Farrand Mark I spectrofluorometer. See Petrus and Attaway (1980) for detailed instrumental settings and attenuations.

Flavor scores were determined by 27 experienced panelists rating reconstituted (approximately 12.0°Brix) samples on a 9-point hedonic scale, where 9 was "like extremely" and 1 was "dislike extremely." Samples were evaluated three at a time in individual

Authors Petrus, Fellers and Anderson are with the Florida Department of Citrus, Scientific Research Dept. and Univ. of Florida Citrus Research & Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850.

booths equipped with red light to eliminate color bias. Sample score sheets contained an area headed "Comments" for panelists' comments. Statistical evaluation of flavor scores and comparison of sample means was by analysis of variance and the method of least significant differences.

Color number was determined with a Hunterlab Color/Difference Meter D45D2.

Degrees Brix was determined with a table-model Bausch and Lomb Refractometer.

Percent acid was determined by titration with standard alkali and calculated as grams anhydrous citric acid per 100 grams.

Limonin was determined by R. L. Mansell (Mansell and Weiler, 1980).

RESULTS & DISCUSSION

TABLE 1 revealed progressively lower color numbers and acid percentages, and higher degrees Brix to percent acid ratios, which are related to maturity standards (Official Rules Affecting the Citrus Industry, 1975; United States Standards for Grades, 1976) and limonin (bitter principle) contents with increasing amounts of added PW (samples 1, 2, 3 and 7). A similar trend existed for samples 4, 5 and 6 having added PW and also dilution (with approximately 12° Brix cane sugar solution). In this system samples 4 through 6 resulted in minimum U.S. Grade A color score of 36. Samples 1, 2, 3 and 7 exhibited a trend to lower mean flavor scores with increasing amounts of PW. Samples 4, 5 and 6 with increasing amounts of PW accompanied by dilution revealed a similar trend.

Fig. 1 is a plot of the mean flavor scores and 95% confidence intervals obtained from the model system samples. The trend of lower mean flavor scores with increasing amounts of added PW is readily apparent, even in diluted samples 4, 5 and 6. From the plot (Fig. 1) and analysis of variance of the data (Table 2) significant differences in flavor preferences were revealed. Comparison of the means (by the method of least significant differences) showed significant flavor differences between any sample combination of 1, 2 or 3 and 4, 5, 6 and 7 (e.g. 1 and 4, 1 and 5, 1 and 6, 1 and 7, etc.). There were no significant differences between samples 1, 2 and 3 or 4, 5 and 6. Samples 1 through 6 were highly significantly different from sample 7.

Table 3 revealed that the number of individual flavor scores below 5 (neither like nor dislike) increased with increasing amounts of added PW. Authentic Florida-produced orange juice had two individual flavor scores below 5 whereas 100% PW had 22 flavor scores below 5 of a total of 27 per sample. Total negative comments also increased with increasing PW concentration, showing 15 negative comments for pure orange juice and 46 negative comments for 100% PW. Samples 1, 2 and 3 were about even in flavor scores below 5 and in negative comments. The summarized comments for samples 1, 2 and 3 were very similar, except

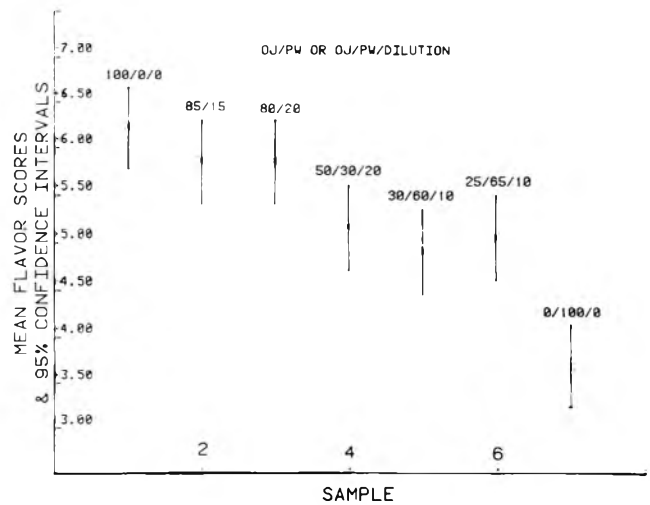

Fig. 1—Mean flavor scores and 95% confidence intervals of various combinations of percent orange juice (OJ), pulpwash (PW) or dilution of samples 1 through 7.

Table 1—Flavor and analytical results of model system samples

Sample	OJ/PW/Dil[a]	Color number	Flavor score[b]	Degrees Brix[c]	% Acid[d]	Brix to acid ratio	Limonin[e]
1	100/ 0/ 0	37.3	6.2	12.12	0.73	16.6	2.1
2	85/ 15/ 0	37.1	5.9	12.12	0.71	17.1	2.8
3	80/ 20/ 0	37.0	5.9	12.21	0.70	17.4	3.2
4	50/ 30/20	36.0	5.1	12.18	0.54	22.6	2.8
5	30/ 60/10	35.8	4.9	12.29	0.56	21.9	4.3
6	25/ 65/10	35.6	5.0	12.29	0.55	22.3	5.5
7	0/100/ 0	35.1	3.7	12.29	0.57	21.6	7.1

[a] Percentage orange juice, pulpwash and dilution of sample, respectively
[b] 9-Point hedonic flavor score: 9 = Like extremely, 5 = Neither like nor dislike, 1 = Dislike extremely.
[c] Degrees Brix corrected for temperature and acid
[d] As citric acid
[e] As determined by Mansell, University of South Florida, Tampa (Mansell and Weiler, 1980)

Table 2—Model system flavor scores – levels of significance as determined using analysis of variance

Sample (Mean flavor score)	1 (6.2)	2 (5.9)	3 (5.9)	4 (5.1)	5 (4.9)	6 (5.0)	7 (3.7)
1 (6.2)	—	NS	NS	***	***	***	***
2 (5.9)	—	—	NS	*	**	*	***
3 (5.9)	—	—	—	*	**	*	***
4 (5.1)	—	—	—	—	NS	NS	***
5 (4.9)	—	—	—	—	—	NS	***
6 (5.0)	—	—	—	—	—	—	***
7 (3.7)	—	—	—	—	—	—	—

* Significantly different at the 95% confidence level
** Significantly different at the 99% confidence level
*** Significantly different at the 99.9% confidence level
NS = Not significantly different at 95% confidence level

Table 3—Model system flavor comments

Sample	OJ/PW/Dil[a]	Mean flavor score[b]	Individual flavor scores below 5	Total negative comments	Summarized comments
1	100/ 0/ 0	6.2	2	15	Very slightly below average-flavored Florida commercial juice, slightly on the bland side
2	85/ 15/ 0	5.9	2	13	Very slightly below average-flavored Florida juice with very slight heated or processed flavor.
3	80/ 20/ 0	5.9	3	15	Same as above with 3 panelists also commenting on presence of nondescript off-flavor.
4	50/ 30/20	5.1	9	22	Too sweet, bland, and watery with 2 nondescript off-flavor comments.
5	30/ 60/10	4.9	11	33	Very bland, moderately too sweet, and slightly too watery. Other comments: atypical orange juice (4 panelists), tastes like pulp-wash (3), peculiar mouth feel (3), and several other diverse negative comments.
6	25/ 65/10	5.0	13	27	Moderately bland, slight heated or processed flavor, slight watery and several other diverse negative comments.
7	0/100/ 0	3.7	22	46	Off-flavored (11 nondescript off-flavor comments), very bland (10 comments), heated (8) product and 17 other diverse negative comments.

[a] Percent orange juice, pulpwash and dilution of sample
[b] For 27 taste panelists/sample

that sample 3 with 20% added PW had three comments on the presence of nondescript off-flavor. Dilutions (with sugar solutions) of samples 4, 5 and 6 were evident in the panelists' comments of too sweet and watery. One-hundred percent PW (sample 7) having the greatest number of negative comments and flavor scores below 5 did not have the severe off-flavor comments that might have been expected, especially as related to bitterness and harshness which Fellers (1984) found in 73% of the PW samples studied. This may be due in part to the PW sample possibly being derived from a late-season variety with lower limonin concentrations in more mature fruit. Fellers (1984) pointed out the significantly better flavor (but still of poor flavor quality) of late-versus early-mid season PW products.

Fig. 2 presents the visible and ultraviolet (VIS/UV) absorption curve obtained from sample 1 (100/0/0). Visible absorption appeared normal with good resolution (Petrus and Attaway, 1980). Ultraviolet absorption appeared normal with slight resolution at 280 nm and a slightly weak 245 nm absorbance. Absorption at 221 nm appeared normal. VIS/UV absorption appeared characteristic of orange juice obtained mainly from the Florida Valencia variety. Sum of absorbance (at 444, 325 and 280 nm) was 2.421 and ratio of absorbance (at 444/325 nm) was 0.137 or about average for Florida-produced orange juice (Petrus and Attaway, 1980).

Fig. 3 presents the VIS/UV absorption curve for sample 4 (50/30/20). Visible absorbance appeared slightly weak and lacked resolution as compared to the curve in Fig. 2. Ultraviolet absorption revealed a well-resolved peak at 283 nm, and a shift from 221 to 218 nm. Qualitatively, the absorption curve was typical of PW addition accompanied by dilution (shift to 218 nm) of the product. However, the sum of absorbance was 2.426 absorbance units or about average for Florida-produced orange juice (Petrus and Attaway, 1980). This resembles orange juice that had been

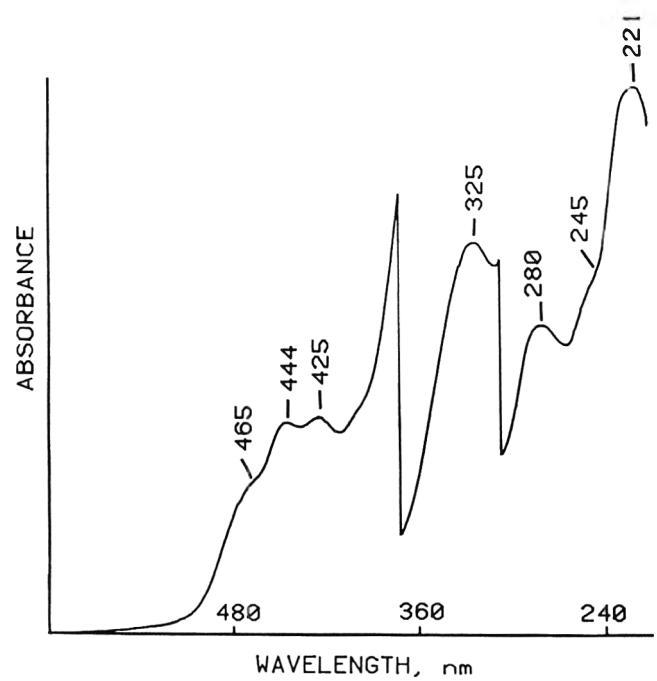

Fig. 2—Visible and ultraviolet absorption curve obtained from sample 1.

carefully adulterated with PW so that the numerical sum of absorbance would remain well within the range observed for an authentic orange juice. The ratio of absorbance was 0.088 and appeared somewhat low indicating PW addition.

Fig. 4 presents the absorption curve obtained from sample 7 (0/100/0). The curve was typical of PW having weak, unresolved visible absorbance and strong ultraviolet ab-

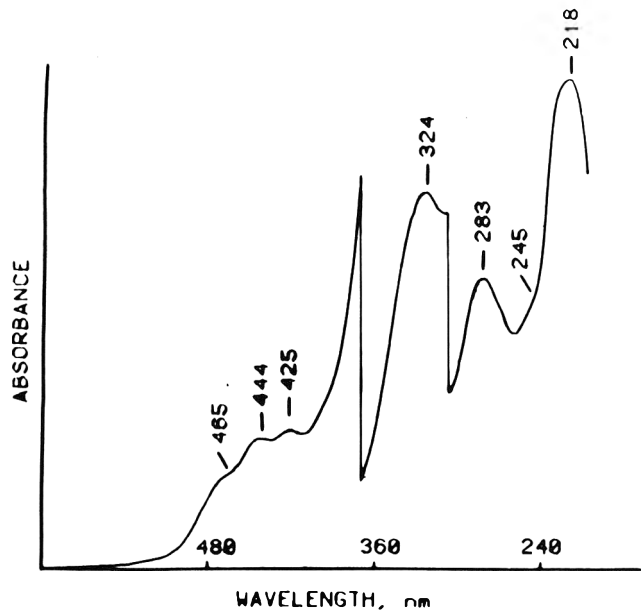

Fig. 3—Visible and ultraviolet absorption curve obtained from sample 4.

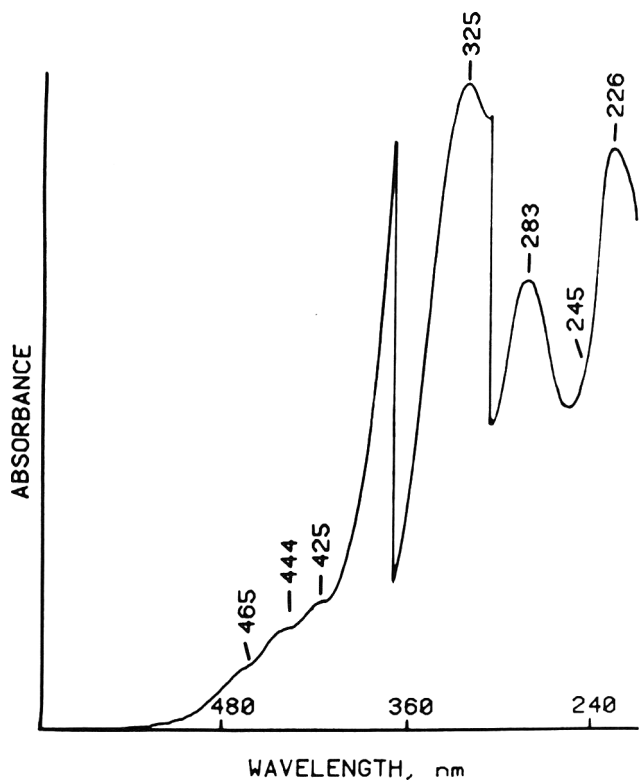

Fig. 4—Visible and ultraviolet absorption curve obtained from sample 7.

sorbance with a well resolved peak at 283 nm. The sum of absorbance was 3.595 absorbance units and ratio of absorbance was 0.041 or about average for 100% PW (Petrus and Attaway, 1980).

Fluorescence excitation spectra obtained from samples 1, 4 and 7 are presented in Fig. 5. The emission maximum was determined and monochromator set at 340 nm. The excitation curve was then obtained by scanning from 200 to 350 nm. Other spectra (Petrus and Attaway, 1980) were also obtained and are important for sample characterization, but for brevity only one (292 nm excitation with 340 nm emission) of the excitation spectra will be discussed.

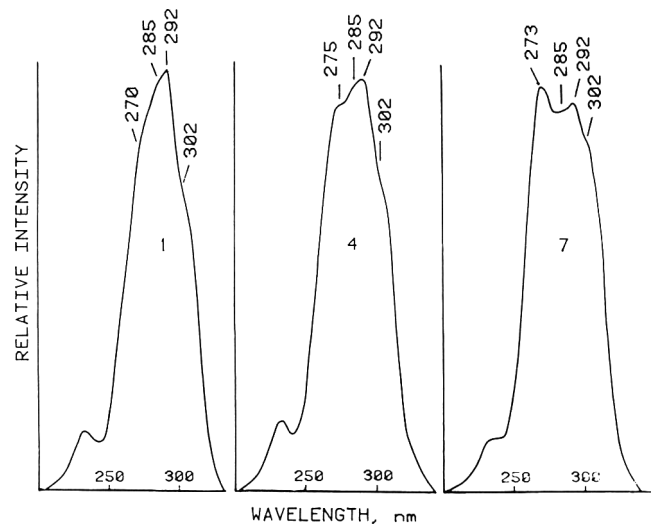

Fig. 5—Fluorescence excitation curves obtained from samples 1, 4 and 7 with emission monochromator at 340 nm.

Qualitatively, fluorescence excitation curve for sample 1 (100/0/0) was characteristic (maximum at 292 nm, slight inflection at 270 nm, and fluorescence decreasing smoothly to an inflection at 302 nm) of authentic Florida-produced orange juice and indicated the product to have been derived mainly from the Valencia variety. Qualitatively both the fluorescence excitation and VIS/UV absorption curves indicated authentic product derived mainly from the Valencia variety.

Qualitatively, the fluorescence excitation curve obtained for sample 7 (0/100/0) was characteristic (maximum at 273 nm, minimum at 285 nm and high 302 nm inflection) of PW containing orange juice, and indicated the product to have been derived mainly from the Valencia variety.

Qualitatively, the fluorescence excitation curve for sample 4 (50/30/20) revealed a strong shoulder at 275 nm. The curve appeared deformed when compared to sample 1. This was typical of PW addition to orange juice products. The qualitative evaluations of the fluorescence and VIS/UV absorption curves were complementary, both indicating PW addition.

Spectra of the remaining model-system samples were similarly evaluated with comparable results.

Analysis of the model system has shown that the flavor, color number, percent acid, degrees Brix to percent acid ratio, limonin, and absorption and fluorescence spectral characteristics of an orange juice product change with increasing amounts of added PW.

Other Types of Adulteration

Florida processors import large quantities of bulk non-Florida concentrated orange juice for manufacturing which may be blended in the production of Florida FCOJ and other products.

Fig. 6 presents the VIS/UV absorption curve of a selected COJFM imported product. The sample showed fairly strong visible absorption, but lacked resolution. Ultraviolet absorption appeared similar to a product obtained from the Valencia orange variety. The sample had an absorbance sum of 2.437 and ratio of 0.157, well within the ranges for pure orange juice (Petrus and Attaway, 1980). However, maximum visible absorption at 425 nm, slightly too much resolution at 279 nm, and a shift to 216 nm (indicating dilution), did not fit the established characteristics (Petrus and

Attawya, 1980). Investigation of the fluorescence curves also indicated Valencia characteritsitcs. The excitation curve (excitation at 295 with emission at 340 nm) presented in Fig. 7 was typical of PW or PW addition, showing a very deformed curve with maximum at 272 nm and a strong shoulder at 302 nm.

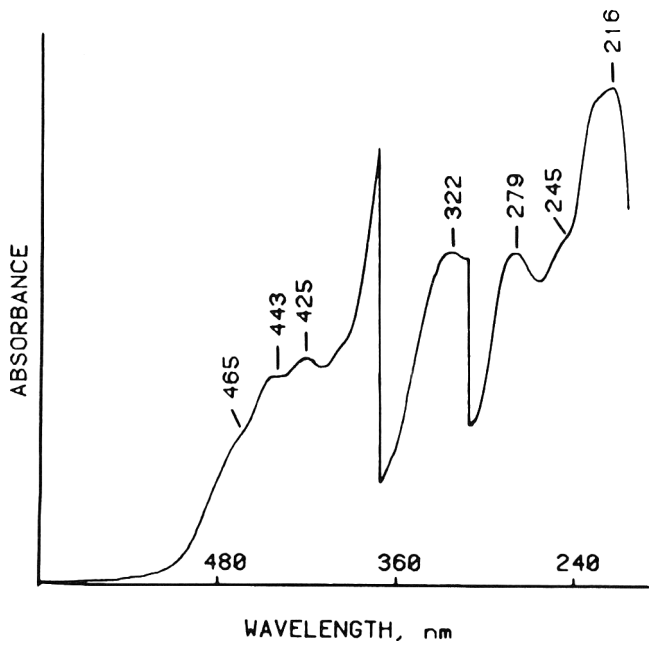

Fig. 6—Visible and ultraviolet absorption curve obtained from bulk concentrated orange juice for manufacturing imported by some Florida processors.

The absorbance sum of 2.437 (about average for pure orange juice, Petrus and Attaway, 1980) may be explained by sample dilution, except for the relatively high absorbance ratio of 0.157. Further investigation produced the fluorescence excitation and emission curves labelled "B" presented in Fig. 8. Curves labelled "A" were normal fluorescence background excitation and emission curves obtained for pure orange juice. Research of the fluorescence characteristics of the colorant turmeric (curcumin) or turmeric-spiked orange juice, produced fluorescence curves almost identical to the "B" curves, with excitation at 435 nm and emission maximum at 520 nm. The relatively high absorbance ratio of 0.157 could then be explained by the addition of color to the product. It was concluded that the imported bulk product (and many others investigated) was adulterated with PW and added colorant.

Fig. 9 revealed another type of adulteration; namely addition of an undeclared preservative. The sample investigated was a single-strength orange juice from concentrate obtained from the U.S. retail market packed outside Florida. Visible absorption appeared normal. Ultraviolet absorbance at 325 nm appeared weak (indicating dilution). Absorbance at 280 and 245 nm were obscured by the strong absorbance at 254 nm. Previous investigations (Petrus, unpublished data) have identified the absorbance at 254 due to added sorbate preservative. Confirmation of the presence of sorbate preservative in this sample was made at the United States Department of Agriculture, Winter Haven, Florida using a high performance liquid chromatography method (Fisher, 1983).

CONCLUSIONS

THE RESULTS showed that the addition of PW (with or without accompanying dilution) to FCOJ, in a model

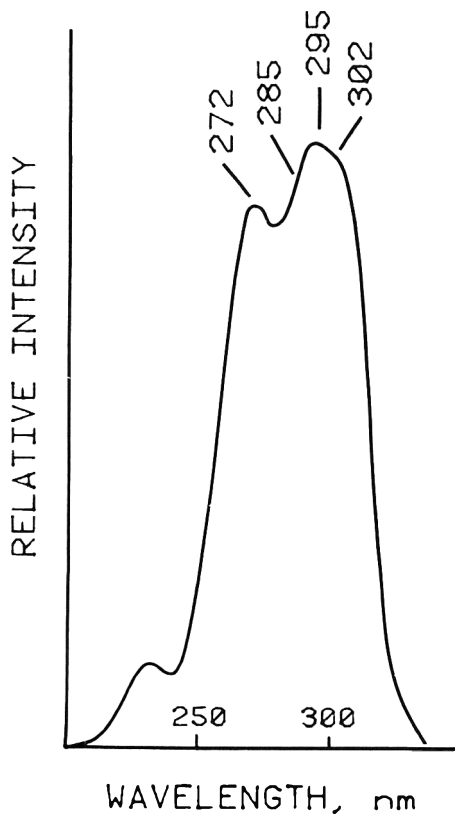

Fig. 7—Fluorescence excitation curve obtained from bulk concentrated orange juice for manufacturing imporated by Florida processors with emission monochromator at 340 nm.

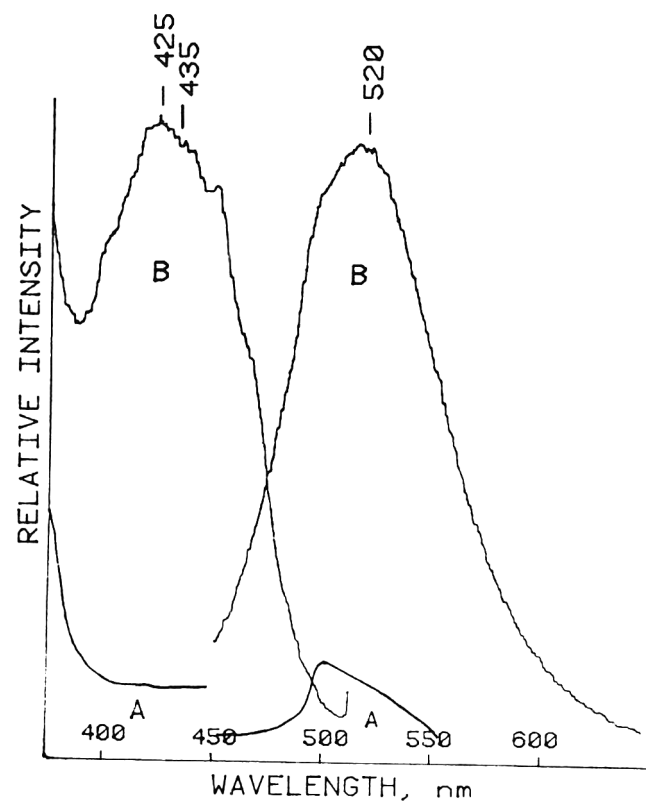

Fig. 8—Turmeric fluorescence excitation (435 nm) and emission (520 nm) curves obtained from bulk concentrated orange juice for manufacturing imported by Florida processors.

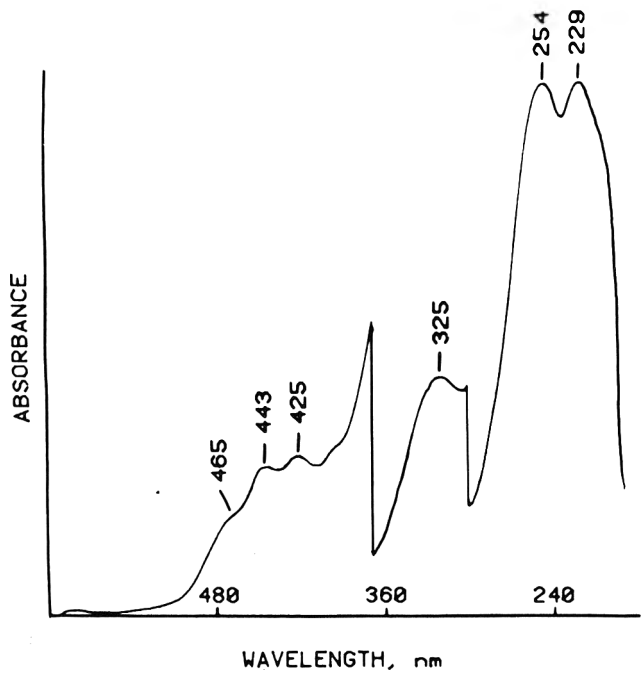

Fig. 9—Visible and ultraviolet absorption curve obtained from retail single-strength orange juice from concentrate showing sorbate preservative absorption at 254 nm.

system, in general lowered the flavor scores, lowered the color numbers and percent acid and increased degrees Brix to percent acid ratios and limonin concentration.

Significant differences between mean flavor scores of samples were evident. Individual flavor scores below 5 (neither like nor dislike) and number of negative comments per sample increased with increasing amounts of added PW to the orange juice regardless of dilution with sugar solutions. It was also observed that addition of 30, 60 and 65% added PW to FCOJ accompanied by dilution with sugar solutions may result in unacceptably flavored products. It was observed in this system that starting with a good-colored product, various combinations of orange juice, PW and dilution still resulted in a U.S. Grade A color score of 36. Visible and ultraviolet absorption, and fluorescence spectral characteristics indicated the presence of PW and dilution of the products in the model system.

Bulk concentrated orange juice for manufacturing imported by some Florida processors was determined to contain added PW and turmeric as a colorant, by VIS/UV and fluorescence characteristics.

A retail SSOJ from concentrate produced outside Florida was found (by VIS/UV absorption characteristics) to contain sorbate preservative. There was no label declaration of the added preservative.

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Preparation of a Yogurt-like Product Containing Egg White

J. C. C. LIN and F. E. CUNNINGHAM

ABSTRACT

The fermentation of liquid egg white by lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, to develop a new yogurt-type product has been studied. Egg white was prepared by pasteurization, blending, and neutralization to minimize antimicrobial activity, and by combining additives to make the media more suitable for growth of lactic acid bacteria. Skim milk, gums (guar gum, CMC, xanthan gum), soymilk, and glucose were studied. Optimum results were obtained when 47.4% egg white was combined with 28.4% alkali treated soymilk, 19.0% skim milk, 1.9% glucose, 2.8% sucrose, 0.5% xanthan gum, and 0.01% vanilla extract. The composition per 100 gram of the final product included 7.52% protein, 0.57% fat, and 62 calories. Microbiological and sensory tests showed the product to be free of pathogens and to have an extended shelf life at refrigeration temperature.

INTRODUCTION

FERMENTATION of egg products for desugaring before drying has long been practiced by the egg industry. However, fermentation of liquid egg white with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for developing a yogurt type product has not been extensively researched.

Stewart et al. (1943) fermented egg white with a pure culture of *Streptococcus*, and noted some flavor changes; however, the inoculation of whole egg with lactic *Streptococci* showed better results (Hopkins et al., 1947). Many suggestions have been made for improving egg white fermentation, such as adding a high inoculum of resting cells (Kaplan et al., 1950), enriching the egg white with yeast extract (Ayres, 1958), and lowering the pH to 5 to achieve better fermentation (Bean et al., 1963).

In order to ferment an egg white product successfully, the antimicrobial activity of egg white must be negated. Seideman et al. (1963) found that when egg white at pH 9 or above was heated to 60°C for 3 min a significant decrease in activity of lysozyme occurred. Nath and Baker (1973) explained that dilute egg white had less antimicrobial activity because the dilution made more nutrients available, not because the concentrations of conalbumin and lysozyme were lowered. According to Yadav and Vadehra (1977), in fresh egg white the physical structure of its protein was probably a primary barrier to bacterial growth, besides the other factors such as lysozyme, avidin, and lack of free water and nutrients.

In a recent study, Cunningham and Francis (1982) diminished the antimicrobial activity of egg white and produced a successful egg-cheddar cheese product by fermenting whole egg or egg white and low-fat milk. Their results pointed out the feasibility of developing a new product through fermentation of egg white.

The objective of this study was to develop a high protein, low calorie yogurt-like snack food containing egg white.

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

MATERIALS & METHODS

Preparation of egg white

Eggs were obtained from Kansas State University's poultry farm and were cleaned, broken, and separated using a hand separator. After the separation of albumen from yolk, the chalazae cords were removed with tweezers and discarded to prevent high lysozyme content (Baker et al., 1959). The pH of the liquid egg white was between 8.9 and 9.3.

Egg white was heat treated in a sterile Erlenmeyer flask fitted with a rubber stopper and a thermometer. A thermomagnetic stirrer (Fisher Thermix) was equipped with a water bath for pasturization. The egg white was stirred vigorously during heating with a magnetic stirring batr. Temperature was increased within 30 min to 60°C and held for 3 min (Cunningham et al., 1965). Following heat treatment, the flask was placed in an ice water bath and cooled to room temperature. Pasteurized egg white was blended for 15 sec in a sterile Waring Blendor (Yaday and Vadehra, 1977), and neutralized by adding lactic acid (85%) drop by drop until a pH of 7.00 ± 0.02 or 6.80 ± 0.02 was obtained. A pH meter (Fisher Accumet pH meter, Model 292) was used for the pH measurement.

Culture and media

Lactobacillus bulgaricus NRRL B-548 and *Streptococcus thermophilus* ATCC 14485 were used as starter cultures, which were maintained in sterile 12% reconstituted skim milk by transferring 1% inoculum every other week. A 2% inoculum of each strain containing from 1.0×10^9 to 3.0×10^9 CFU/mL was prepared for this study.

Glucose enrichment

Aliquots (80 mL) of treated egg white (pH 7.0) were dispensed into sterile beakers (100 mL) and separated into two parts. One part was mixed with 3.2 mL sterile glucose solution (50% w/v) to obtain a 2% glucose enrichment, the other aliquot, without glucose served as controls. The 2% pure cultures were added to both parts and incubated at 37°C for 24 hr. Portions of egg white were removed periodically (4 hr) and plated on MRS agar and Lee's agar (Sandine et al., 1976), and the pH of the solution was measured also.

Addition of liquid skim milk to egg white

Skim milk powder was obtained from Mid-America Farms (Springfield, MO) and reconstituted to 12% total solid skim milk, pasteurized at 71°C for 15 sec, cooled to room temperature and mixed with treated egg white (pH 6.8). Samples were prepared in which the ratios of egg white to the skim milk were as follows: 100:0, 80:20; 60:40; 50:50; 40:60; 20:80; 0:100.

Starter cultures were inoculated into each group and mixed well and incubated at 37°C. After 6 to 20 hr fermentation, pH, viscosity (determined by a Brookfield viscometer RVF type), and appearance of the egg-product were monitored.

Addition of gums as texture improver

Three different gums were used individually in this study to adjust product firmness. Sodium carboxymethylcellulose (CMC, E.I. Du Pont De Nemours and Co. Grade: p-75-L), guar gum, and xanthan gum (Kelco Co.) were used at 0.1%, 0.5%, and 1.0% level. Treated egg white was mixed with pasteurized skim milk at a ratio of 50:50 (v/v). Gum powder was added and stirred until completely dissolved. Starter cultures were added to the mixture. After 6 hr fermentation at 37°C, samples were refrigerated for 4 hr, then evaluated for appearance of the curd, pH, and viscosity.

Addition of soymilk

Nitrogen-packed 1-lb bags of soybeans were obtained from Arrowhead Mill Inc. (Hereford, TX). Soymilk was prepared according to the method Hackler and co-workers (1965) and heated at 93°C for 60 min and cooled to 37°C. The treated egg white (pH 6.8) was mixed with soymilk in the following proportions: 80:20; 60:40; 50:50; 40:60; 20:80. Two percent glucose was added to each set of samples, starter cultures were added, and samples were tested after fermentation.

Addition of skim milk and soymilk

The treated egg white (pH 6.8), reconstituted skim milk (12% total solids), and heated soymilk were prepared as previously described and combined in the proportions shown in Table 1. All sets of samples contained 2% glucose with or without 0.5% xanthan gum. Starter cultures were added to each set and samples were incubated at 37°C for 6 hr, before noting pH, viscosity, and appearance of curd.

Addition of skim milk and alkali treated soymilk

Egg white was pasteurized and acidified with citric acid (32.6%, w/w) to pH 6.8 and mixed with skim milk (12% total solids) and alkali treated soymilk. Alkali treated soymilk was prepared using Badenhop and Hackler's (1970) method. Blends of egg white:skim milk:soymilk were prepared as follows: (1) 50:10:40; (2) 50:20:30; (3) 50:30:20. Sample treatments were the same as previously described.

Addition of flavoring and sweetener

The addition of skim milk and alkali treated soymilk study was modified by adding 0.01% vanilla extract (alcohol 35%, Virginia Dare Extract Co., Inc., Brooklyn, NY) and 3% sucrose as a sweetener.

Sensory evaluation

Three sets of samples were prepared containing a combination of 47.4% egg white, 28.4% soymilk, 19.0% skim milk, 1.9% glucose, 2.8% sucrose and 0.01% vanilla extract. One set of samples contained alkali-treated soymilk; a second set contained traditional soymilk (both sets contained 0.5% xanthan gum). A third set of samples was the same as the first set except that they contained 1.0% added xanthan gum rather than 0.5%.

The taste panel was composed of six panelists experienced in dairy product evaluation in the Animal Sciences & Industry Dept., KSU. The panelists were asked to rate the samples on a nine-point hedonic scale in which 9 = "like extremely" and 1 = "dislike" extremely," and to write comments about the product to reflect acceptability. The samples were presented in random order, and coded with random three-digit numbers according to suggestion published by IFT Sensory Evaluation Division (1981). All data were analyzed using the analysis of variance procedures of Snedecor and Cochran (1980).

Composition analysis

The composition of samples containing alkali treated soymilk and 0.5% xanthan gum were analyzed by AOAC methods (1980) to determine the content of moisture, protein, fiber, ash, and fat. The carbohydrate value was calculated by subtraction.

Table 1—Ratio of treated egg white (pH 6.8), reconstituted skim milk (12% total solids), and heated soymilk (93°C for 60 min) for sample preparation

Sample	Egg White %	Skim Milk %	Soymilk %
1	50	10	40
2	50	20	30
3	50	30	20
4	50	40	10
5	60	10	30
6	60	20	20
7	60	30	10
8	70	10	20
9	70	15	15
10	70	20	10
11	80	10	10

Test for salmonella and product stability

The samples containing alkali-treated soymilk and 0.5% xanthan gum were diluted 10X in lactose broth, transferred into tetrathionate broth, then incubated for 24 hr at 37°C. Samples were streaked onto brilliant green agar and incubated at 37°C for 24 hr to test for *Salmonella* (Difco Manual, 1977). After 7, 14, and 21 day's storage at refrigeration temperature (4°C), the flavor, appearance, pH, and mouth feel were also examined.

RESULTS & DISCUSSION

Growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in egg white

Growth rate curves of *L. bulgaricus* and *S. thermophilus* in egg white are shown in Fig. 1, 2 and 3. Viable cells of *L. bulgaricus* NRRL B-548 (Fig. 1) increased rapidly during 4 to 16 hr incubation and the pH dropped significantly after 8 hr. The addition of 2% glucose did improve growth and acid production. The final pH of the fermentation egg white with 2% glucose was 4.8. The numbers of viable cells of *S. thermophilus* ATCC 14485 decreased during 24 hr fermentation (Fig. 2). Adding 2% glucose to egg white did not improve growth or acid production. However, when the 2 strains were combined (Fig. 3) the total numbers of *S. thermophilus* increased after 16 hr. This probably was due to the pH drop in the medium caused by *L. bulgaricus*, making the environment more suitable for *S. thermophilus* to grow. Similar results were reported by Bean et al. (1963), who suggested that egg white be adjusted to pH 5 to achieve better fermentation.

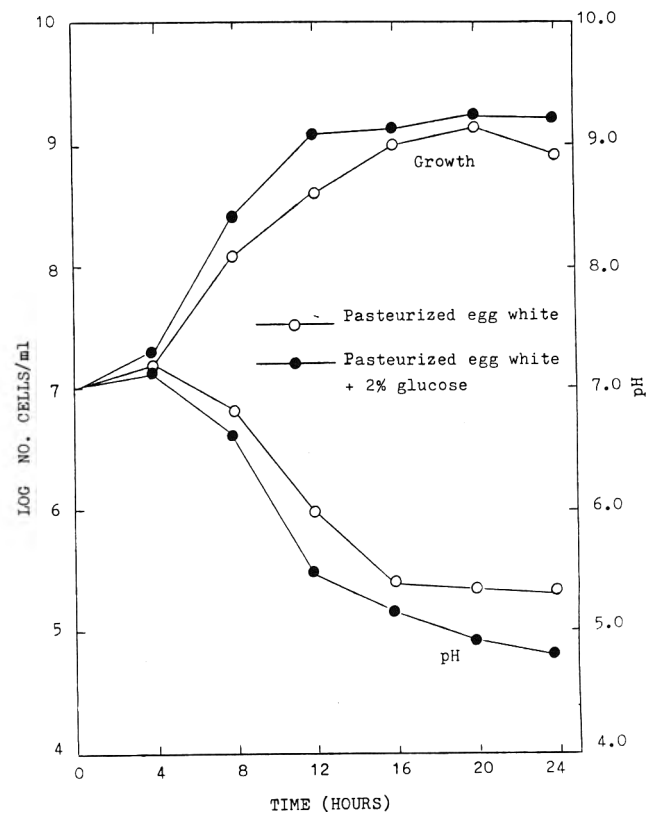

Fig. 1—Growth curve and acid production of L. bulgaricus NRRL B-548 in pasteurized, neutralized egg white at 37°C.

Addition of liquid skim milk to egg white

The results of combining egg white with skim milk at different ratios and fermenting the mixtures are shown in Fig. 4 and Fig. 5. Samples 5 and 6 showed a better curd formation than the other blends. After 10 hr incubation there was no significant improvement in the appearance, or viscosity of the test samples, but the pH values dropped slightly. Fig. 5 shows the viscosity of the fermented egg white-skim milk blends. Only samples 6 and 7 became viscous. After 10 hr incubation, samples 6 and 7 showed a drop in viscosity (Fig. 5), probably due to the low pH which caused formation of whey.

Since the cultures employed in this study are customarily used for making yogurt, it is understandable that samples with more skim milk had lower pH and higher viscosity. In order to develop a suitable product, containing at least 50% egg white, some method must be used to increase viscosity and improve curd formation in the fermented product.

Table 2—Influence of gum on viscosity, flavor, and pH of egg white/skim milk:50/50 fermented at 37° C for 6 hr[a]

Gum	Level %	Viscosity (cps)	Flavor	pH
Guar	0.1	700	plant odor	4.9
Guar	0.5	1300	strong plant odor	4.8
Guar	1.0	4000	strong plant odor	4.9
CMC	0.1	800	odorless	4.9
CMC	0.5	300	odorless	4.8
CMC	1.0	1200	odorless	4.9
Xanthan	0.1	900	yogurt-like flavor	4.7
Xanthan	0.5	1500	yogurt-like flavor	4.7
Xanthan	1.0	2500	xanthan gum's flavor	4.8

[a] 2% L. bulgaricus and 2% S. thermophilus were inoculated for the fermentation.

Addition of gums to improve texture

The results of adding gums to egg white viscosity, flavor, and pH are shown in Table 2. Adding gums at 1% gave higher viscosities, but when the level was reduced to 0.5% only xanthan gum produced a product with satisfactory viscosity. The pH of the samples with added gum ranged from 4.7 - 4.9.

Addition of soymilk to egg white

The fermentation of combined soymilk and egg white resulted in only a slight pH drop, however, the more soymilk added, the lower the final pH (Fig. 6). In sample 5, the final pH was 4.3 which was the lowest value achieved. The high soymilk content also produced a firmer product. In Fig. 7, sample 5 showed a very high viscosty; however, when the soymilk content was less than 50%, the products were watery. In order to improve viscosity and lower pH, a combination of egg white, soymilk, and skim milk were subsequently used.

Addition of skim milk and soymilk to egg white

The pH values and viscosities of egg white combined with skim milk, soymilk, and 2% glucose after fermentation for 6 hr at 37°C are shown in Table 3. The egg white content affected the final acidity and viscosity. The samples containing 50% egg white achieved low pH and satisfactory consistency. Adding 0.5% xanthan gum did improve the curd and pH. Although the samples 1, 2, 3, and 1A, 2A,

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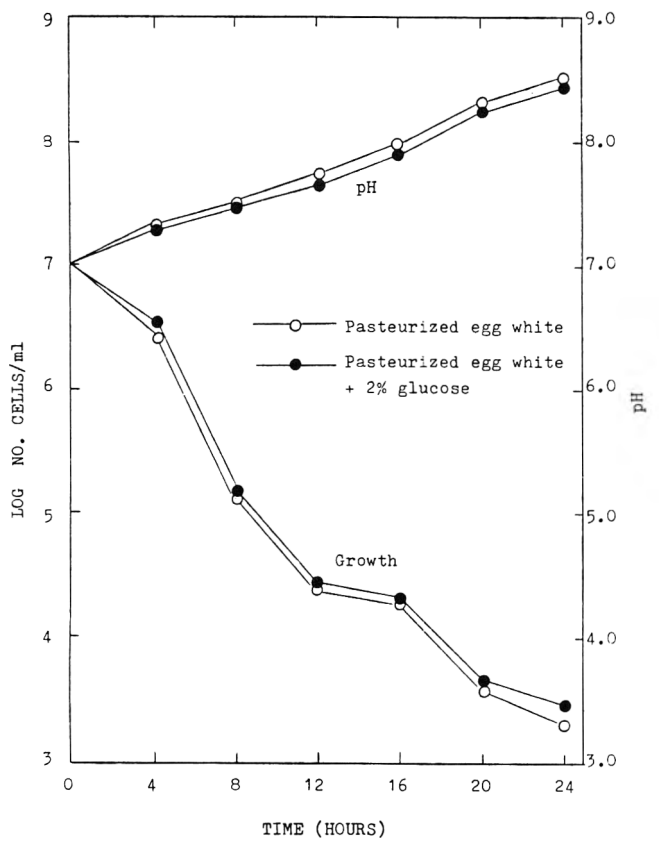

Fig. 2—Growth curve and acid production of S. thermophilus ATCC 14485 in pasteurized, neutralized egg white at 37° C.

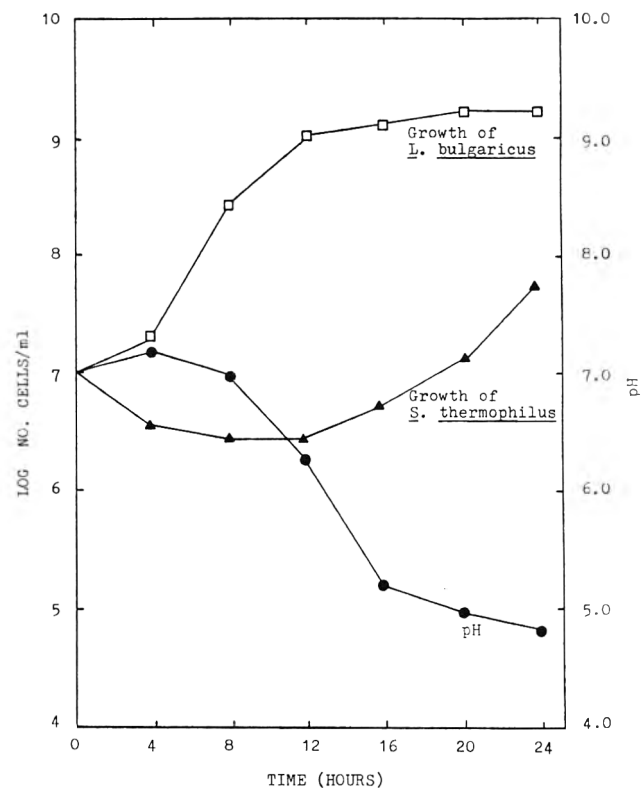

Fig. 3—Growth curve and acid production of L. bulgaricus NRRL B-548 and S. thermophils ATCC 14485 in pasteurized neutralized egg white with 2% glucose at 37° C.

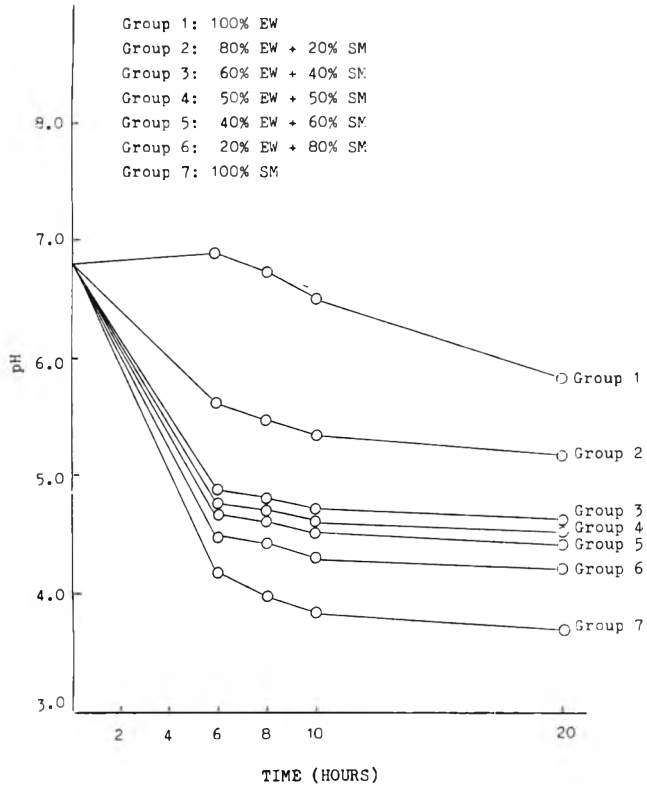

Fig. 4—pH value of the combination of egg white (EW) and skim milk (SM) when fermented with 2% L. bulgaricus and 2% S. thermophilus at 37°C.

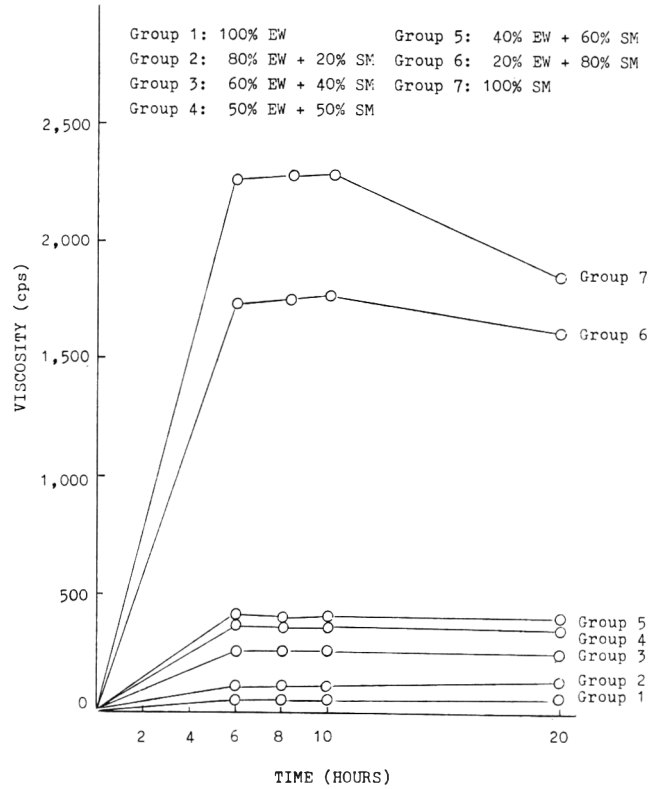

Fig. 5—Viscosity of the combination of egg white (EW) and skim milk (SM) when fermented with 2% L. bulgaricus and 2% S. thermophilus at 37°C.

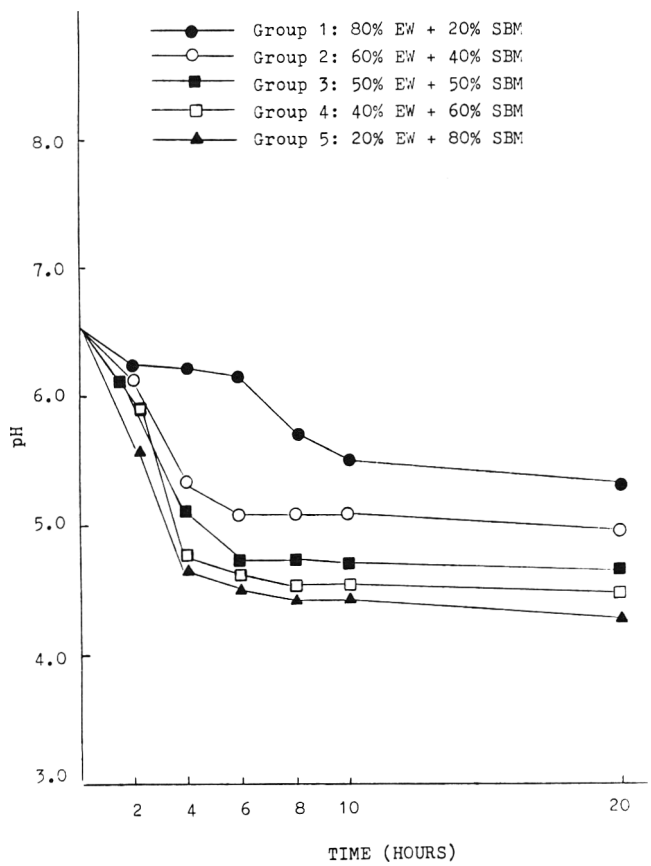

Fig. 6—pH value of the combination of egg white (EW), soymilk (SBM) and 2% glucose fermented by 2% L. bulgaricus and 2% S. thermophils at 37°C.

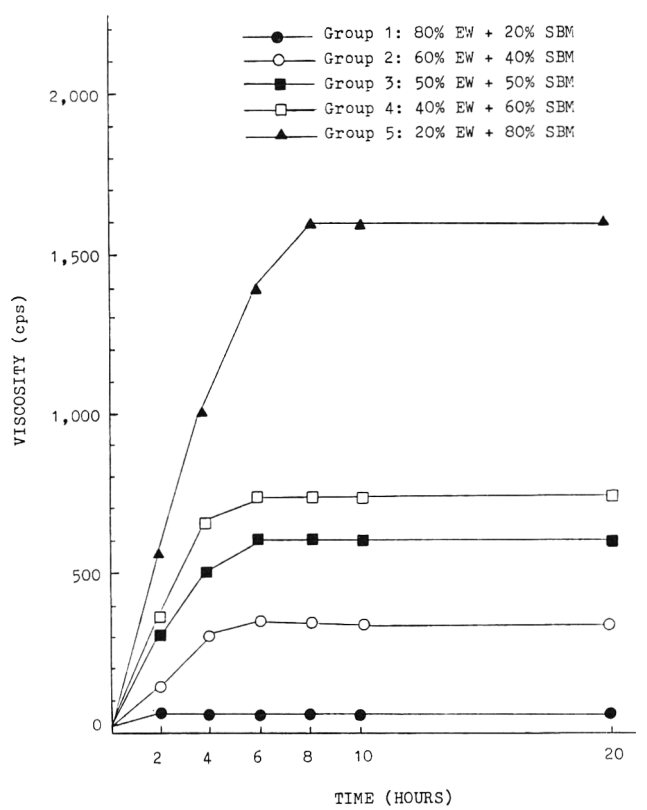

Fig. 7—Viscosity of the combination of egg white (EW), soymilk (SBM) and 2% glucose fermented by 2% L. bulgaricus and 2% S. thermophilus at 37°C.

Table 3—pH value and viscosity of fermentation of egg white (EW) with the combination of skim milk (SM), soymilk (SBM) and 2% glucose at 37°C for 6 hr[a]

Sample	Combination			Results	
	EW%	SM%	SBM%	pH	Viscosity (cps)
1	50	10	40	4.9	1,500
2	50	20	30	4.9	1,000
3	50	30	20	4.9	700
4	50	40	10	5.0	450
5	60	10	30	5.2	475
6	60	20	20	5.1	550
7	60	30	10	5.2	375
8	70	10	20	5.4	250
9	70	15	15	5.5	220
10	70	20	10	5.2	330
11	80	10	10	5.9	150
1A[b]	50	10	40	4.7	2,200
2A	50	20	30	4.6	2,150
3A	50	30	20	4.7	1,200
4A	50	40	10	4.8	725
5A	60	10	30	5.0	1,200
6A	60	20	20	4.9	1,150
7A	60	30	10	5.1	525
8A	70	10	20	5.1	425
9A	70	15	15	5.1	425
10A	70	20	10	5.1	425
11A	80	10	10	5.5	400

[a] 2% L. bulgaricus and 2% S. thermophilus were inoculated for the fermentation
[b] "A" series represent the addition of 0.5% xanthan gum.

3A in Table 3 showed lower pH and better viscosity than the other samples, the mouth feel of those products were astringent. In order to eliminate these objectionable characteristics, citric acid was used as an acidifying agent for egg white (Galluzzo et al., 1974) and soymilk was treated with NaOH (Badenhop and Hackler, 1970).

Addition of skim milk and alkali-treated soymilk to egg white

Fermentation of samples containing egg white, skim milk and alkali treated soymilk resulted in products free of the soy flavor (Table 4). The higher pH and lower viscosities were due to the sodium hydroxide treatment of the soymilk.

Sensory evaluation

Sensory evaluations for flavor of 3 sets of samples of the egg white fermented product described in Materials & Methods, are shown in Table 5. The first set, prepared with 28.4% alkali treated soymilk, had a higher panel score than the other samples; the second set made with traditional soymilk was scored down for flavor; the third set was the same as the first set except that 1.0% of xanthan gum was added.

Composition analysis

A yogurt-like egg product was prepared, containing egg white (47.4%), alkali treated soymilk (28.4%), skim milk (19%), sucrose (2.8%), xanthan gum (0.5%) and vanilla extract (0.1%). The product contained 7.52% protein, 6.74% carbohydrates (mainly mono- and di-saccharides), 0.57% fat, 0.26% fiber, and 0.77 ash. There were 62 calories per 100g product.

Test for *samonella* and product stability

Salmonella tests of the new fermented product were all negative. The storage test at refrigeration temperature (4°C) showed no significant difference in flavor, appearance, pH, and mouth feel after 7 and 14 days. A slight syneresis after 21 day's storage was observed as a small amount of whey-like liquid on top of the product. These tests indicate that the quality of the product can be assured for 3 wk of storage.

Table 4—pH value and viscosity of egg white[a] *(EW) combined with skim milk (SM), soymilk*[b] *(SMB) and 2% glucose fermented*[c] *at 37°C for 6 hr*

Sample	Combination			Results	
	EW%	SM%	SBM%	pH	Viscosity (cps)
1	50	10	40	4.9	1,100
2	50	20	30	5.0	700
3	50	30	20	4.9	750
1A[d]	50	10	40	4.8	1.400
2A	50	20	30	4.7	1,450
3A	50	30	20	4.8	1.000

[a] Egg white was acidified by citric acid.
[b] Soymilk was treated by Badenhop and Hackler's methods (1970).
[c] 2% L. bulgaricus and 2% S. thermophilus were inoculated for the fermentation.
[d] "A" series represent the addition of 0.5% xanthan gum.

Table 5—Average sensory scores of new egg white fermented product[a]

Samples	Panel score
1st set[b]	6.3[e] ± 1.2[f]
2nd set[c]	4.8 ± 1.6
3rd set[d]	3.7 ± 0.8

[a] Score 9 for sample was "liked extremely," score 5 was "neither liked nor disliked," score 1 was "disliked extremely."
[b] Prepared with 28.4% alkali treated soymilk.
[c] Prepared with 28.4% traditional soymilk.
[d] Modified group 1 with 1.0% xanthan gum.
[e] Mean value (n = 6).
[f] Standard deviation of mean (n = 6).

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Impedimetric Estimation of Coliforms in Dairy Products

R. FIRSTENBERG-EDEN, M. L. VAN SISE, J. ZINDULIS, and P. KAHN

ABSTRACT

An impedance method was developed for the estimation of coliforms in raw and pasteurized milk, heavy cream, and ice cream mix. Impedance detection times (IDTs) in CM, a medium developed for the impedimetric detection of coliforms, were compared to confirmed Violet Red Bile Agar (CVRBA) plate counts. Correlations of 0.91-0.95 between the two methods were obtained for the four dairy products. The line equations of these relationships were similar for all products. An IDT shorter than 9 hr was indicative of coliform levels >10/mL, while an IDT longer than 12 hr was indicative of levels <10/mL. The impedimetric estimation of coliforms provided the same information as CVRBA counts from dairy products, offering a savings in time and labor.

INTRODUCTION

THE OCCURRENCE of coliforms in dairy products has received considerable attention. Although the standard methods of coliform enumeration are well established, the applicability of the fecal indicator concept itself in foods is still being discussed (Krumperman, 1983). This is due in part to the ubiquitous nature of the coliforms in the environment, which places some constraints on their significance in foods. Nevertheless, the food industry has a need to monitor the microbial safety of its products and has adopted the coliform test as one means of fulfilling this need.

The different methods used to monitor coliforms in foods have several problems. The errors of the plate count are well known (Fowler et al., 1978), and the use of Violet Red Bile Agar (VRBA), the recommended medium for dairy products (Marth, 1978), further suffers from the requirement to discriminate colonies on the basis of size. The effect of elevated temperature on stressed coliforms as a result of the pour plating procedure is another problem. This problem has been avoided with the use of membrane filtration. However, vacuum filtration may itself be a potential cause of stress. The MPN procedure has fewer stress-related problems, but is logistically awkward. While several methods exist to avoid these problems (Ray and Speck, 1978; Brodsky et al., 1982), they also add to the complexity of the procedure. This complexity is an important consideration since coliform tests are frequently done in stages; i.e., presumptive, confirmed, completed, and the everyday user needs to monitor many samples in as short a time as possible.

Several alternatives to the standard methods of coliform analysis are currently available. One method employs a hydrophobic grid-membrane filtration (Brodsky et al., 1982). A second method detects enterotoxigenic *E. coli* by DNA colony hybridization (Hill et al., 1983). Another method combines MPN with impedance measurements (Martin and Selby, 1980).

Recently, a new coliform medium (CM) was developed specifically for use with impedance measurements (Firstenberg-Eden and Klein, 1983). The impedance method with CM provides earlier coliform detections than standard methods and is specifically designed to monitor large numbers of samples with less labor than standard methods. CM provides better impedance signals than conventional coliform media and is more selective than lauryl tryptose broth and Violet Red Bile Agar. Tests with meat indicate a favorable comparison between detections in CM and plate counts on VRBA that were confirmed by inoculation into Brilliant Green Bile broth.

The dairy industry would appear to benefit from a shortened, automated test for coliforms. The purpose of this study was to test the feasibility of using CM with an impedimetric protocol to monitor coliforms in several dairy products, and to compare the impedance method with the standard presumptive and confirmed coliform tests.

The authors are affiliated with Bactomatic, A Division of Medical Technology Corp., P.O. Box 3103, Princeton, NJ 08540.

MATERIALS & METHODS

Samples

Pasteurized heavy cream and pasteurized milk were purchased from local markets. Commercial samples of ice cream mix were obtained directly from a manufacturer. Whole raw milk samples from 14 New Jersey farms were collected from a local dairy. Raw milk was incubated at an abuse temperature of 18°C, 25°C, or 32°C. Pasteurized cream and milk were held at room temperature, and ice cream mix was held at 32°C. All products were temperature abused for different lengths of time (several hours to 3 days), to obtain a wide range of coliform concentrations. The correlation curves comparing impedance detection times to plate counts included a minimum number of 50 independent samples for each of the products tested in this study.

Media

Violet Red Bile Agar (VRBA) (Difco) was used for counting coliforms. Brilliant Green Bile Broth (BGB) (Difco) was used to confirm typical colonies from the VRBA plates. CM, described by Firstenberg-Eden and Klein (1983), was used for the impedimetric detection of coliforms in raw and pasteurized milk and ice cream mix. CM, with 0.1M Tris buffer (final concentration, pH 7.0), was used for the impedimetric detection of coliforms in pasteurized cream.

Plate count procedure

The procedure used for counting coliforms on VRBA followed that of Marth (1978). Two or more dilutions of each sample were plated. The plates were incubated at 35°C for 24 hr. After counting, ten of the typical dark red colonies, 0.5 mm or more in diameter, were randomly selected for confirmation. Individual colonies were transferred from the VRBA plates to BGB tubes using a sterile Pasteur pipet. A colony could easily be picked up inside the tip of the pipet and could then be blown out into the BGB tube. If none of the ten colonies produced gas in BGB within 48 hr, ten more colonies were selected for confirmation. The fraction of positive confirmations was multiplied by the total VRBA count to arrive at the corrected VRBA count (CVRBA).

Impedance measurement

Instrumentation. Impedance measurements were carried out on the BACTOMETER® Microbial Monitoring System M120SC (Bactomatic, A Division of MTC, Princeton, NJ). This instrument has been described by Firstenberg-Eden (1983) and Firstenberg-Eden and Tricarico (1983). The conductance signal (Gsol) was monitored in this study.

Sample preparation. A 1:10 dilution of each sample of raw and pasteurized milk was made in a bottle containing 90 mL CM. Pasteurized heavy cream was diluted similarly in mCM. Ice cream mix was diluted 1:10 in a bottle containing 45 mL CM. The bottles were shaken using 25 complete up and down motions of about 1 ft in 7 sec and incubated for 3 or 4 hr in a 35°C waterbath.

Sample loading. After the 3 or 4 hr incubation period, each diluted sample was again shaken, and 1.5 mL were aseptically dispensed into duplicate module wells. Once the modules were inserted into the instrument, continuous automatic data collection was started. Impedance was monitored for 20–24 hr at 35°C.

Data analysis. The impedance detection times (IDTs) for the duplicate wells showing a dye change indicative of coliforms (Firstenberg-Eden and Klein, 1983) were averaged. All IDTs represent the sum of the 3–4 hr incubation time and the instrument detection time. Linear regression analysis was performed with averaged IDTs and averaged CVRBA plate counts for each of the four dairy products. At selected coliform levels, 95% confidence limits were calculated from the scatter around the line.

RESULTS & DISCUSSION

Raw milk

The raw milk samples obtained from the 14 local farms had counts in the range of 20–500 coliforms/mL. Higher coliform counts were obtained after the sample was temperature abused. When 10 typical colonies of each plated sample were confirmed in BGB, gas production from all 10 colonies occurred in only 39% of the samples. The remainder of the samples were divided between 0 and 90% BGB confirmation. This low percentage of BGB confirmation was probably caused by the presence of noncoliforms which were able to produce colonies comparable in color and size to those of coliforms in VRBA (Hartman, 1960; Rosen and Levine, 1970; Firstenberg-Eden and Klein, 1983). The distribution of confirmations obtained suggests that a better prediction of coliform levels in raw milk would be obtained by multiplying the VRBA counts by the percentage of BGB confirmations, than by relying on VRBA counts alone. The result of this correction was called the CVRBA plate count. The correlation obtained between the impedimetric method and coliform plate counts ($r = 0.93$, $n = 89$) suggests that the estimation of coliforms by the impedance method and by CVRBA plate counts was similar (Fig. 1). Thus, a concentration of 10^3 coliforms/mL in raw milk was detected in about 7 hr. Applying the sample classification method reported by Firstenberg-Eden and Klein (1983) to a specification of 10^3 coliforms/mL, samples with detections in 6.1 hr or less contained unacceptable coliform levels. Samples with detections between 6.1 and 8.0 hr were close to 10^3 coliforms/mL but were not definitively categorized.

Pasteurized milk

Pasteurized products contained very low numbers of coliforms. Classification of these products above or below a specified level of coliforms was simpler than enumeration. Neither impedimetric detection nor colony formation on VRBA was observed in the majority of samples tested. Therefore, they were classified as "clean" by both methods. In seventy of 100 unabused-pasteurized samples, typical colonies in VRBA plates were not observed nor did the impedimetric method show their presence. In twelve samples late IDTs (>12 hr) were observed, and no colonies appeared on the plates. With temperature abuse the concentration of coliforms in these products was elevated to a level at which CVRBA plate counts and IDTs were compared with high correlation ($r = 0.88$, $n = 123$) (Fig. 2). Abused and unabused pasteurized milk samples were classified according to the method of Firstenberg-Eden and Klein (1983). If a specification of 10 coliforms/mL is chosen (Jay, 1978), samples with detections in 9.5 hr or less contained unacceptable coliform concentrations. Samples with detections after 12.5 hr were acceptable. Only one sample of approximately 200 abused and unabused samples was misclassified. Although the majority of the colonies from the VRBA plates (60%) had 100% confirmation, 15% of the samples did not give a positive reaction in BGB. This suggests that numbers of coliforms in these samples were not properly assessed from the VRBA plates, and confirmation of typical colonies from VRBA was necessary for this product.

Errors in estimating numbers of bacteria from plates were increased when the numbers of colonies on the plates were below 30. This was expected since samples with low cell numbers show larger variation about the true mean density than samples with high cell numbers (Velz, 1951). Similar variation was associated with the impedance method. An impedimetric procedure employing bottles which can hold a large volume of medium and sample was adopted to try to reduce the variation. Impedance was monitored directly in the bottles with wire electrodes. An analogous strategy (increased sample volume) was used with plate counts by dispensing ten mL volumes among three plates. When this method was employed with 97 samples of pasteurized milk, the correlation rose from 0.88 in the modules to 0.93 in the bottles (Fig. 3). Agreement between the two methods improved greatly for samples with less than

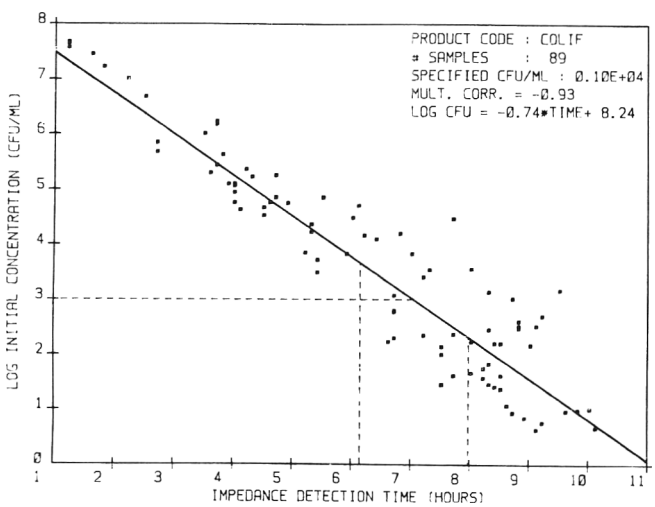

Fig. 1—Scattergram relating impedance detection times in modules and corrected violet red bile agar plate counts for raw milk samples.

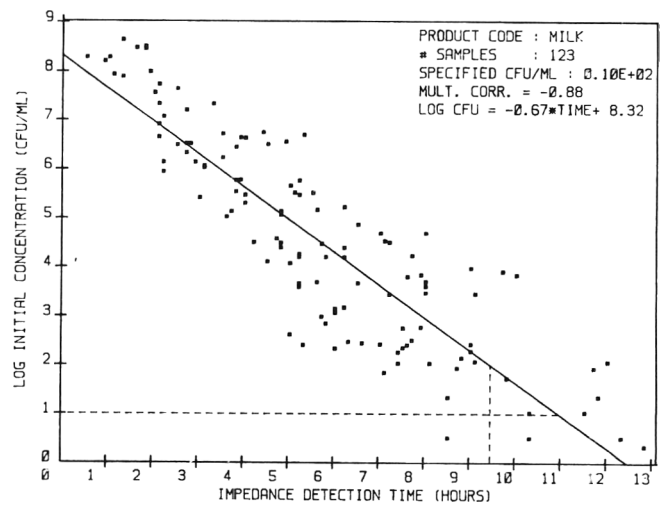

Fig. 2—Scattergram relating impedance detection times in modules and corrected violet red bile agar plate counts for pasteurized milk samples.

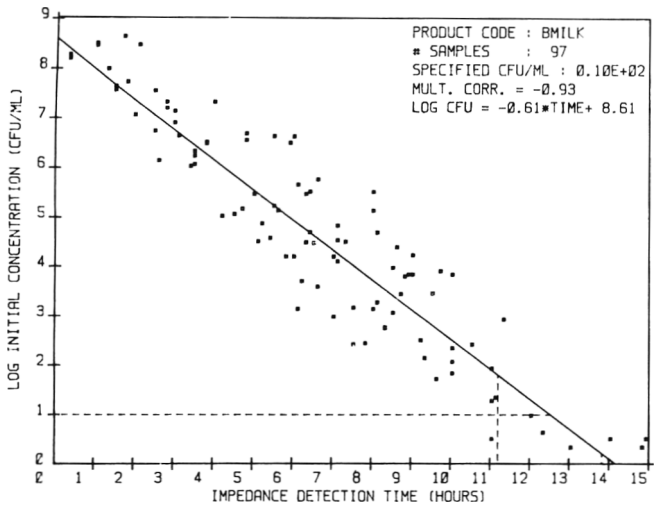

Fig. 3—Scattergram relating impedance detection times in bottles and corrected violet red bile agar plate counts for pasteurized milk samples.

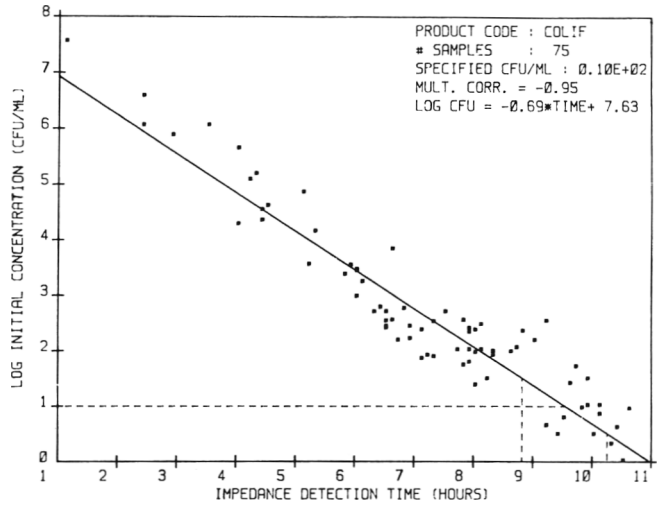

Fig. 4—Scattergram relating impedance detection times in modules and violet red bile agar plate counts for ice cream mix.

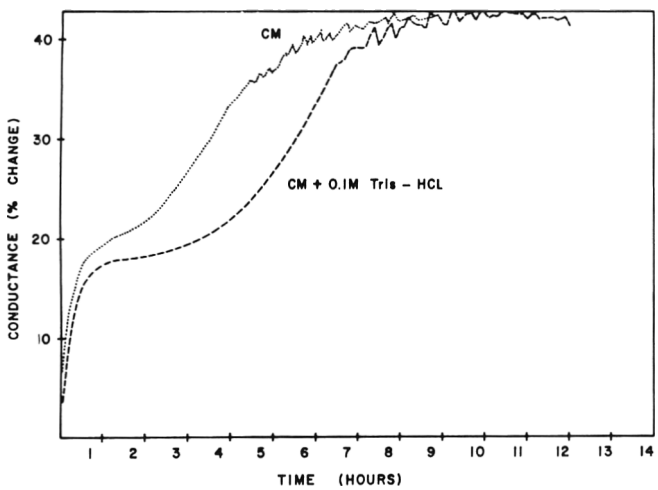

Fig. 5—Impedance curves from pasteurized heavy cream in CM and CM + 0.1M Tris-HCl showing the improved baseline in the modified medium.

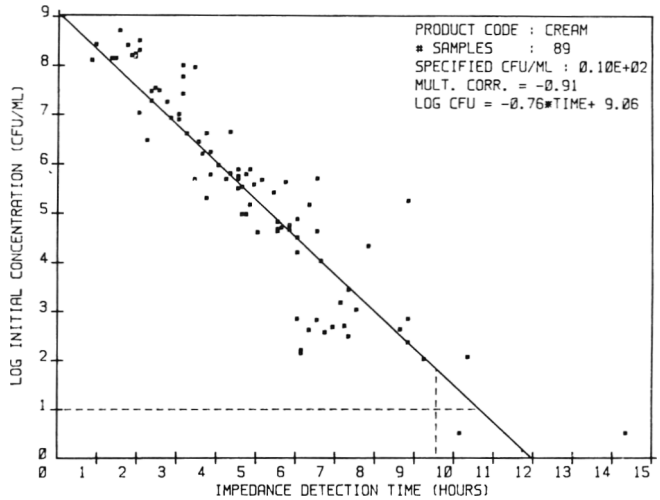

Fig. 6—Scattergram relating impedance detection times in modules and violet red bile agar plate counts for pasteurized heavy cream samples.

10^2 CFU/mL (Fig. 2 and 3), indicating that some of the sampling error associated with low coliform levels was reduced by using larger sample volumes. The 3-4 hr incubation step used with small sample volumes could be omitted when the bottle method is used.

Ice cream mix

With the ice cream mix, as with the pasteurized milk, very low numbers of coliforms were found in the product. Therefore, all the unabused samples could easily be classified as having above or below 10 coliforms/g. Twelve of the 79 samples examined contained no coliforms as determined by plate counts on VRBA and the impedimetric method. Linear regression analyses of 75 samples with coliforms showed a correlation of 0.95 for the two methods (Fig. 4). Ice cream mix with a coliform concentration above 10/mL could be screened out within 8.9–10.4 hr. At least 90% of the colonies which appeared on the VRBA plates from the ice cream mix were confirmed as coliforms in BGB. Confirmation for this product was therefore unnecessary.

Heavy cream

The pasteurized heavy cream necessitated a medium modification to alleviate a drift problem in the impedance curves (drift refers to a deviation from a horizontal baseline, before IDT) (Fig. 5). The addition of 0.1 M Tris buffer at pH 7.0 to CM improved the impedance curves. The reason for the reduction in drift upon the addition of Tris is not known, although it has been shown that Tris increases cell membrane permeability (Irvin et al., 1981). The addition of Tris buffer to the medium could have altered the permeability of the fat globule membranes in the product. Tris, also neutralizes negative charges in solution (Schindler and Teuber, 1978). These effects could be responsible for the change in the impedimetric drift.

As with the other pasteurized dairy products, most (70–80%) of the heavy cream samples contained very low (<1.0/mL) levels of coliforms. Impedimetric detection of the organisms did not occur, and changes in the color of CM were not observed. Eighty-nine samples, most of which were temperature abused, contained more than 1.0 organism/mL, and detection times were obtained with these samples. Linear regression analysis of these samples showed a correlation of 0.91 for the two methods (Fig. 6). As with the ice cream mix, confirmation of coliforms in BGB was found to be unnecessary because 66% of the samples had 100% confirmation, and 20% of the samples had 90% confirmation.

SUMMARY

ALL PASTEURIZED DAIRY PRODUCTS tested in this study had low coliform concentrations (<1 coliform/mL). It was therefore difficult to assess coliform numbers by either plate counts on Violet Red Bile Agar (VRBA) or impedimetric detections in CM. These samples were categorized above or below a specified level of coliforms.

Temperature abuse of the pasteurized products and raw milk increased the coliform numbers. High correlation between impedimetric detection times (IDTs) in CM and plate counts on VRBA were obtained with these samples, and similar line equations were obtained for all the products tested. An IDT shorter than 9 hr was indicative of coliform levels >10/mL, while an IDT longer than 12 hr was indicative of levels <10/mL. Since there was error associated with both methods, i.e., scatter of points around the regression line, a sample with coliforms detecting between 9 and 12 hr was not definitively classified above or below 10 coliforms/mL. Higher levels of coliforms were usually found in raw milk. An IDT shorter than 6 hr indicated coliform levels $>10^3$/mL.

The confirmation of typical colonies from VRBA plates in BGB was necessary when a product was first examined. Confirmation was found to be unnecessary in pasteurized heavy cream and in ice cream mix. Raw milk and pasteurized milk required confirmation and correction of the VRBA plate counts to obtain a true correlation between impedance detection times and plate counts.

Some of the sampling errors associated with low coliform numbers could be reduced by the use of larger sample volumes. Bottles containing 10 mL of sample improved the sensitivity of the impedance method.

The high correlation between IDTs in CM and CVRBA plate counts indicates that impedance can provide the same coliform estimation as confirmed results from VRBA for raw and pasteurized milk, ice cream mix, and pasteurized cream. A savings in time and labor is possible with the impedance method.

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Storage of New Zealand Jack Mackerel (*Trachurus novaezelandiae*) In Ice: Chemical, Microbiological and Sensory Assessment

J. M. RYDER, D. H. BUISSON, D. N. SCOTT, and G. C. FLETCHER

ABSTRACT

Physical, sensory, microbiological and chemical analyses were carried out on jack mackerel during 23 days of storage in ice. Sensory results indicate that jack mackerel used in this trial had a shelf-life of 7 days. Aerobic plate counts never exceeded 10^6/g flesh during the first 11 days. The K value reached 20% after 7 days. Trimethylamine, total volatile base, pH and thiobarbituric acid analyses were not good indicators of changes in quality during the shelf-life. Proximate analyses were carried out on representative samples of the fish.

INTRODUCTION

JACK MACKEREL (*Trachurus novaezelandiae* and *T. declivis*) are sufficiently common and widespread throughout N.Z. waters to allow an annual sustainable yield estimated at 50,000–187,000 tonnes (Robertson and Eggleston, 1979). Potential markets exist for quality chilled and frozen jack mackerel (Best, 1979) in a number of countries such as Japan, West Germany and Korea. However, quality criteria such as fat content, size and freshness need to be ascertained and met.

Virtually no studies have investigated the changes occurring during storage in ice of species of the genus *Trachurus*. Ehira (1976) considered horse mackerel (*Trachurus japonicus*) as part of his biochemical study on the freshness of fish and concluded from investigations of the nucleotide breakdown that horse mackerel had a shelf-life of 3–6 days.

The purpose of this study was to determine the shelf-life of jack mackerel in ice by use of sensory, microbiological and chemical assessment. Further, it was intended to identify those analyses which could be used to monitor changes in jack mackerel during its shelf-life in ice.

MATERIALS & METHODS

Sample preparation

Jack mackerel used in this study were caught in November 1981 at a depth of 80m in Area 010 (Lat. 37° 42′ S Long. 177° 40′ E) by purse seining. They were taken from the deck immediately and packed into fish bins with an equal volume of ice. The fish were transported to our laboratories and were less than 2 days old on arrival. The fish were then placed in an insulated container and the ice:fish ratio maintained for the duration of the trial.

Sampling

Days of ice storage were counted from the day of catching. Fish were first sampled at day 0, on the boat, when the physical characteristics and raw sensory attributes were determined. Samples for microbiological and chemical analysis were also taken and held at −60°C until reaching the laboratory. Subsequently, fish were sampled every 2–3 days until day 23. At each sampling time, microbiological analyses were carried out on 5 randomly chosen fish. These were then filleted and 3 of the fillets were used for cooked sensory analysis while the remaining 2 were left in reserve at −30°C.

Authors Buisson, Scott, and Fletcher are affiliated with the Division of Horticulture and Processing, Dept. of Scientific & Industrial Research, Private Bag, Auckland, New Zealand. Author Ryder, is also affiliated with DSIR, but may be reached at the Dept. of Food Science & Technology, Nutrition & Dietetics, Univ. of Rhode Island, Kingston, RI 02881.

Sensory raw assessment was carried out on a further 5 randomly chosen fish which were then weighed, sexed, measured to the fork of the tail and filleted. Five fillets from these fish were used for cooked sensory assessment while the remaining 5 were used for chemical analysis.

Proximate analysis

The protein, ash, oil and moisture contents were determined for 6 fish of the storage trial according to Vlieg (1982). Samples were taken at day 4.

Sensory analyses

Attributes of raw fish: The fish were examined for changes in general appearance. This included describing the eyes, surface slime, surface color, elasticity and gills. The gill odor was also described. Each fish was described by at least 3 people experienced in fish quality evaluation.

Attributes of cooked fish: Fillets were skinned by hand and a sample (approximately 3 cm × 5 cm) which included a portion of dark meat was removed from the anterior dorsal region of each of 8 fish. Samples were placed in individual 250 ml lidded casserole dishes, steamed for 15 minutes in a water bath and presented in these dishes to the panelists.

The sensory evaluation panel consisted of 8 members of the research staff who were experienced in sensory evaluation of fish. Panelists, seated at individual tasting booths, were initially required to describe the odor, flavor and texture for one cooked sample from a list of terms provided or others that seemed appropriate, and then rate each descriptive term used for intensity, according to the 4 point category scales of Vaisey Genser et al. (1977). The degree of liking was rated using a 5 point hedonic scale (5 = like very much; 4 = like slightly; 3 = neither like nor dislike; 2 = dislike slightly; 1 = dislike very much). Mean scores for intensity and degree of liking were determined for each term.

Microbiological analyses

Two samples were taken from the anterior dorsal region of each of 5 fish. Firstly, 10 cm^2 skin was swabbed to enumerate surface microorganisms. The swabs were shaken in 100 mL peptone water (0.1% Difco). Secondly, using sterile scalpels and forceps, the skin was removed and approximately 10g flesh excised. This was homogenized in peptone water using a sterile shaft (TP 18/2N) on an Ultra-Turrax T 45 disperser to give a 10-fold dilution (w/w) which was used to enumerate microorganisms in the flesh.

Enumeration of total aerobes was by the pour plate method using 1 mL of appropriate dilutions (0.1% peptone water) in Medium B of Simudu and Hasuo (1968). Enumeration of sulphide producing bacteria was by the same method using the Peptone-Iron agar of Sumner and Gorczyca (1981). Colony-forming units (C.F.U.) were counted after 4 days aerobic incubation at 25°C, and the logarithmic mean calculated. Black colonies on the Peptone-Iron agar were recorded as sulphide-producers.

Chemical analyses

Skinned fillets from each of 5 fish were packed in individual plastic bags and frozen at −30°C until analysis.

Frozen skinned fillets were sampled in the anterior dorsal region for nucleotide analysis. (The remainder of each fillet was vacuum packed with nitrogen gas flushing and left to thaw overnight at 4°C). Five grams frozen muscle were homogenized with 50 mL chilled 0.6M perchloric acid at 0°C for 1 min using an Ultra-Turrax disperser. The homogenate was centrifuged at 3000 × *g* for 10 min and the pH of 10 mL supernatent was adjusted to 6.5–6.8 with 0.1M potassium hydroxide. After standing at 4°C for 30 min, the

neutralized extract was made to 20 mL, filtered and stored at −70°C until analyzed by high pressure liquid chromatography. Thawed extracts were passed through 0.45 μm Millipore filters and 20 μL aliquots were injected in duplicate on to a Waters μ Bondapak C18 column. The mobile phase was 0.04M potassium dihydrogen orthophosphate and 0.06M dipotassium hydrogen orthophosphate with a flow rate of 2 mL/min. Eluted compounds were detected by their absorbance at 254 nm. Baseline separation of adenosine 5'-triphosphate (ATP) and its degradation products was accomplished with in 20 min (Ryder, 1984). K value (the proportion of hypoxanthine and inosine in the total pool of ATP and its degradation products) was determined according to Saito et al.. (1959).

Thawed fillets were diced finely using a sharp knife and mixed thoroughly. Twenty gram subsamples from the diced fillets were subsequently used for determination of trimethylamine (TMA) and total volatile bases (TVB) using the Modified Dyer Picrate method and steam distillation method respectively (Analytical Methods Committee, 1979). A 20 gram subsample was used for the determination of the thiobarbituric acid number (TBA No) by the method of Vyncke (1970). Ten grams diced fillet were homogenized with 20 mL distilled water and the pH of the resulting suspension measured at ambient temperature.

Table 1—Proximate composition of jack mackerel[a]

	Mean	Range	Standard deviation
Length (cm)	37.2	34 –40	2.1
Weight (kg)	0.72	0.58– 0.86	0.11
Sex	All female		
Protein (% w/w)	20.0	18.9 –20.9	0.7
Oil (% w/w)	2.0	1.1 – 4.2	1.1
Ash (% w/w)	0.9	0.8 – 1.0	0.1
Moisture (% w/w)	77.1	74.4 –78.5	1.5

[a] six fish were used.

RESULTS & DISCUSSION

Proximate analyses

Proximate analyses for 6 fish are shown in Table 1 and agree closely with results of Vlieg (1982). The fat content is low, as would be expected in fish caught in months other than August, when fat content rises to approximately 14% w/w (Vlieg, 1982).

Physical characteristics

Of the 35 fish examined, the mean weight of jack mackerel for this trial was 639g (range 479–883g) and the mean fork length 36.4 cm (range 31.5–40 cm). These results, and the area and method of catching suggest the fish were *T. novaezelandiae* (Robertson, 1977). Eighty three percent of the fish were female.

Sensory analyses

Raw fish. Changes in the attributes of the raw fish during storage in ice are shown in Table 2. During the 16 days of sensory assessment the fish underwent noticeable changes in the eyes, surface slime, gill appearance and gill

Table 2—Changes in appearance, odor and texture of raw jack mackerel during storage in ice[a]

		Days in ice								
		0	1	2	5	7	9	12	14	16
Eyes	Pupil	Black	VS misty	VS misty	S misty	Cloudy	Cloudy	Cloudy	Cloudy	Reddened
	Cornea	Clear	Clear	—	—	—	—	—	Bloodshot	Reddened
	Shape	Convex	Convex	Flattening	Flat	Flat	Flat	S sunken	Sunken	Sunken
Slime	Consistency	None	None	None	Watery	Watery	—	Watery	Thin	Thick
	Color	None	None	None	Clear	S cloudy	—	Brown	Brown	Brown
Elasticity		V firm	Rigid	Firm	M firm	M firm	M firm	M firm	Firm	Resilient
		Pliable	(Rigor)	(No rigor)		Elastic	Resilient	L resilient		
Gills-	Color	Rose red	Wine red	Scarlet	Brown/red	Brown/red	Brown	—	Dark brown	Dark brown
Appearance	Mucus	Thin	Thin	Cloudy brown	Thick	—	Brown	Bloody	Dark brown/thick	Thick
	Bleach	—	—	Slight	Mottled	Mottled	—	—		
Gills-		Seaweedy	Fresh	Briney	Rancid	Oily	Rancid	S fruity	Strong stale	Rancid
Odor		Neutral	Celery	Salty	Oily	Rancid	Oily	Briney	Rancid	Stale
				Metallic	Salty	Briney	Stale	Metallic	Sweet	Rotting
					Metallic				Briney	

[a] Each descriptor was used by 1 to 3 panelists, where V = very; M = moderately; S = slightly; VS = very slightly and L = less.

Table 3—Changes in odor, flavor and texture of cooked jack mackerel during storage in ice

	Days in ice													
	2		5		7		9		12		14		16	
Odor	Briney[a]	(4.0)[b]	Briney	(3.6)	Fishy	(3.0)	Toffee	(4.0)	Fishy	(3.0)	Sour	(1.8)	Sour	(1.5)
	Sweet	(4.3)	Fishy	(3.5)	Oily	(2.2)	Meaty	(4.0)	Briney	(3.0)	Fishy	(2.0)	Stale	(1.5)
	Oily	(3.0)	Oily	(2.2)			Sour	(2.0)	Sour	(1.5)	Cardboard	(1.5)	Smokey	(1.5)
							Fishy	(3.3)			Smokey	(3.6)		
Flavor	Meaty	(4.3)	Meaty	(3.8)	Meaty	(3.5)	Oily	(2.0)	Flat	(2.5)	Sour	(2.0)	Sour	(1.2)
	Sweet	(4.5)	Neutral	(3.0)	Oily	(1.8)	Astringent	(2.0)	Sour	(2.3)	Oily	(2.0)	Salty	(2.0)
	Bland	(3.3)			Salty	(4.5)	Sour	(2.6)					Metallic	(2.0)
Texture														
Moistness	Moist	(4.2)	Moist	(4.2)	Moist	(4.3)	Moist	(4.0)	Dry	(1.5)	Dry	(2.0)	Dry	(1.5)
Mouthfeel	Soft	(5.0)	Soft	(3.6)	Soft	(3.7)	Mealy	(2.3)	Soft	(3.6)	Sticky	(1.0)	Mushy	(1.7)
	Mealy	(2.7)	Firm	(3.5)	Mealy	(2.0)	Fibrous	(2.5)	Mealy	(2.0)	Soft	(2.8)	Sticky	(1.5)
			Short	(3.0)					Sticky	(1.0)	Chewy	(1.5)		

[a] Descriptive terms shown were used by at least 2 panelists and had a mean intensity of greater than 2.0. Terms are in decreasing order of intensity.
[b] Figures in brackets represent mean scores for degree of liking where: 5 = like very much; 1 = dislike very much.

odor. After 7 days the eyes had changed from convex with a black pupil to flat with a cloudy pupil. In the same period a slightly cloudy slime had developed on the surface of the fish. The gills were initially rose-red with a thin mucus but after 5 days were brown-red and bleached in parts with a thick brown mucus. The gills developed oily and rancid odors after only 5 days in ice.

The changes that occurred in the eyes and gills of jack mackerel during its shelf-life have potential for use in the quality assessment of whole jack mackerel.

Cooked fish. Table 3 shows the changes in the attributes of cooked fish during storage in ice. Fresh jack mackerel (stored up to 2 days in ice) were described by the panel as: briney, sweet and oily in odor; meaty, sweet and bland in flavor; and soft, mealy and moist in texture. The oily odor and flavor, and the mealy texture were disliked by some panelists. These attributes, however, are characteristic of jack mackerel throughout its shelf-life and cannot be regarded as spoilage characteristics.

After 7 days the fish had not developed any spoilage characteristics and on average the odor, flavor and texture were liked as indicated by degree of liking scores (Table 3).

After 9 days the first sourness was detected in the odor and flavor. This, along with an astringent flavor and fibrous texture, were attributes disliked by the panel. From 12 to 16 days sourness became progressively more intense and the texture became dry, soft and sticky.

The shelf-life was taken to be the time elapsed prior to the development of spoilage characteristics disliked by the panel. Thus the jack mackerel used in this trial had a shelf-life of 7 days in ice.

Microbiological analyses

Fig. 1 shows the results of the microbiological analyses. All plate counts exhibited approximately logarithmic increase throughout the trial, with the exception of the surface aerobic plate count (APC).

Recommendations set by the International Commission on Microbiological Specifications for Foods (ICMSF) are met during the shelf-life, in that the flesh APC did not exceed 10^6/g wet weight in more than 2 of 5 samples and never exceeded 10^7/g. The last sampling time these recommendations were met was day 9.

Counts of sulphide-producing bacteria have been used as indicators of fish spoilage (Scott et al., 1983). Fig. 1 shows that during the first 16 days of storage in ice, the percentage of sulphide-producers in jack mackerel was relatively constant at about 1% of the APC for both surface and flesh counts. This percentage increased to 12% on the surface and 5% in the flesh on day 23.

Other workers have found that sulphide-producing bacteria often constitute a major proportion of the microflora of spoiling fish (Chai et al., 1968; Jensen et al., 1980; Sumner and Gorczyca, 1981), the predominant sulphide producing bacterium being *Alteromonas putrefaciens* (Levin, 1968). In this trial the relatively low numbers (1.1×10^3/g) of sulphide-producers at day 9, when jack mackerel had begun to spoil according to sensory evaluation, indicate that *A. putrefaciens* did not play a major role in the spoilage of jack mackerel.

Chemical analyses

Nucleotide analyses. Fig. 2 shows the levels of ATP breakdown products at the time of capture (day 0) through to the end of the trial at day 23. ATP, adenosine 5′ diphosphate (ADP), and adenosine 5′ monophosphate (AMP) concentrations are not shown because they were consistently found to be present at very low concentrations (less than 0.2 μmoles/g wet weight flesh).

The concentrations of both inosine and hypoxanthine increased gradually over the period. During the shelf-life, the rate of these increases were too slow to be useful as indices of freshness.

Inosine 5′ monophosphate (IMP) concentrations decreased relatively rapidly during the same period but the decline slowed after 12 days. This is similar to changes of IMP concentrations found in gurnard by Vyncke (1980) and in other species as reviewed by Martin et al. (1978). IMP concentration may be of value as an indicator of freshness in jack mackerel stored in ice during the shelf-life period.

Fig. 3 shows a near linear increase of the K value of jack mackerel during the 23 day storage in ice from 2% to over 60%. By the end of the shelf-life, the K value had reached 20%. This compares favorably with Japanese work on horse mackerel (*Trachurus japonicus*) where K values of approximately 10% after 2 days in ice and 28% after 10 days in ice were obtained (Ehira, 1976). This worker reported a limit for high quality fish (destined for sashimi use) of 20% and noted that by 60% fish were unfit for human consumption. The K value for jack mackerel used in this trial exceeded 20% after 7 days when also sensory analyses indicated the end of the shelf-life. This supports the use of the 20% K value as an indicator of acceptance for sashimi grade fish, and demonstrates its potential as an indicator of freshness of jack mackerel during its shelf-life.

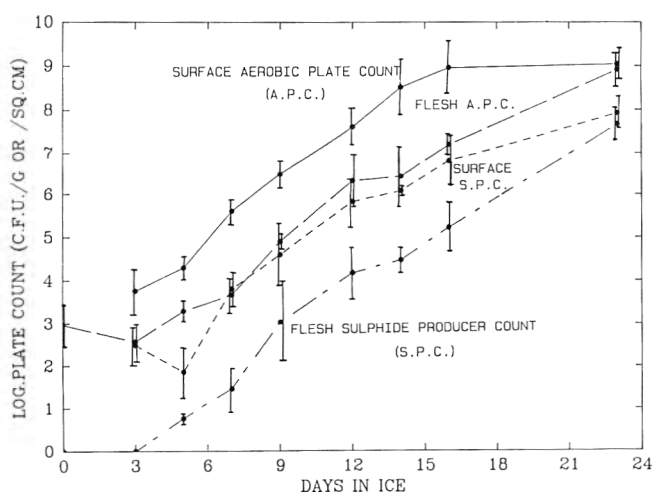

Fig. 1—Changes in microbial counts of jack mackerel during storage in ice.

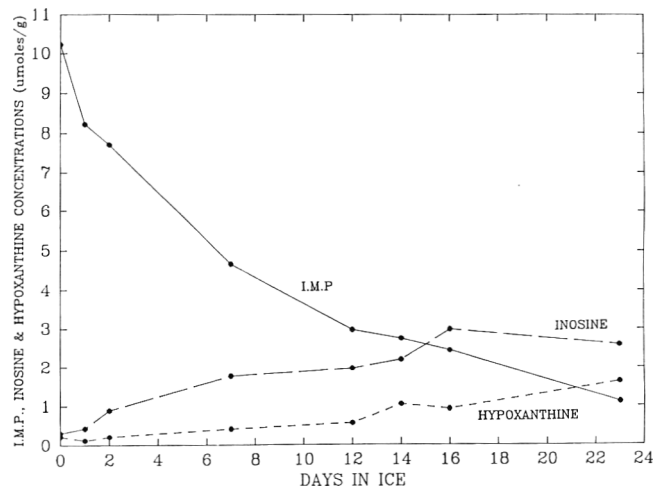

Fig. 2—Changes in inosine monophosphate, inosine and hypoxanthine concentrations in jack mackerel flesh during storage in ice. Each point represents the mean of 5 samples.

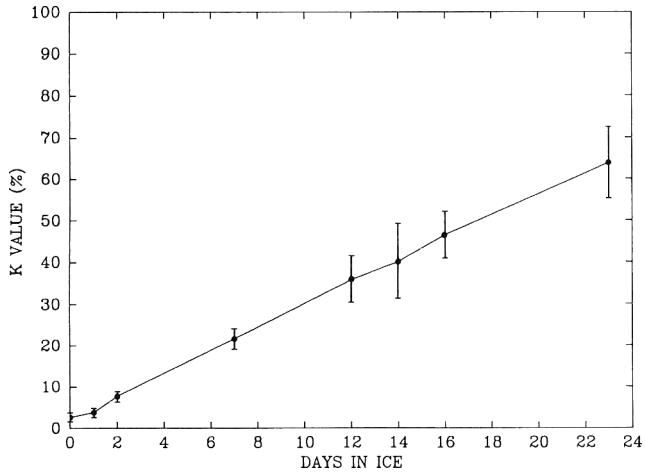

Fig. 3—Changes in K value of jack mackerel flesh during storage in ice. Each point represents the mean of 5 samples.

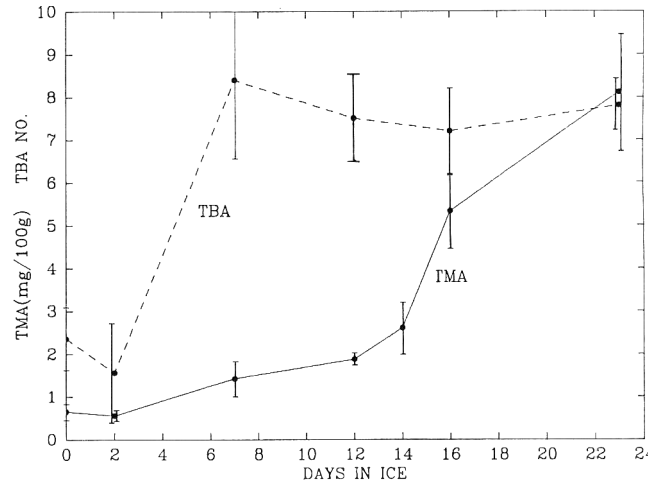

Fig. 4—Changes in trimethylamine concentration and thiobarbituric acid number in jack mackerel flesh during storage in ice. Each point represents the mean of 5 samples.

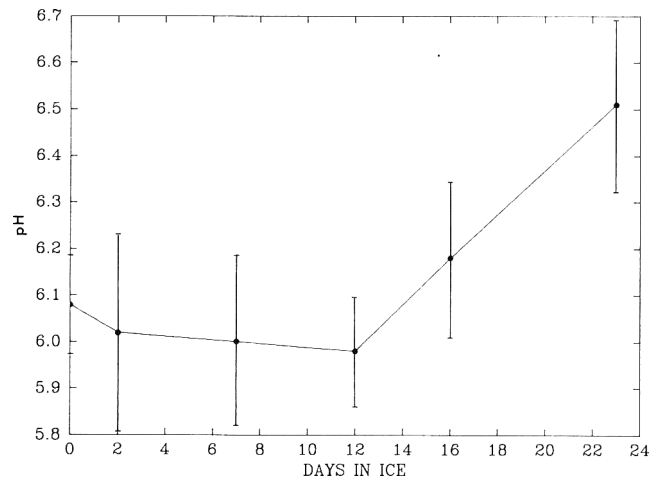

Fig. 5—Changes in pH of jack mackerel flesh during storage in ice. Each point represents the mean of 5 samples.

Trimethylamine and total volatile base analyses. The TMA levels remained low (less than 2 mg TMA-N/100g wet weight flesh) during the shelf-life of jack mackerel, but increased rapidly after day 12 (Fig. 4). This increase in TMA concentrations correlated with the APC of 10^6/g wet weight flesh at day 12, emphasizing the use of TMA as an indicator of the onset of bacterial spoilage rather than an indicator of freshness.

TVB concentrations were determined at day 0 and day 12. During this period they had risen from 21.9 to 28.3 mg TVB-N/100g wet weight flesh. This minimal increase suggests that measurement of TVB concentrations is not a good indicator of freshness.

Thiobarbituric acid number. The increase in TBA No. from day 2 to day 7 (Fig. 4) coincided with the detection of oily odors and flavors in the sensory evaluation of the cooked fish, and the development of rancid and oily odors of the gills. However, these oily odors and flavors were not indicative of spoilage during the initial period of storage. Therefore, the increase in TBA No. did not imply loss of acceptability and so was not useful for assessing the end of the shelf-life.

pH measurement. The pH changes with time during storage in ice, are shown in Fig. 5. During the initial storage period, the pH was consistently low (less than pH 6.1). After 12 days it increased linearly reaching pH 6.5 at the end of the trial. The observed increase in pH was probably due to production of basic volatiles by bacteria during the spoilage process. Purse-seining is a relatively gentle method of catching and fish were taken directly from the net for sampling. This would have resulted in high concentrations of glycogen immediately postmortem and subsequently high levels of lactic acid explaining the low initial pH (MacCullum et al., 1967).

CONCLUSIONS

THE SHELF-LIFE of whole jack mackerel stored in ice was found to be 7 days. On the 9th day of storage the fish had developed spoilage characteristics which were disliked by the panel. Oily odor, oily flavor and mealy texture are characteristic of cooked jack mackerel and not indicative of spoilage.

The microbiological counts did not exceed the limits recommended by the ICMSF until day 12, well after the shelf-life determined by sensory evaluation. Sulphide producing bacteria were a small percentage of the microflora during the trial and thus did not play a major role in the spoilage of jack mackerel.

The loss of sensory quality in this species was not determined by bacterial spoilage so much as by the changes due to autolytic degradation. Thus the determination of IMP concentration and K value, which are primarily measures of autolytic degradation, were good indicators of loss of freshness during the shelf-life of jack mackerel in ice. The bacterial metabolic end products (TVB and TMA), as found previously by Spinelli et al. (1964), were less useful as objective measurements of freshness.

The pH was not a good indicator of early storage changes and TBA No. could not be used to determine loss of acceptability or end of shelf-life.

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Effects of Vacuum and Atmospheric Freeze-Drying on Quality of Shrimp, Turkey Flesh and Carrot Samples

OSEI BOEH-OCANSEY

ABSTRACT

To assess a technique of freeze-drying at atmospheric pressure, the quality of shrimp, turkey flesh and carrot freeze-dried by the conventional method in vacuo, was compared to that of the same products freeze-dried under atmospheric conditions. Shrinkage, ability to rehydrate, water retention, and color change as well as sensory analyses were used as indices to evaluate the effect of processing conditions. The atmospheric process induced greater quality loss than freeze-drying in vacuo. However, process techniques to improve upon the quality of the atmospheric freeze-dried products are discussed.

INTRODUCTION

INTEREST in freeze-drying at atmospheric pressure has increased in recent times. The underlying objective has been to reduce the cost of the freeze drying operation by eliminating vacuum creating equipment.

Meryman (1959) pioneered research in this area by freeze-drying histological samples at −30°C using drying agents in a fixed bed at atmospheric pressure. Other researchers including Lewin and Mateles (1962), Woodward (1963), Sinnamon et al. (1968) also attempted with varying degrees of success to freeze dry food samples without vacuum. The general observation was that this approach necessitated very long drying periods. Malecki et al. (1969, 1970) attempted to reduce drying time by reducing sample sizes and using fluidized beds. However, very low temperatures (−34°C) were required in subsequent trials with apple juice in order to overcome melting. Some amount of interstitial melting is tolerated in practice so far as quality is not sacrificed while allowing a considerable reduction in drying time and operational costs (Boeh-Ocansey, 1979). Other workers such as Heldman and Hohner (1974), Ku et al. (1977), Zarkarian and King (1978) have provided information on the analysis of the mechanisms involved in the freeze-drying process.

In this work, a method of atmospheric freeze-drying in a fluidized bed of adsorbant particles was developed. In order to assess the process, a study of quality was undertaken comparing food samples freeze-dried in vacuo to the same products freeze-dried at atmospheric pressure.

MATERIALS & METHODS

THREE FOOD TYPES – carrot, shrimps and turkey chest muscles – of, respectively, 89.5%, 82.5% and 72.0% water content (wet weight basis) were cut to units weighing (on the average) 2.1g, 1.5g and 2.0g, respectively. The carrot samples were cut into discs 22 mm diameter, 4 mm thick and frozen at −35°C. Commercially quick frozen shrimp (10 mm maximum diameter) and turkey flesh pieces 36 x 72 x 6 mm^3 were stored in a freezer at −35°C. Two hundred grams of each frozen sample were dried to constant weight in a freeze-drier (SERAIL RP3V) which, under optimum conditions, operates at a vacuum of 0.3 mm Hg, or in an atmospheric drier (Boeh-Ocansey, 1983). The atmospheric drier consisted principally of a cylindrical column, a refrigeration unit and an air blower. The drying chamber, a vertical cylindrical column 10 cm in diameter and 100 cm high, contained 1 kg of alumina particles (0.4 mm average diameter) in which the food sample to be dried was immersed. This column was joined below to a similarly shaped arrangement linked to the refrigeration unit and thereby constituting a heat exchange system (cooling section). Air from the blower (Fougal HD40A), at a flow rate one and one-half times the minimal fluidizing velocity of the alumina particles (0.11 m/s), is introduced into the drying chamber after having been cooled in the lower column. The air, making its exit at the upper end of the drying chamber, is purified by passing it through a dust separator or cyclone before it is recycled back to the blower. Drying was followed by measuring changes in the weight of food products with time. The temperature of the drying medium was monitored continuously using copper-constantan thermocouples while an electrolytic hygrometer (Beckman) was used to measure the relative humidity of the air. During drying the relative humidity of the air was maintained at all times below 20 ppm. At the end of drying, immersed food products were recovered by the help of a cylindrical sieve placed inside the drying chamber – an arrangement that did not hinder the free circulation of fluidizing material during drying. The dried food products were thereafter stored in a freezer at −35°C until they were required for quality assessment. The following determinations were made to monitor quality changes.

Shrinkage

Using carrot discs, changes in thickness were measured; a coefficient of shrinkage, r, was defined as the ratio of the difference in thickness of the sample to its initial thickness ($r = \Delta L/L_o$).

Rehydration

Ten grams of the freeze-dried product were immersed in 200 mL distilled water for varying periods of time. Excess water was drained off using filter paper. By weighing, the quantity of water absorbed was determined. A rehydration curve was established by plotting the amount of water absorbed in grams per unit weight of dry matter as a function of rehydration time, t_R. A rehydration coefficient, R, representing the ratio of water absorbed to unit weight of initial freeze-dried sample was determined.

Water retention

Twenty grams of sample were placed in a tubular-shaped metal gauze whose neck was fixed to a centrifuge tube in a manner as to allow exudation of water during centrifuging at varying speeds for 10 min. (The centrifuge used was a Jouan-Paris model K-63F.). The exuded water was weighed.

Exudation of water results when, during centrifugation, the internal capillary forces of the food material are not sufficient to overcome the centrifugal force applied. When the weight of water extracted upon centrifuging food samples, and expressed as a percentage of the food's initial water content, was plotted against the corresponding centrifugal force, or more simply against the corresponding rotational speed (rpm), the resulting diagrams illustrate the food material's ability to retain water.

Color measurements

Fat-soluble pigments in the shrimp and carrot samples (carotenoids) were extracted using the method of Moore, Ely and Macleod recommended by the Association of Vitamin Chemists (Joslyn, 1970). In the presence of 70 mL petroleum ether (60-70°) and 100 mL ethyl alcohol (95%), 10g of the raw, thawed or rehydrated product were disintegrated in a Waring Blendor. The mixture was transferred into a separating funnel where 50 mL petroleum ether was first added followed by 25 mL ethyl alcohol. The mixture was shaken carefully, releasing pressure periodically. After allowing the

Author Boeh-Ocansey is affiliated with the Dept. of Food Science & Technology, Univ. of Nigeria, Nsukka, Nigeria.

layers to settle, the aqueous phase, containing a lot of suspended matter, was transferred into another separating funnel where the remaining fat soluble fraction was extracted by washing twice with 50 mL (90%) methyl alcohol. The ether extract was washed three times, each time with 30 mL 3% sodium sulphate solution. The layers having settled, the aqueous portion was decanted and the ether extract was dried by adding a teaspoonful of anhydrous sodium sulphate. The extract was stored in a cold room.

Depending on the pigment intensity, the extract was either diluted with petroleum ether or concentrated using a rotary vacuum evaporator. Absorption spectra were then plotted using a scanning spectrophotometer in the wavelength range of 550-350 nm for the carrot extract and 350-600 nm for the shrimp extract.

For turkey chest muscles, the water soluble pigments were extracted by macerating 10g of sample in 70 mL distilled water at 10°C. The extract and residue were separated by centrifuging. The pigment extract was concentrated at low temperatures under vacuum as above and absorption spectra plotted over the range 320-500 nm.

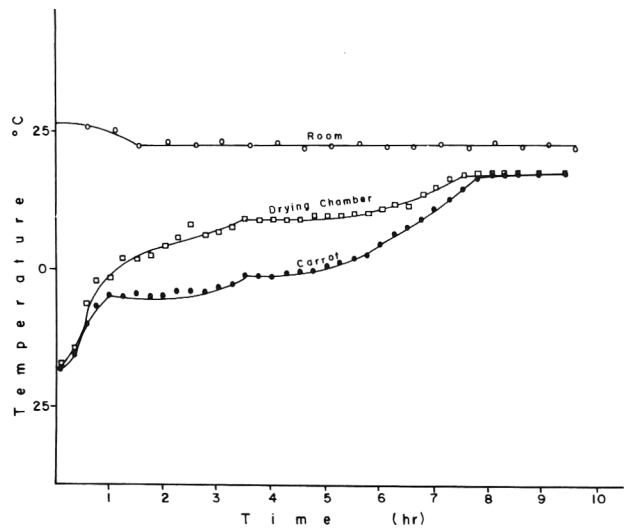

Fig. 1—Variations of temperature during freeze-drying under vacuum.

Sensory evaluation

A taste panel comprising 10 experienced subjects was organized to compare the freeze-dried products to their fresh, raw or thawed analogues. All the different samples of the same food product were cooked in an identical manner, with due regard to traditional cooking practices. Samples of each product were presented simultaneously to the panel members in well-lit (white light) partitioned booths. The test subjects were asked to evaluate the quality attributes of each sample with respect to texture, color, flavor, appearance and overall quality on a 5-point hedonic rating scale where 5 denoted like very much, and 1 represented dislike very much. Variance was analyzed and Student's t-test was used to compare the mean scores for the samples. Since sample sizes were not greater than 10, test samples were combined to obtain normal distributions and significance of the equality of means tested at 5%.

RESULTS & DISCUSSION

FIG. 1 PRESENTS the temperature profiles during a typical freeze-drying operation under a vacuum of 0.3 mm Hg where the only source of heat was ambient temperature, constant at +23°C. The initial temperature of drying chamber and food sample (center) was −20°C. During the drying operation, the temperature of the carrot disc rose in parallel with the rising temperature of the chamber except for a period when it appeared to have stabilized around −5°C. The temperature difference between the drying chamber and carrot remained relatively constant at 10°C for about 2½ hr. Towards the end of drying the difference decreased gradually while food sample temperature rose to that of the drying chamber.

Fig. 2 presents curves for both atmospheric and vacuum drying of carrots. Atmospheric drying at a constant temperature of −10°C or less required very long periods (exceeding 20 hr), whereas the drying time for the vacuum process was around 10 hr. In order to improve upon the rather slow drying rates observed at low temperatures, a temperature programming technique simulating the temperature evolution under vacuum (Fig. 1) was adopted for the drying operation at atmospheric pressure such that at least 50% of the total water in the food sample was removed at temperatures around −10°C; about 70-80% of total water was removed at −5°C; 90% total water removed around 0°C; and the remaining 10% removed at temperatures greater

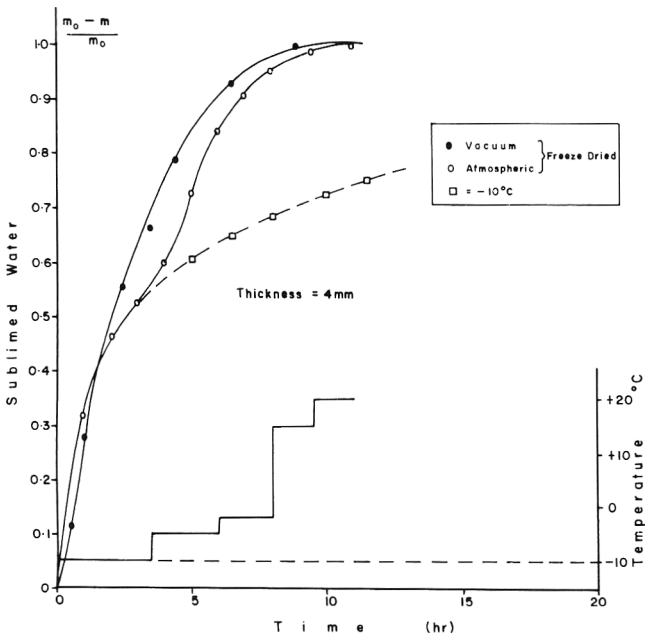

Fig. 2—Freeze-drying of carrots in vacuo and at atmospheric pressure by temperature programming and at constant temperature (−10°C). Lower curve is the temperature history.

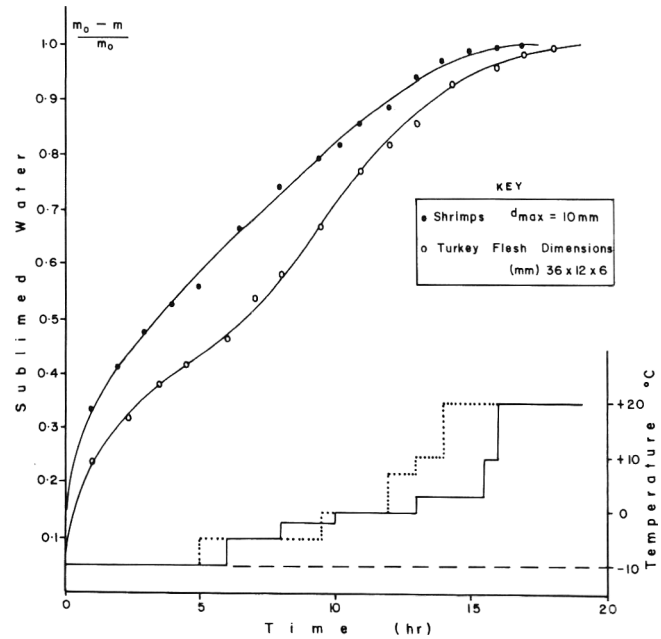

Fig. 3—Freeze-drying of shrimp and turkey flesh at atmospheric pressure by temperature programming technique. Lower curves are the temperature history.

Table 1—Biophysical and biochemical analyses of frozen and freeze-dried food samples

	Carrots			Shrimp			Turkey flesh		
		Freeze-dried			Freeze-dried			Freeze-dried	
Test	Frozen	Vacuum	Atmospheric	Frozen	Vacuum	Atmospheric	Frozen	Vacuum	Atmospheric
Shrinkage[a]									
r_1	–	0.44	0.74	–	–	–	–	–	–
r_2	–	0.28	0.36	–	–	–	–	–	–
Rehydration									
R	–	0.21	0.08	–	0	0	–	–	–
$t_{R(min)}$	–	27	24	–	10	15	–	–	–
Color content (relative)									
B-carotenes	1.00	0.99	0.98	–	–	–	–	–	–
astacene	–	–	–	1.00	0.33	0.27	–	–	–
Water-soluble pigments	–	–	–	–	–	–	1.00	0.63	0.56

[a] r_1 = after freeze drying; r_2 = after rehydration; — = not determined

than 0°C but never exceeding 20°C. The programming was undertaken in a stepwise gradual fashion avoiding severe and sharp increments. Temperature changes were made especially when the drying rates tended to fall. This temperature programming technique permitted a drying time of 11 hr for carrots and around 17 hr for shrimp and turkey flesh (Fig. 3) to be obtained.

Quality indices

The principal results of the objective quality tests are presented in Table 1.

Shrinkage is a parameter measured by comparing the final volume of product to the initial volume or in a simpler way, by following linear changes in the product's dimensions. Owing to its more regular shape, carrot was used as the ideal product for studying shrinkage after freeze-drying (r_1) and after rehydration of the freeze-dried product (r_2); freeze-drying at atmospheric pressure induced greater shrinkage (r_1 = 0.74) than freeze-drying in vacuo (r_1 = 0.44). However, upon rehydration the products regained much of their original form: r_2 = 0.28 for atmospheric and r_2 = 0.36 for vacuum freeze-drying.

The rehydration coefficient R measured the ability of the dried product to reconstitute well. Rehydration was judged complete when the water content of the raw product was obtained. All the freeze-dried products reconstituted well upon rehydration ($R \leqslant 0.21$) and in good time, t_R was between 10–27 min.

The absorption spectra used for the evaluation of color are shown in Fig. 4, 5, and 6. B-carotene, the principal pigment in carrots exhibited a peak at 450 nm (Fig. 4). The absorption spectrum for shrimp pigments (Fig. 5) was characterized by a peak at 475 nm and a minimum at 390 nm; the principal pigment in shrimp is astacene. A peak at 407 nm and a minimum at 368 nm characterized the absorption spectrum (Fig. 6) of the water-soluble pigments of turkey flesh where myoglobin and its derivatives constitute the major coloring matter. Large changes in pigment concentration can be followed by visual or qualitative comparison of absorption spectra but for quantitative evaluation of major changes more precise procedures are required. Goldblith et al. (Rey, 1964) proposed that the ratio of absorbance of astacene at 475 nm (peak) to that at 390 nm (minimum) gave a measure of shrimp color which was independent of pigment concentration. This reasoning was applied to the measurement of carrot pigmentation where a color index was defined as the ratio of absorbance at 450 nm to that at 465 nm. Similarly, the color of turkey flesh was evaluated quantitatively, using a color index of the ratio of absorbance at 410 nm to that at 370 nm. Table 1

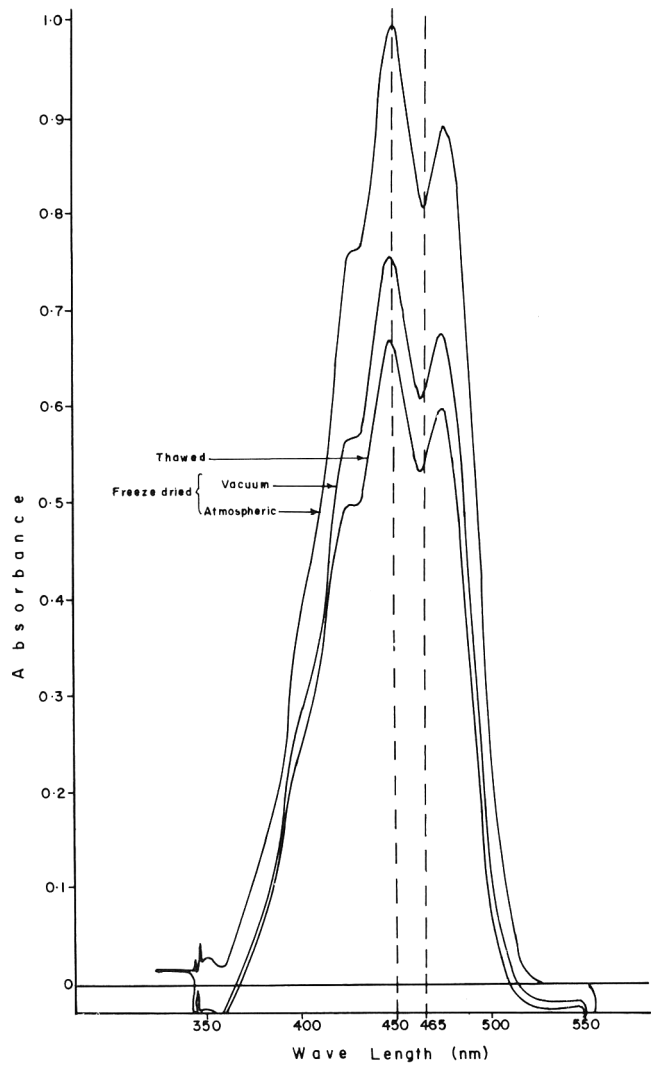

Fig. 4—Spectral analyses of fat-soluble carrot pigments.

presents color retention data of samples, expressed as the color content of sample relative to that of the frozen analogue. The results demonstrate that while carrot pigments were hardly affected by freeze-drying conditions, shrimp pigments were most sensitive, with color degradation of about 73%, computed as 100 less percent color content. For turkey flesh, color loss was 44%. Atmospheric freeze-drying consistently produced slightly higher color loss than drying under vacuum.

In applications of centrifugation, such as the method described above for the determination of a food material's ability to retain water, the centrifugal force, $F_{(g)}$, is commonly expressed in multiples of the standard force of gravity. The centrifugal force varies with the speed of rotation (n, revolutions per minute) and with the radial distance from the center of rotation, d. When d is measured in inches, the centrifugal force is computed from the relationship (Perry and Chilton, 1973): $F_{(g)} = 1.42n^2d \times 10^{-5}$. Water retention plots, which present mass of exuded water as a function of rotational speed, are shown in Fig. 7 and 8 for carrot and shrimp, respectively, from which the following observations can be made. The fresh food retained practically all its water even when a higher centrifugal force, corresponding to 2700 rpm, was applied. Samples freeze-dried in vacuo retained water better than samples freeze-dried at atmospheric pressure, or samples thawed.

Unlike the carrot samples, initially frozen at −35°C and stored at the same temperature, the shrimp and turkey flesh samples were obtained commercially quick frozen then stored at −35°C. These conditions were good for promoting ice crystal formation and growth, which in turn could have induced changes in the food samples' internal structure. But, since samples of the same food type were purchased at the same time and stored at the same temperature for the same length of time prior to drying and experimentation, it is safely assumed for purposes of comparison that any damages inflicted on the internal structure of samples were of equal magnitude in all samples of the same food product. The ability of a freeze-dried material to retain water when rehydrated reflects changes in the bonding relations between the macromolecules which constitute the material's dry matter and water. Reconstituted water does not recombine with the dry matter in a manner akin to that occuring in the raw fresh food prior to freezing. The water no longer forms an inherent part of the food materials microstructure, it simply fills the porous structure created as a result of the sublimation of ice-crystals. Such water is held by relatively weaker internal capillary forces. Consequently, it is more easily extracted upon centrifuging. It is reasonable to infer from Fig. 7 and 8 that freeze-drying at atmospheric pressure induced interstitial melting to a greater degree than freeze-drying under vacuum.

In Table 2 are presented the results of the taste panel assessment of the quality of the food samples: shrimp and turkey flesh were judged unacceptable when freeze-dried

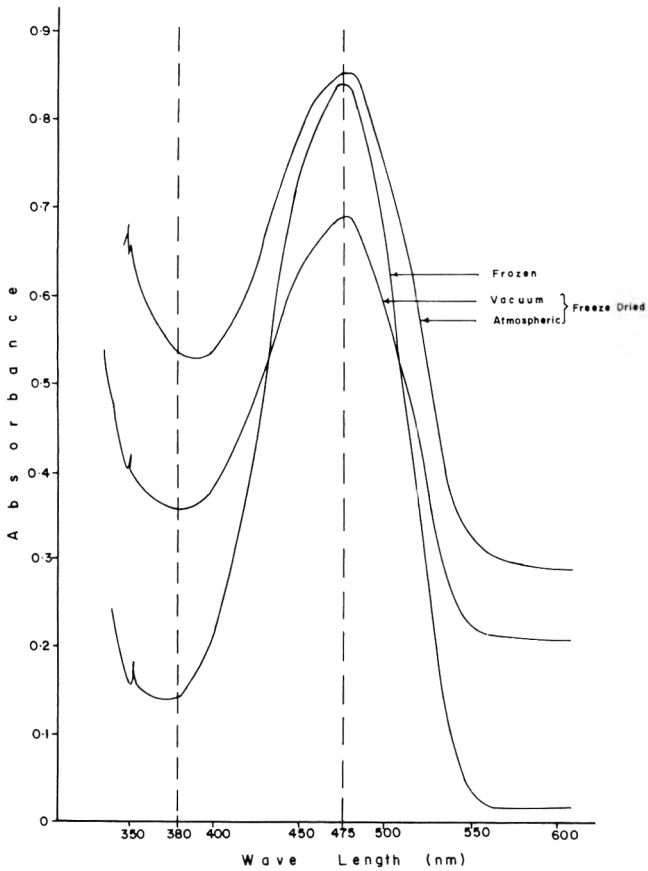

Fig. 5—Spectral analyses of fat-soluble shrimp pigments.

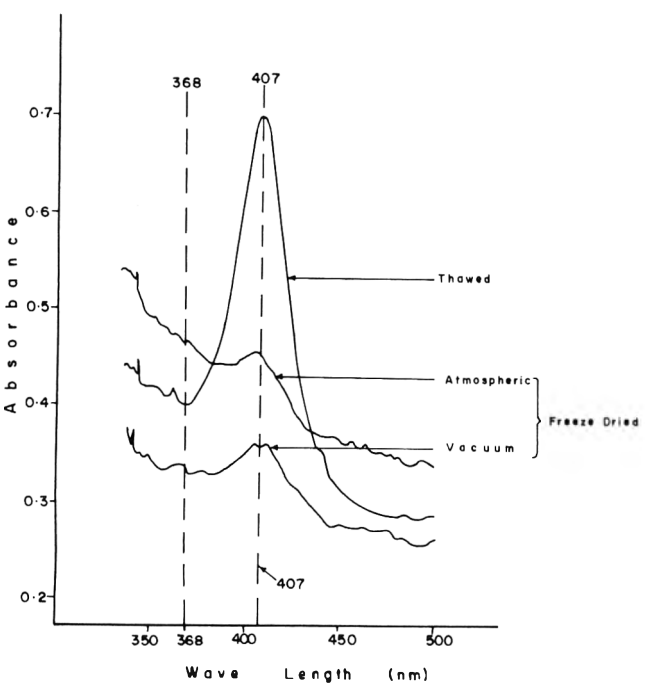

Fig. 6—Spectral analyses of water-soluble pigments of turkey flesh.

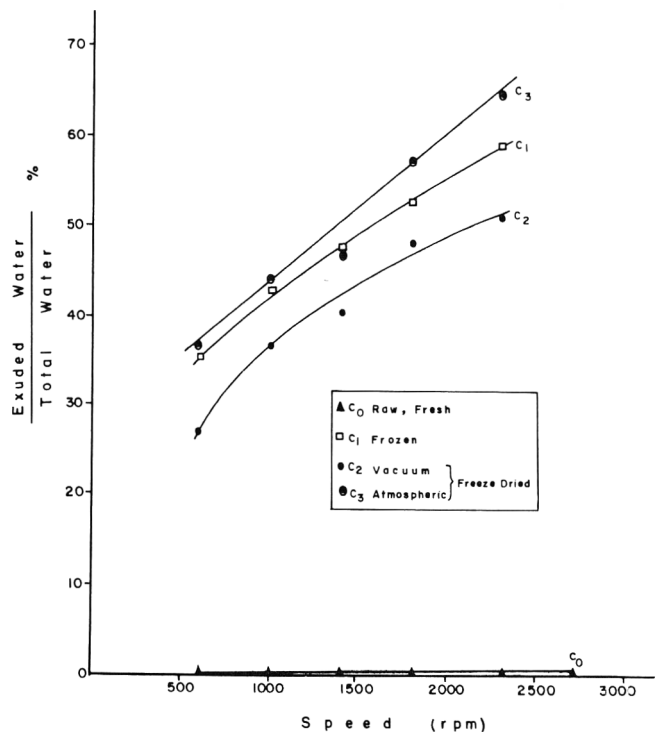

Fig. 7—Water retention plots for fresh, frozen and freeze-dried carrot samples.

Table 2—Mean scores[d] for individual quality attributes and t-test analysis comparing atmospheric-dried products to frozen/vacuum-dried analogues

Product	Quality	Sample			
		Fresh	Frozen	Freeze dried	
				Vacuum	Atmospheric
Carrot	Appearance	3.40	4.10	3.20	3.20[a]
	Color	3.90	3.90	3.40	3.50
	Texture	4.60	3.50	2.80	2.80[a]
	Flavor	4.30	2.60	2.10	2.30
	Acceptability[c]	4.05	3.53	2.88	2.95
Shrimp	Appearance	–	3.30	4.05	1.50[ab]
	Color	–	3.40	4.15	1.50[ab]
	Texture	–	3.50	3.75	2.40[b]
	Flavor	–	3.20	3.45	1.90[ab]
	Acceptability[c]	–	3.35	3.85	1.83
Turkey flesh	Appearance	–	4.20	3.30	2.10[ab]
	Color	–	4.20	3.50	1.80[ab]
	Texture	–	4.10	3.50	2.70[ab]
	Flavor	–	3.85	3.30	3.00
	Acceptability[c]	–	4.09	3.40	2.40

[a] Denotes significant difference at 5% level (p = 0.05) when atmospheric freeze-dried product is compared to frozen sample.
[b] Denotes significant difference at 5% level (p = 0.05) when atmospheric freeze-dried product is compared to vacuum freeze-dried sample.
[c] Means of taste panel acceptability scores for cooked carrots, shrimp and turkey flesh prepared from fresh, frozen and freeze-dried samples.
[d] Scale: 5 = like very much; 1 = dislike very much

by the technique at atmospheric pressure. The mean scores for the individual quality attributes, appearance, color, texture and flavor are also presented in Table 2 for the fresh, frozen and freeze-dried samples. Also presented in Table 2 are the results of a t-test analysis comparing the mean scores of the individual quality attributes for the frozen/vacuum freeze-dried products and their analogues freeze dried under pressure. Appearance, color and texture were the quality attributes of the atmospheric freeze-dried product which markedly affected its acceptability score. Since appearance is a derived property very dependent upon color, color and texture could be identified as the primary attributes of quality that affected the acceptability scores directly.

Degradation of color was caused mainly by oxidation of pigments and interstitial melting affected texture. Evidence of interstitial melting were shrinkage and inability to retain water both of which were observed to be greater for the atmospheric-dried product than for the vacuum dried sample. Oxidation also had adverse effects on flavor. Drying under an inert gas atmosphere such as nitrogen or helium would necessarily obviate the adverse effects of oxidation and a better programming of drying temperatures would also minimize interstitial melting. Even if drying times for the atmospheric freeze-dried products became appreciably longer than for the vacuum dried samples, a cost analysis would still show the process to be acceptably competitive as Woodward (1961) has indicated.

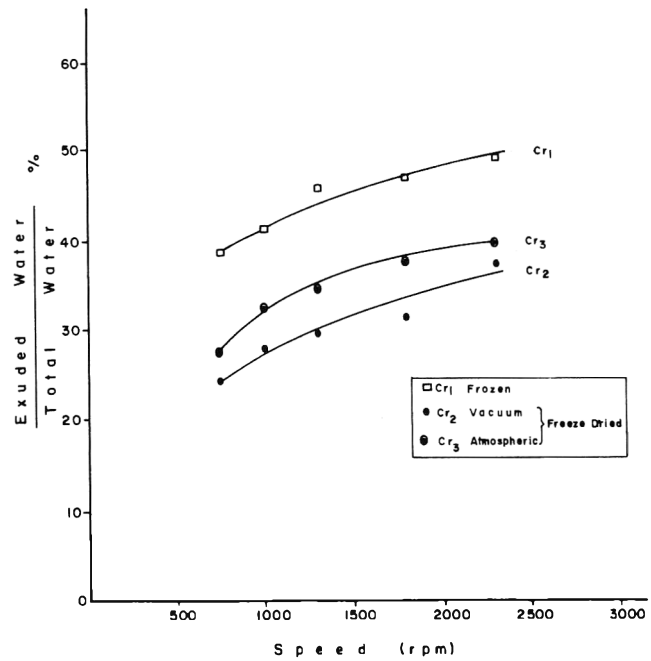

Fig. 8—Water retention plots for frozen and freeze-dried shrimp samples.

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A Comparison of Nonmeat Proteins, Sodium Tripolyphosphate and Processing Temperature Effects on Physical and Sensory Properties of Frankfurters

J. T. KEETON, E. A. FOEGEDING, and C. PATANA-ANAKE

ABSTRACT

Frankfurters, with and without 0.5% sodium tripolyphosphate (STPP), containing 3.5% vital wheat gluten (VWG), calcium reduced nonfat dry milk (RNFDM) and soy protein concentrate (SPC) were processed to an internal temperature of 72° or 82°C and compared to an all-meat control. Processing yields, textural profile analysis (TPA) and sensory textural attributes were not different among protein treatments, but SPC and VWG contributed slight-to-moderate off-flavor. VWG and SPC franks were acceptable, but slightly less desirable than the control and RNFDM treatments. Franks processed to 82°C were more desirable, but at the expense of reduced yields. STPP did not affect yields, but increased sensory firmness and TPA fracturability and hardness. VWG, RNFDM and SPC were comparable to the control for most traits studied, but alterations in spice formulation are needed to improve VWG and SPC flavor.

INTRODUCTION

MOST NONMEAT PROTEIN BINDERS and extenders used in sausages, such as frankfurters and bologna, are derived from soybeans or milk and serve to enhance some functional characteristic of the product while reducing costs. Proteins from blood, cottonseeds, eggs, peanuts, single-cell organisms, sunflowers and wheat have been incorporated into meat formulations with varying degrees of success. Several studies utilizing soy proteins as binders in frankfurters or similar products have found them to be compatible with chopped meat systems (Smith et al., 1973; Sofos and Allen, 1977; Sofos et al., 1977; Terrell and Staniec, 1975; Terrell et al., 1979). Among the plant proteins currently marketed, vital wheat gluten (VWG) also appears to offer potential as a nonmeat binder in these products. It is approved by the USDA for binding nonspecific meat loaves, poultry rolls and boneless meat pieces from the same muscle group. However, its incorporation into finely comminuted meats is limited by the lack of information concerning its functional characteristics in these types of products. Aref and Tape (1966) concluded that VWG and dried egg albumen were satisfactory binders for meat pieces because of their ability to produce intact turkey loaves with good slicing qualities. Siegel et al. (1979) used beef semitendinosus pieces to test the adhesion properties of various nonmeat proteins and found VWG and egg white to exert the strongest binding force in the presence of 8% sodium chloride (NaCl) and 2% sodium tripolyphosphate (STPP). In a similar study, Terrell et al. (1982) observed plasma protein and egg albumin to have higher viscoelastic properties than isolated soy protein (ISP) or VWG at 72°C, but when VWG was heated to 93°C, it had the highest viscoelastic value. Hand et al. (1981) reported restructured beef steaks containing 3.6% VWG or 3.2% ISP to be similar in overall desirability, but slightly less desirable than all-meat controls. The addition of seasonings (containing 0.44% NaCl, 0.25% STPP and 0.31% hydrolyzed vegetable protein) increased overall desirability of all samples, but the control remained slightly more desirable. Randall et al. (1976) reported increased product yields in a meat emulsion by substituting up to 80% of the beef component with devitalized wheat gluten, ISP and egg white, but undesirable textural changes were apparent at the 40% replacement level.

Among the treatments that alter textural and flavor characteristics of meat products, alkaline phosphates appear to be the most practical from the standpoint of labeling and economic considerations. Hamm (1970) reported polyphosphates to increase water-binding capacity (WBC) in meat products as a result of: an increase in pH and ionic strength; chelation of divalent metal ions; binding to the myofibrillar proteins and dissociation of actomyosin. Trout and Schmidt (1983) have shown that WBC in meat increases until the total ionic strength is greater than 0.6 and the pH more the 6.0. They also concluded that disodium phosphate, tetrasodium pyrophosphate and sodium tripolyphosphate were the most effective for increasing ionic strength, pH and WBC in meat. Puolanne and Ruusunen (1980) found these effects to be enhanced when combined with 1-2% NaCl. Combinations of NaCl and alkaline phosphates have been reported to act synergistically in beef and pork products to improve WBC, stabilize color and to increase meat particle binding and cooking yields (Ellinger, 1972; Huffman et al., 1981; Moore et al., 1976, Neer and Mandigo, 1977; Rongey and Bratzler, 1966; Sherman, 1961a, b; Swift and Ellis, 1957). Sensory characteristics of the products from these studies were also enhanced. Schwartz and Mandigo (1976) found a 0.75% NaCl/0.125% STPP combination to be optimum for use in restructured pork while Pepper and Schmidt (1975) reported a 2.0% NaCl/0.5% phosphate combination to have the highest cook yield in beef rolls. Shults et al. (1972) reported a blend of 75% STPP and 25% sodium hexametaphosphate (SHMP) to be the most effective combination for increasing water-holding capacity (WHC) in beef samples, while Vollmar and Melton (1981) found processing yields of hams to be greatest with a blend of 95% STPP and 5% SHMP. For wieners, Puolanne and Matikkala (1980) determined that a combination of 2.0% NaCl/0.3% pyrophosphate increased the pH to 6.1 and thus enhanced WBC.

Because of the limited information available concerning the use of VWG and alkaline phopshates in comminuted meats, this study was initiated to determine the effects of incorporating VWG, soy protein concentrate (SPC) calcium reduced non-fat dry milk (RNFDM) and STPP in a frankfurter product. Two processing temperature end points were selected to evaluate the thermosetting properties of the nonmeat proteins.

MATERIALS & METHODS

FROZEN BONELESS BEEF, 80% lean pork, 50% lean pork and 80% pork fat trimmings were purchased from local suppliers. Meat trimmings were ground through a 0.79 cm plate, packaged in freezer wrap, frozen at −30°C and used within 1 month. Percent moisture and fat (AOAC, 1980) were determined on all raw materials and

Author Keeton is affiliated with the Dept. of Animal Science, 346 Kleberg Center, Texas A&M Univ., College Station, TX 77843-2471. Authors Foegeding and Patana-Anake are affiliated with the Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27695-7624.

13.6 kg batches formulated on a 40/60 beef/pork basis. Frankfurter formulations were calculated on the basis of a finished product analysis of 54% moisture, 28% fat, 10–13% total protein and 5–8% nonmeat ingredients such as NaCl, dextrose, plant proteins and spices. Treatments consisted of VWG, SPC and RNFDM, substituted at 3.5% of the final product weight (Table 1 and 2), with an all-meat frankfurter serving as a control. Since STPP is the predominant polyphosphate in most phosphate blends and has been shown to have a large influence on WBC (Shults et al., 1972; Trout and Schmidt, 1983), its use was deemed appropriate for this study. Each formulation was made with and without 0.5% STPP and processed to an internal temperature of 72°C. Additional formulations without STPP were processed to 82°C and the experiment replicated four times. Vital wheat gluten was obtained from four processors (Henkel Corp., Minneapolis, MN; Industrial Grain Products, Montreal, Quebec; Manildra Milling Corp., Shawnee Mission, KS; Mid West Solvents, Atchison, KS) and equal portions blended to form a composite sample. Soy protein concentrate (GL-301, Griffith Laboratories, Alsip, IL), calcium reduced nonfat dry milk (Savortex, Mid America Farms, Springfield, MO) and sodium tripolyphosphate (Flavorphos, Flavorite Laboratories, Inc., Horn Lake, MS) did not require additional blending. Tempered (−2° to −1°C) beef and lean pork, salt, STPP (if required) and one-half the ice were chopped in a Hobart VCM-40 bowl chopper for 3 min followed by a rest period of approximately the same duration to allow for partial extraction of the salt-soluble proteins. The remainder of the ingredients were then added and chopped 3 min to an end point temperature of approximately 16°C with 1 min intermittent rest intervals to allow for hydration of the nonmeat proteins. The finished meat batter was stuffed into 26 mm No Jax® casing (Union Carbide Corp., Chicago, IL) from a Vemag (Robert Reiser Co., Inc., Boston, MA) vacuum stuffer, linked 13.3 cm in length and randomized by treatments on smoke sticks prior to processing in a Dry-Sys smokehouse. Heating rates were monitored by a potentiometer, fitted with iron-constantan thermocouples placed in the geometric center of at least two or more samples. Franks were processed using a step-wise heating schedule to an internal temperature of 72°C or 82°C, and then showered with cold water to 38°C. After chilling overnight to 4.5°C, samples were peeled, vacuum packaged in Cryovac® pouches (clear std gauge, P850, W.R. Grace and Co., Duncan, SC) and held at 2°C for further analyses. Since the primary factors of this investigation were textural and sensory characteristics rather than microbiological quality during storage, the samples were held no longer than two weeks before evaluation. Processing losses and yields were based upon the chilled, peeled weights prior to vacuum packaging.

Chemical analyses and emulsion stability

Raw emulsion and cooked products samples were analyzed for percent moisture and fat (AOAC, 1980). Emulsion stability (ES) (Townsend et al., 1968) was determined by measuring the moisture and fat released from a 30g sample after incremental heating in a water bath to simulate the smokehouse schedule. Triplicate samples were heated in polycarbonate centrifuge tubes and covered with aluminum foil to avoid excessive evaporation.

Sensory and textural evaluation

A descriptive sensory panel consisting of 15 members evaluated four samples per session using a structured, 8-point attribute scale. Springiness, firmness, flavor intensity, off-flavor and desirability were the descriptors considered and intensity of each increased with corresponding numeric values, e.g., 1 = not springy . . . 8 = extremely springy. Panelists were initially selected on the basis of triangle tests (AMSA, 1978) and previous experience with judging frankfurter products. Four training sessions were held at which a broad range of frankfurters were compared to a standard item. Each attribute was discussed and testing initiated after the panelists agreed with the scale on the ballot. Panelists varied in age from mid-20's to mid-50's. Partitioned booths with controlled lighting (red filter) accommodated panel members and these were located adjacent to the test kitchen. Samples were prepared by steeping 7 min in hot water (approx 92°C), draining the liquid and holding on a warming tray in covered sauce pans for no longer than 30 min. Sections approximately 6 cm in length, were served individually and order of presentation randomized among panelists. Tap water was supplied to the panelists for rinsing between samples.

Texture profile analysis (TPA), as described by Bourne (1978), was performed with the Instron Universal Testing Machine (Model 1130, Instron Corp., Canton, MA). Four, 13 mm frankfurter sections/sample were equilibrated to room temperature after heating and compressed to 25% of their original height through a two-cycle compression sequence. Force-time curves were obtained at a crosshead speed of 100 mm/min and chart speed of 200 mm/min. Texture variables from the force and area measurements were: F_F/g = force required to fracture; F_1/g = maximum force required for the first compression; A_1/g = total energy required for the first compression; F_2/g = maximum force required for the second compression; A_2/g = total energy required for the second compression; springiness = height that the sample recovered between the end of the first compression and start of the second; gumminess = $F_1 \times A_2/A_1$; chewiness = $F_1 \times A_2/A_1 \times S$; and cohesiveness = A_2/A_1.

Statistical analysis

Treatments were arranged in a split-plot design and randomized within each replication. Data were analyzed with the general linear model program of the Statistical Analysis System (Helwig and Council, 1979) and mean separation was accomplished by the Waller-Duncan K-ratio T test.

RESULTS & DISCUSSION

REPLICATION EFFECTS were different ($P < 0.05$) for some parameters measured, but interactions between main effects of nonmeat proteins, processing temperature end point and STPP were not different ($P > 0.05$). Data for all protein treatments were therefore pooled to allow for within treatment comparisons of end point temperature and addition of STPP (Table 3).

Kalin (1979) reported that VWG proteins coagulate irreversibly at approximately 85°C yielding a firm, resilient gel. Therefore, frankfurters were heated to an 82°C end point to determine if the thermosetting properties of nonmeat proteins resulted in textural changes in the product. In comparison, STPP was incorporated into formulations to evaluate yield and textural parameters at normal processing temperatures (72°C).

Means for the ES test are shown in Table 3. The volumes of water and total fluid released during heating of meat

Table 1—Composition of nonmeat binders used in frankfuter formulations

	Vital wheat gluten	Soy protein conc	Ca reduced nonfat dry milk
Protein	70.0%[a]	67.5%[b]	36.0%
Moisture	5.0-8.0	5.0	4.5
Fat	0.5-1.5	0.5	1.5
Ash/Carbohydrate	20.5-24.5	7.5	59.6[c]

[a] Protein = (N X 5.7) on a moisture free basis or 75.0-80.0% crude protein.
[b] Protein = 71.0% on a moisture free basis.
[c] Lactose = 51.0%; Minerals = 8.2%; Calcium = 0.4%.

Table 2—Formulations for all meat and protein added frankfurters[a]

Ingredients	Control kg	With protein kg
Beef lean trim (80/20) lean:fat ratio	4.36	4.17
Regular pork trim (50/50)	3.73	2.91
Fat pork trim (20/80)	2.81	3.33
Added water	3.33	3.69
Protein source (VWG, RNFDM or SPC), 3.5%	—	0.48
	g	g
NaCl, 2.25%	306.8	306.8
Dextrose, 2.0%	272.7	272.7
$NaNO_2$, 156 mg/kg meat	1.7	1.6
Ne erythorbate, 546 mg/kg meat	6.0	5.7
Spices, 6.23 g/kg	85.1	85.1

[a] All treatments were made with and without 0.5% sodium tripolyphosphate.

batters were not different among the protein treatments. Fat release from RNFDM samples was greater than other protein treatments indicating a reduced capacity for RNFDM to stabilize the lipid phase. Percent ES yields were greatest ($P < 0.05$) for VWG and RNFDM, intermediate for SPC and least for the all-meat control. Heating to 82°C increased moisture loss and total fluid release, but not lipid release, thus decreasing yield by 3.68%. Conversely, the addition of STPP decreased moisture loss and total fluid released while increasing yields by 4.95%. Heating to 82°C and the addition of STPP did not affect lipid release. Although the ES test does not duplicate the heating environment of commercial processing conditions, potential changes in meat systems as a result of treatments may be monitored. ES trends indicate some advantage in frankfurter yields with the addition of 3.5% VWG, RNFDM or SPC. However, the consequence of increasing processing temperature to 82°C appears detrimental to yields, while the addition of STPP may be advantageous.

Chemical analyses of processed frankfurters are given in Table 3. Percent moisture of the raw meat batter and finished product yields were not different among protein treatments. Yields from the ES test for protein treatments were similar to those from processing, except for the control. Moisture losses were greater for RNFDM and SPC treatments, followed by VWG and least for the all-meat control. Fat losses, however, were less for each protein treatment than for the control. This difference can be seen by combining the moisture and fat values to give 81.95, 78.39, 78.75 and 73.23% for the control, VWG, RNFDM and SPC treatments, respectively. The lower values for the nonmeat proteins appear to be the result of the protein's inability to prevent water loss from the product or a reduced capacity in the meat protein's ability to retain this constituent. In a similar study, Smith et al. (1973) observed increased emulsion stability and decreased fatting-out of frankfurters containing 34–35% fat and 3.5% SPC. These researchers attributed this stability to reduced interfacial tension between the protein additive and oil droplets when examined microscopically. However, in their study, 3.5% of the protein additive was added to the same meat block, but in this study, 3.5% of the meat block was substituted with the appropriate nonmeat protein. This in effect reduced the total meat protein available for interacting with the lipid and moisture components. These observations indicate that a critical muscle protein level is required when using nonmeat protein sources. Percent yield was not different among treatments and was most likely due to the residual protein and carbohydrate remaining after fat and moisture losses. Randall et al. (1976) reported that drip losses were

Table 3—Emulsion stability and chemical analyses means[a] of franks by protein, temperature and phosphate treatments

		Protein (3.5%)			Endpoint temp		Sodium tripolyphosphate (0.5%)	
	Control	VWG	RNFDM	SPC	72°C	82°C	Without	Added
Emulsion stability (ES)								
Volume (mL) of water released/100g	9.95[b]	8.07[b]	7.42[b]	9.19[b]	6.69[c]	10.62[b]	6.69[b]	2.23[c]
Volume (mL) of lipid released/100g	0.34[c]	0.34[c]	0.78[b]	0.36[c]	0.48[b]	0.43[b]	0.48[b]	0.31[b]
Total (mL) of fluid released/100g	10.29[b]	8.40[b]	8.11[b]	9.56[b]	7.18[c]	10.99[b]	7.18[b]	2.53[c]
ES Yield (%)	86.53[c]	89.08[b]	88.73[b]	87.51[b,c]	89.80[b]	86.12[c]	89.80[c]	94.75[b]
Chemical analysis (Product)								
Moisture, raw (%)	56.38[b]	56.12[b]	56.23[b]	55.62[b]	56.12[b]	56.05[b]	56.12[c]	56.73[b]
Moisture, cooked (%)	50.18[b]	49.33[b,c]	48.99[c]	48.39[c]	50.16[b]	48.54[c]	50.16[c]	50.76[b]
Fat, cooked (%)	31.77[b]	29.06[c]	29.76[c]	29.34[c]	29.67[b]	30.29[b]	29.67[b]	28.80[c]
Yield (%)	88.73[b]	88.82[b]	89.35[b]	88.62[b]	89.64[b]	88.23[c]	89.64[b]	89.79[b]

[a] n = 8 for protein source; n = 16 for endpoint temperature; n = 12 for phosphate treatment.
[b,c] Means in rows and within treatments followed by the same superscript are not significantly different (P>0.05).

Table 4—Texture profile analysis and sensory evaluation means[a] by protein, temperature and phosphate treatments

		Protein (3.5%)			Endpoint temp		Sodium tripolyphosphate (0.5%)	
	Control	VWG	RNFDM	SPC	72°C	82°C	Without	Added
Textural profile analysis (TPA)								
Fracturability (F_F), N[f]	54.87[b]	61.40[b]	66.79[b]	60.31[b]	56.39[c]	65.20[b]	56.39[c]	62.86[b]
First bite hardness (F_1), N	84.26[b]	82.45[b]	80.93[b]	97.62[b]	79.53[c]	93.09[b]	79.53[b]	87.07[b]
Second bite hardness (F_2), N	59.62[b]	60.09[b]	59.93[b]	68.13[b]	57.18[c]	66.71[b]	57.18[c]	63.53[b]
Cohesiveness (A_2/A_1)	0.18[b]	0.19[b]	0.19[b]	0.18[b]	0.18[b]	0.19[b]	0.18[b]	0.19[b]
Springiness (S), mm	10.96[b]	11.15[b]	11.39[b]	10.60[b]	11.09[b]	10.95[b]	11.09[b]	10.78[b]
Gumminess $F_1 \times A_2/A_1$	15.39[b]	15.56[b]	15.30[b]	17.79[b]	14.65[c]	17.38[b]	14.65[b]	17.15[b]
Chewiness $F_1 \times A_2/A_1 \times S$	166.54[b]	173.85[b]	175.69[b]	188.22[b]	161.81[c]	190.33[b]	161.81[b]	188.04[b]
Sensory panel evaluation[e]								
Springiness	4.01[b]	4.23[b]	4.61[b]	4.18[b]	4.14[b]	4.38[b]	4.14[b]	4.45[b]
Firmness	3.83[b]	3.85[b]	4.55[b]	3.89[b]	4.03[b]	4.03[b]	4.03[c]	4.40[b]
Flavor intensity	4.99[b,c]	5.00[b,c]	5.17[b]	4.77[c]	4.89[b]	5.08[b]	4.89[b]	4.88[b]
Off-flavor	1.61[d]	3.81[b]	1.40[d]	3.41[c]	2.55[b]	2.57[b]	2.55[b]	2.25[b]
Desirability	5.15[b]	2.92[c]	5.58[b]	3.21[c]	3.97[c]	4.48[b]	3.97[b]	4.31[b]

[a] n = 8 for protein source; n = 16 for endpoint temperature; n = 12 for phosphate treatment.
[b,c,d] Means in rows and within treatments followed by the same superscript are not significantly different (P>0.05).
[e] Sensory scale intensity increased with numeric value, e.g. 1 = Not springy. . . 8 = Extremely springy.
[f] N = Newtons.

largely accounted for by the nonlipid fraction of meat emulsions containing up to 80% of VWG, ISP or egg white binders. Further study of these components is warranted to identify the influence of various nonmeat proteins. Processing to an 82°C endpoint did not affect percent fat, but decreased percent moisture 1.62% and product yield by 1.41%. Similar results were observed for the ES test. The addition of STPP reduced moisture losses, but with a correspondingly greater fat loss resulting in no change in overall yield. Although the ES test indicated that greater yields were possible with STPP addition, this observation probably represents the difference in moisture retention due to stoppered, plastic centrifuge tubes versus a porous cellulose casing.

As shown in Table 4, TPA values for the protein treatments were not different from the control. In studies with high levels of textured soy protein (25-30%, Sofos et al., 1977) or VWG (40%, Randall et al., 1976), textural quality decreased. Increasing the temperature endpoint to 82°C, resulted in higher values for force to fracture (F_F) first and second bite hardness (F_1, F_2) gumminess and chewiness. STPP addition increased the force to fracture (F_F) and second bite hardness, but had no effect on other TPA parameters. Franks with STPP appeared to have stronger skin formation which would have contributed to their resistance to fracture.

Sensory panel evaluations (Table 4) were not different for springiness or firmness of frankfurters containing VWG, RNFDM and SPC. These observations agree with those values from TPA. Flavor intensity was evaluated at the same spice level because the addition of nonmeat proteins often tends to contribute a bland or off-flavor to meat products. VWG and RNFDM flavor intensity was not different ($P > 0.05$) from the control, nor was the SPC different ($P > 0.05$) from the control and VWG. However, flavor intensity was greater for RNFDM franks in comparison to the SPC treatment. Off-flavor was slight to moderate for VWG franks, slightly detectable with SPC and at the threshold level for RNFDM and control treatments. Overall desirability tended to follow the same trend as flavor with VWG and SPC franks being slightly desirable and RNFDM and control franks moderately desirable. Hand et al. (1981), Seideman et al. (1982a, b) and Terrell et al. (1979) also reported VWG, SPC or ISP at levels greater than 3.0% to reduce flavor and desirability of restructured poultry steaks, restructured beef steaks and frankfurters, respectively. Since off-flavor, rather than textural differences was the primary factor influencing desirability scores for VWG and SPC franks, spice formulations could be developed to make these products more compatible with meat systems. The higher end point temperature increased TPA fracturability (brittleness), hardness, gumminess and chewiness while STPP increased fracturability and hardness. Processing to 82°C increased desirability and the addition of STPP increased frank firmness, respectively, but neither had an effect on other sensory parameters. This is in agreement with Puolanne and Terrell (1983) and Rongey and Bratzler (1966) who also reported increased firmness in sausages with the addition of phosphates. Although STPP did not enhance most sensory properties in this study, its inclusion could be advantageous for increasing resiliency or "bite" of frankfurters.

Correlation coefficients for selected sensory and texture profile values are given in Table 5. No correlations were significant between TPA and sensory evaluations for the nonmeat protein treatments and only small, but significant values were observed for heating end points. For STPP, fracturability (F_F) accounted for 53 and 55% of the variation in sensory firmness and springiness, respectively. However, because of the low correlations, the TPA and sensory panel appear to be measuring different textural attributes.

Table 5—Selected correlation coefficients for texture profile analysis and sensory evaluation

		Sensory evaluation parameters	
Treatments	TPA parameters	Springiness	Firmness
Endpoint temp	Fracturability (F_F), N[a]	0.45**	
	Springiness (S), mm		−0.38
STPP	Fracturability (F_F), N[a]	0.74**	0.73**
	Gumminess ($F_1 \times A_2/A_1$)	0.41*	
	Chewiness ($F_1 \times A_2/A_1 \times S$)	0.53**	

[a] Newtons
* $P \leq 0.05$
** $P \leq 0.01$

CONCLUSIONS

ES TESTS indicated that the addition of 3.5% VWG, RNFDM or SPC to frankfurter formulations could increase yields. However, actual processing yields were not different even though moisture and fat losses were greater for each of the protein treatments (except for VWG moisture). Maintenance of processing yields was attributed to the residual nonmeat protein and carbohydrate components, but further investigation is needed to identify the influence of these components for binding moisture and fat. No textural differences were apparent among protein treatments, but SPC decreased flavor intensity slightly while VWG and SPC contributed some off-flavor. VWG and SPC franks were acceptable, but less desirable overall than all-meat or RNFDM franks. Since off-flavor appears to influence the desirability of VWG and SPC treatments, consideration should be given to altering the spice formulation to improve desirability and expand the potential application of these proteins. Franks processed to 82°C were rated more desirable by the sensory panel, but at the expanse of reduced yields. This high temperature would also be, undesirable from the standpoint of increased energy consumption. No thermosetting effects were noted for VWG, SPC or RNFDM. Addition of STPP increased ES yields by 4.95%, but did not affect processing yields. Sensory firmness and TPA fracturability and hardness were increased by STPP, but other textural and sensory parameters were not affected. Based on these results, other phosphate blends and nonmeat proteins should be investigated to determine their effects on physical and sensory properties of frankfurters.

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Electrical Stimulation, Hot-Boning and Prerigor Cookery Effects on Lamb Longissimus Tenderness

D. J. STOLARZ, J. D. THOMAS, E. E. RAY, and M. CARDENAS

ABSTRACT

Paired halves of 32 lamb carcasses were either electrically stimulated (ES) or not (NES), then assigned to one of the following treatments: (1) hot-boned, cooked prerigor, frozen and reheated (HB-PRC); (2) conventionally chilled and boned, cooked, frozen and reheated (CB-ARC); (3) hot-boned, frozen and cooked (HB); and (4) cold-boned, frozen and cooked (CB). Electrical stimulation lowered ($P < 0.05$) peak force (PF) of chops from CB-ARC, HB and CB treatments. Nonstimulated HB-PRC chops had a lower ($P < 0.05$) PF than ES, HB-PRC chops. Lower ($P < 0.05$) compression values were noted for HB-PRC and CB-ARC chops than for HB and CB chops. Electrical stimulation reduced ($P < 0.05$) PF regardless of cooking method. Chops cooked in the microwave had lower ($P < 0.05$) work values than chops cooked in the convection oven.

INTRODUCTION

THE MEAT INDUSTRY is highly labor and energy intensive and recent research has focused on methods that reduce labor and energy input. Because of the realization that postmortem factors such as electrical stimulation, hot-boning and prerigor cookery influence meat palatability more than antemortem factors (Cross, 1979), the use of alternative processing methods has been studied. Accelerated processing has been shown to increase product turnover, reduce chill time, conserve space, reduce product shrinkage and reduce labor costs (Henrickson, 1975).

Accelerated processing includes electrical stimulation, hot-boning and prerigor cookery. Numerous studies involving the effects of these methods upon muscle tissue and the feasibility of their commercial use have been conducted (Kastner et al., 1973; Crystall and Hagyard, 1976; Gilbert and Davey, 1976; Ray et al., 1980a and b, 1982, 1983; Berry et al., 1981). Most research in this area has been with beef as opposed to lamb.

This study was conducted to examine the effects of electrical stimulation, hot-boning and prerigor cookery on the tenderness of lamb loin chops cooked or reheated by three different methods as measured by Instron® textural analyses.

MATERIALS & METHODS

Carcass preparation

Thirty-two market lambs of similar weights and ages were slaughtered. Carcasses were split longitudinally. One half of each carcass was electrically stimulated (ES) with 100 impulses (1 second on, 1 second off, A.C., 100V, 1-2 amps) while the paired half was not electrically stimulated (NES). Electrical stimulation was done 45 min post-exsanguination. One stimulator probe was inserted medial to the scapula and thoracic vertebrae and one probe inserted into the semimembranosus muscle parallel to the femur bone. Loin roasts (13th rib to anterior portion of ilium) from the paired halves were then assigned to one of four treatments: (1) hot-boned, cooked prerigor, frozen and reheated (HB-PRC); (2) conventionally chilled (24 hr, 2°C) and boned, cooked, frozen and reheated (CB-ARC); (3) hot-boned, frozen and cooked (HB); and (4) conventionally chilled and boned, frozen and cooked (CB). Weights, before and after treatment, for all experimental groups of loin roasts were recorded.

All authors are affiliated with the Animal & Range Sciences Dept., New Mexico State Univ., Box 3-I, Las Cruces, NM 88033.

Water bath cooking schedule

Roasts from HB-PRC and CB-ARC treatments (treatments 1 and 2) were put in cooking bags and heated in a water bath according to a 6-hr schedule. Initial water temperature was 46°C. The temperature was increased to 51.5°C at the end of the first hour, to 57°C at the end of the second hour, to 62.5°C at the end of the third hour, to 68°C at the end of the fourth hour and to 79°C at the end of the fifth hour. Thermocouples were inserted into the geometric center of each roast. Internal temperatures of the roasts were continually monitored with a potentiometer (Honeywell®). Roasts were removed from the water bath upon reaching an internal temperature of 68°C. Cooking times and cooked weights were recorded. Roasts from all treatments were vacuum packaged and frozen in a blast freezer (−29°C).

Sample preparation

Each of the 64 loins was cut while frozen into six 1-inch-thick chops, thawed overnight (1.1°C), and the bones removed. Frozen, thawed and boneless weights were recorded. Thaw loss and percentage thaw loss were determined. The first two successive chops removed from the rib end of each loin were cooked or reheated in a Blodgett®, forced air convection oven at an operating temperature of 149°C, the next two on a Farberware® broiler, and the last two in a microwave oven (Amana® Touchmatic, 482.6W cooking power). Precooked chops (treatments 1 and 2) were reheated to an internal temperature of 49°C while the uncooked chops were heated to an internal temperature of 68°C. Temperatures were monitored with a Koch® meat thermometer, inserted in the geometric center of each chop. Percentage cooking loss for each chop was determined. Percentage cooking losses during precooking and reheating for chops from treatments 1 and 2 were combined to produce total cooking loss. Percentage cooking loss during cooking of chops from treatments 3 and 4 was also designated as total cooking loss. Chops were cooled to room temperature. Three cores (1.3 cm) were obtained from one chop for each cooking or reheating method for Instron® (Model 1122, 500 kg load cell) Warner-Bratzler Shear analysis. A 1-centimeter-thick sample was also obtained from one chop for each cooking or reheating method for Instron compression analysis.

Instron analysis

Warner-Bratzler measurements. A blade, with a blunt-edged triangular hole containing the core, was pulled past the blade guide at a rate of 250 mm/min. Force-deformation curves were recorded with a load scale setting of 10 kg and 500 mm/min chart speed. Integrator values were recorded to determine total work done. Each core for Warner-Bratzler testing was sheared three times. Parameters evaluated included peak force (PF) and total amount of work needed to shear the core or Warner-Bratzler Work Done (WBWD).

Compression measurements. A 0.70 cm-diameter rod was driven 0.80 cm into the 1.0 cm thick samples at a compression rate of 50 mm/min (CPFA). Fibers were aligned perpendicular to the rod travel. The rod was withdrawn and driven to the same depth into the damaged area (CPFB). A force-deformation curve was recorded for both compressions with a 10 kg load scale setting and 200 mm/min chart speed. Integrator values for each penetration were recorded to determine work done.

Each compression sample was evaluated on three separate areas. Parameters evaluated for compression testing included peak force for each compression, (CPFA, CPFB) and work done for both compressions (WCA, WCB). Other parameters evaluated were cohesiveness, which was the ratio of WCB to WCA, and chewiness, which was obtained by multiplying cohesiveness of CPFA.

Statistical analysis

Data were analyzed by analysis of variance of treatment by cooking method by stimulation (4 x 3 x 2 factorial). Means were separated by least significant difference (Snedecor and Cochran, 1973).

RESULTS & DISCUSSION

Electrical stimulation by treatment

An electrical stimulation by treatment interaction was noted for PF, CPFA, CPFB, chewiness, WBWD, WCA and WCB (Tables 1, 2, and 3). The three-way interaction of electrical stimulation by treatment by cooking method was not significant for these parameters. Therefore, the data presented in Tables 1 and 2 were pooled over cookery method and the data in Table 3 were pooled over treatment. Electrical stimulation lowered ($P < 0.05$) PF of CB-ARC, HB and CB chops (Table 1). Peak shear force values have been demonstrated to be an indicator of myofibrillar toughness (Bouton et al., 1975). Hence, reduced peak force values for chops from electrically stimualted CB-ARC, HB and CB treatments indicate that electrical stimulation decreased myofibrillar toughness when combined with after rigor cookery, hot-boning or cold-boning. Nonstimulated chops from HB-PRC treatment had a lower ($P < 0.05$) PF than electrically stimulated HB-PRC chops, indicating that ES may not be necessary when cooking prerigor meat. It is likely that ES, which increases the rate of rigor onset, combined with heat induced sarcomere contraction, caused thermal denaturation of the muscle proteins to occur while the sarcomeres were in a highly contracted state. Conversely, NES samples did not have any of the stimulated-induced shortening and, therefore, the sarcomeres were longer at the time of protein denaturation.

Chops from the nonstimulated, CB treatment had the highest ($P < 0.05$) PF values. The application of hot-boning, prerigor cookery and electrical stimulation, alone or in combination, produced lower ($P < 0.05$) PF values.

Warner-Bratzler work done (Table 1), the total work needed to shear the sample, was higher ($P < 0.05$) for electrically stimulated CB chops than for electrically stimulated HB-PRC and CB-ARC chops indicating that prerigor and after rigor cookery used in combination with ES produced a more tender product than when using ES in conjunction with the conventional processing method. Nonstimulated, CB chops had the highest ($P < 0.05$) WBWD and ES lowered ($P < 0.05$) WBWD of CB-ARC, HB and CB chops. This concurs with PF data and it appears that ES should be used in combination with postrigor cookery,

Table 1—Least squares means of Warner-Bratzler parameters for electrical stimulation by treatment

Parameter	Treatment			
	HB-PRC[f]	CB-ARC[g]	HB[h]	CB[i]
Peak force (kg/1.27 cm)				
Electrical stimulation (ES)	2.11[a]	2.10[a]	2.63[b]	2.95[b]
Nonelectrical stimulation (NES)	1.71[c]	3.81[d]	4.16[d]	5.37[e]
Warner-Bratzler work done (joules)				
ES	17.70[ab]	18.25[b]	20.53[bc]	23.40[c]
NES	14.47[a]	30.31[d]	33.21[d]	43.04[e]

[abcde] Means within parameters in same row or same column with a different superscript letter differ ($P < 0.05$).
[f] Hot-boned, cooked prerigor, frozen, reheated.
[g] Conventionally chilled and boned, cooked, frozen, reheated.
[h] Hot-boned, frozen, cooked.
[i] Cold-boned, frozen, cooked.

Table 3—Least squares means of peak force (PF) and Warner-Bratzler work done (WBWD) for electrical stimulation by cooking method

Parameter	Cooking method		
	Convection	Farberware	Microwave
Peak force (kg/1.27 cm)			
Electrical stimulation	2.56[a]	2.42[a]	2.37[a]
Nonelectrical stimulation	4.39[b]	3.56[c]	3.34[c]
Warner-Bratzler work done (joules)			
Electrical stimulation	21.32[b]	20.58[ab]	18.01[a]
Nonelectrical stimulation	36.02[d]	28.58[c]	26.17[c]

[abcd] Means within parameters in same row or same column with a different superscript letter differ ($P < 0.05$).

Table 2—Least squares means of compression parameters for electrical stimulation by treatment

Parameter	Treatment			
	HB-PRC[e]	CB-ARC[f]	HB[g]	CB[h]
Peak Force, First Compression (kg)				
Electrical Stimulation (ES)	3.17[a]	2.49[b]	3.93[c]	4.12[cd]
Non-electrical Stimulation (NES)	2.89[a]	2.80[ab]	4.40[d]	3.82[c]
Peak Force, Second Compression(kg)				
ES	2.71[b]	2.16[a]	3.46[cd]	3.53[cd]
NES	2.45[ab]	2.35[a]	3.73[d]	3.34[c]
Chewiness				
ES	1.40[b]	1.11[a]	1.93[cd]	2.01[cd]
NES	1.27[ab]	1.13[a]	2.06[d]	1.83[c]
Cohesiveness				
ES	0.44[b]	0.44[b]	0.49[c]	0.49[c]
NES	0.44[b]	0.40[a]	0.48[c]	0.48[c]
Work Done, First Compression(joules)				
ES	7.55[b]	5.28[a]	9.75[cd]	9.85[d]
NES	6.48[ab]	6.44[ab]	11.82[e]	8.04[bc]
Work Done, Second Compression (joules)				
ES	3.34[bc]	2.32[a]	4.74[d]	4.76[d]
NES	2.81[ab]	2.57[a]	5.40[d]	3.81[c]

[abcd] Means within parameters in same row or same column with a different superscript letter differ ($P < 0.05$).
[e] Hot-boned, cooked prerigor, frozen, reheated.
[f] Conventionally chilled and boned, cooked, frozen, reheated.
[g] Hot-boned, frozen, cooked.
[h] Cold-boned, frozen, cooked.

hot-boning and cold-boning processing procedures. Physical disruption of myofibrils resulting from massive contractions during electrical stimulation may have resulted in lower values for Warner-Bratzler tenderness measurements (Savell et al., 1978). Contracture bands and broken or stretched sarcomeres have been noted for electrically stimulated samples by numerous workers (Dutson et al., 1980; George et al., 1980; Will et al., 1980).

Table 2 presents least squares means of compression parameters for electrical stimulation by treatment interactions. Peak force for the first and second compressions was significantly lower ($P < 0.05$) for HB-PRC and CB-ARC chops than for HB and CB chops, indicating that cooking, freezing and reheating increased tenderness over the more conventional treatments of freezing and cooking. A lower ($P < 0.05$) CPFA value was noted for electrically stimulated, HB chops than for nonstimulated, HB chops. Thaw rigor and its severe toughening effect on muscle is the major disadvantage to freezing hot-boned, prerigor meat (Marsh et al.,1 968). These data indicate that electrical stimulation may be used to reduce tenderness disadvantages due to thaw rigor. These results agree with other studies which have indicated a tenderness advantage using electrical stimulation in conjunction with hot-boning (Gilbert et al., 1976; Bouton et al., 1980; Kastner et al., 1980; Rashid et al., 1983). The carcass conditioning period may be reduced or eliminated when hot-boning electrically stimulated carcasses because there was a rapid decline in pH and a rapid depletion of muscle adenosine triphosphate (Savell et al., 1977; Whiting et al., 1981). Other studies indicated electrical stimulation did not produce any tenderness improvement in hot-boned meat (Kastner et al., 1980; Ray et al., 1982; Lyon et al., 1938).

Bouton and Harris (1972a) reported that chewiness values were strongly influenced by the connective tissue component of tenderness. Lower chewiness values indicate greater connective tissue degradation. Bouton and Harris (1972b) reported a reduction in chewiness values with increasing cooking time. Chewiness values (Table 2) were lowest ($P < 0.05$) for the HB-PRC and CB-ARC samples. This was probably due to the slow, moist heat cooking cycle.

Cohesiveness values indicate the structural damage that occurs during compression. Samples with high cohesiveness values have undergone less damage and, therefore, are more likely to return to their original shape and require more work for the second compression. Hayward et al. (1980) reported that blade tenderized steaks had lower cohesiveness values than nonblade tenderized steaks. The HB-PRC and CB-ARC had lower cohesiveness values than their HB and CB counterparts. This again indicates that the slow moist heat cooking cycle caused more structural damage.

The Warner-Bratzler data (Table 1), except for the electrically stimulated HB-PRC treatment, consistently showed a significant ($P < 0.05$) advantage in tenderness due to electrical stimulation. However, the compression data (Table 2) did not show this electrical stimulation advantage. Instead, the compression data separated the HB-PRC and CB-ARC from the HB and CB treatments. Obviously, the Warner-Bratzler shear and the compression rod were measuring two different parameters of tenderness. Bouton et al. (1975) reported that shear values were strongly influenced by the myofibrillar proteins while Bouton and Harris (1972a) reported that compression measurements were most strongly influenced by connective tissue. It can be concluded that electrical stimulation caused an increase in myofibrillar tenderness while the slow, moist heat cookery of the HB-PRC and CB-ARC treatments caused an increase in connective tissue tenderness.

Lower ($P < 0.05$) WCA and WCB values were noted for electrically stimulated, HB-PRC and CB-ARC chops than for electrically stimulated, HB and CB chops. Nonstimulated, HB chops had the highest ($P < 0.05$) WCA and WCB values, most likely because of thaw rigor of hot-boned roasts. The lower ($P < 0.05$) WCA for electrically stimulated, HB chops indicated the tenderness advantage of electrical stimulation used in conjunction with hot-boning. A decrease in tenderness of hot-boned meat has been reported by several authors (Gilbert and Davey, 1976; Cross et al., 1979; Ray et al., 1980a, 1983; Berry et al., 1981; Griffin et al., 1981; Lyon et al., 1983). On the contrary, McLeod et al. (1973) reported that cooked, hot-boned lamb legs were more tender than cooked, cold-boned lamb legs. In the present study, nonstimulated, CB chops had lower ($P < 0.05$) WCA and WCB values indicating that electrical stimulation may not be necessary when using the conventional processing method.

It is apparent that low temperature, long duration cooking of hot-boned lamb roasts yields products of acceptable tenderness. Previous studies evaluating prerigor cookery using a high temperature, short duration heating schedule have shown a decrease in tenderness of meat samples (Ray et al., 1980a and b, 1982; Berry et al., 1981; Griffin et al., 1981). The use of bone-in cuts in this study, rather than boneless cuts used in other studies, may have affected the tenderness because the skeletal restraint may have inhibited fiber shortening. Furthermore, slow, low temperature cooking may have resulted in less severe rigor development during heating (Ray et al., 1983). Although lower ($P < 0.05$) CPFA, CPFB, chewiness, WCA and WCB values were noted for electrically stimulated, CB-ARC chops than for electrically stimulated, HB-PRC chops, hot-boning and

Table 4—Least squares means for certain parameters for chops cooked in the convection oven, on the farberware broiler or in the microwave oven

Parameter	Cooking method		
	Convection	Farberware	Microwave
Peak force, First compression (kg)	3.68[a]	3.30[b]	3.39[b]
Peak force, Second compression (kg)	3.18[a]	2.83[b]	2.90[b]
Chewiness	1.68[a]	1.51[b]	1.56[ab]
Work done, First compression (joules)	9.19[a]	7.42[b]	7.86[b]
Work done, Second compression	4.15[a]	3.35[b]	3.67[b]

[ab] Means within parameters in same row with a different superscript letter differ ($P < 0.05$).

Table 5—Least squares means for total cooking loss for electrical stimulation by cooking method by treatment interaction (ES X CM X TRT)

	Electrical stimulation			Nonelectrical stimulation		
	Cooking method					
Treatment	CONV[h]	FW[i]	MW[j]	CONV	FW	MW
HB-PRC[k]	26.32[d]	27.87[de]	19.01[bc]	28.79[de]	26.34[d]	19.75[bc]
CB-ARC[l]	26.85[d]	27.34[d]	18.00[ab]	27.49[d]	27.56[de]	17.15[ab]
HB[m]	32.80[fg]	37.84[g]	19.24[bc]	34.60[g]	26.78[d]	22.38[c]
CB[n]	29.64[def]	32.55[fg]	19.00[bc]	31.38[efg]	35.28[g]	14.92[a]

[abcdefg] Means in same row or same column with different superscript letter differ ($P < 0.05$).
[h] Convection oven.
[i] Farberware broiler.
[j] Microwave oven.
[k] Hot-boned, cooked prerigor, frozen, reheated.
[l] Conventionally chilled and boned, cooked, frozen, reheated.
[m] Hot-boned, frozen, cooked.
[n] Cold-boned, frozen, cooked.

prerigor cookery processing methods should be feasible because of reduced energy costs (Ray et al., 1980a).

Electrical stimulation by cooking method

An electrical stimulation by cooking method interaction was noted for PF and WBWD and means for those parameters are shown in Table 3. Electrically stimulated chops cooked by all three methods had lower ($P < 0.05$) PF values than nonstimulated chops cooked by the same methods. Hence, electrical stimulation increased tenderness of chops, regardless of the cooking method used. Nonstimulated chops cooked in the convection oven had a higher ($P < 0.05$) PF than nonstimulated chops cooked on the Farberware® and in the microwave oven. This was probably caused by use of forced air in convection oven cooking which may have increased moisture losses and decreased tenderness. These data are in agreement with other research which have shown that microwave cooked meat can compare favorably in tenderness with conventionally cooked meat (Ruyack and Paul, 1972; Korschgen et al., 1976; Baldwin et al., 1979).

Data for WBWD followed a trend similar to PF data. Electrical stimulation lowered ($P < 0.05$) WBWD of chops cooked by all three methods. Electrically stimulated and nonstimulated chops cooked in the microwave oven had lower ($P < 0.05$) WBWD values than ES and NES chops cooked in the convection oven, respectively. Thus, precooked and nonprecooked chops would be most tender when reheated or cooked in a microwave oven or on a Farberware® grill.

Cooking method

Means for main effect differences for cooking method are shown in Table 4. These means are presented only for parameters in which there were no significant interactions due to electrical stimulation or treatment. Higher ($P < 0.05$) CPFA, CPFB, WCA and WCB values were obtained for chops cooked in the convection oven than for those cooked by either of the other methods.

Cooking loss

An ES by cookery method by treatment interaction was noted for total cooking loss (Table 5). Chops within the same treatments, regardless of electrical stimulation, had less ($P < 0.05$) total cooking loss when cooked in the microwave oven than when cooked by the other methods. On the contrary, numerous studies have recorded higher cooking losses for meat cooked in a microwave oven than for meat cooked by conventional methods (Baldwin et al., 1979; Moore et al., 1980; Griffin et al., 1981). The low total cooking loss for microwave cooked samples was probably a result of the short cooking time. Other studies have shown that cooking time of meat could be reduced by heating with microwaves (Moore et al., 1980; Griffin et al., 1981).

Few differences within cooking method and treatment between ES and NES samples were noted. Nonstimulated HB chops cooked on the Farberware and nonstimulated CB chops cooked in the microwave oven had lower cooking losses ($P < 0.05$) than their electrically stimulated counterparts. Other researchers have found no effect of electrical stimulation on percent cooking loss (Riley et al., 1980; Griffin et al., 1981; Ray et al., 1983; Rashid et al., 1983). Savell et al. (1978) also noted a higher percent cooking loss for electrically stimulated meat than for nonstimulated meat. Electrical stimulation did not have a consistent effect on cooking loss of meat.

Electrically stimulated, HB-PRC and CB-ARC chops cooked on the Farberware had less ($P < 0.05$) total cooking loss than electrically stimulated, HB and CB chops cooked on the Farberware. Also, total cooking loss was less ($P < 0.05$) in the convection oven for nonstimulated, HB-PRC and CB-ARC than for the HB and CB chops. These data indicate that precooking treatments may decrease cooking losses.

CONCLUSIONS

PEAK FORCE and WBWD values for ES chops from CB-ARC, HB and CB treatments indicate that electrical stimulation produced a tenderness advantage when utilizing postrigor cookery, hot-boning and cold-boning but not when utilizing prerigor cookery. Alternative processing methods, pre- or postrigor cookery, hot-boning (each with or without electrical stimulation) and electrical stimulation with cold-boning produced more desirable results than when conventionally chilling and boning. Electrical stimulation increased tenderness of chops regardless of cooking method used. Low temperature, long duration cooking and then reheating of hot-boned and conventionally chilled and boned lamb roasts yielded products of improved tenderness. Because it appeared that electrical stimulation was required to enhance tenderness of postrigor cooked (CB-ARC) chops and not prerigor cooked (HB-PRC) chops, hot-boning followed by prerigor cookery should be more feasible as a result of reduced energy costs (Ray et al., 1980a).

Precooked and nonprecooked chops were most tender when they were reheated or cooked on the Farberware broiler or in the microwave oven. Furthermore, total cooking loss was lowest for chops cooked in the microwave oven. The advantages noted for microwave cookery are most likely due to decreased cooking time.

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Aeration and Its Influence on the Microbial Sequence in Cacao Fermentations in Bahia, with Emphasis on Lactic Acid Bacteria

F. M. L. PASSOS, A. S. LOPEZ, and D. O. SILVA

ABSTRACT

Three aeration treatments on cacao fermentations in Bahia produced a basically similar microbial sequence which was influenced by the time and intensity of aeration. An initial, low temperature, semi-anaerobic phase dominated by yeasts and lactic acid bacteria, was superceded by aerobic and thermophyllic bacteria when the fermentations gained heat after aeration. Delay in aeration retarded this change in flora while over aeration accelerated it and, in addition, provoked a second lactic acid bacteria phase towards the end of fermentation. *Lactobacillaceae* were predominant during the anaerobic period and *Streptococeaceae* under aerated conditions. This marked, lactic acid bacteria population during the early stages of fermentation is not usual and would account for the lactic and acetic acids produced during this period.

INTRODUCTION

CACAO is one of the many foods whose characteristic flavor is developed by a curing process which involves a series of natural fermentations occurring in the mucilagenous pulp surrounding the seed. The sequence and duration of these fermentations are governed by the aeration and physicochemical conditions of the process which, in turn are determined by the procedures, and receptacles.

Two main stages are recognized: an early anaerobic fermentation during which yeast convert the sugars of the pulp to alcohol, and a succeeding aerobic fermentation when acetic acid bacteria oxidize the alcohol formed, first into acetic acid and then to CO_2 and H_2O. Interposed between these two is what has hitherto been considered a transient lactic acid bacterial fermentation of little consequence (Roelofsen, 1958).

The products of these fermentations diffuse into the bean and provoke biochemical changes which result in the production of flavor precursors. Some unchanged compounds from the pulp that remain in the seed after curing, themselves participate in the flavor. In excess, these compounds may cause off-flavors undesirable in the manufacture of chocolate. Typical of this is the acid flavor of the cacao from Brazil and Malaysia that carries over into final manufactured product. This is one of the reasons for manufacturers' preference for West African cacao and discount prices on Brazilian cacao on international markets.

Acetic acid had been found to be the chief agent of acidity of Brazilian cacao (Lopez, 1982c). This is often accompanied by excessive quantities of lactic acid (Lopez, 1982a) which, being nonvolatile, would be retained in the finished product (Rohan and Stewart, 1965; Seiki, 1977).

Acid production is dependent on aeration (Biehl, 1965; Quesnel, 1968). Acidity increases with increasing aeration up to a certain point after which further increases in aeration result in a reduction in acidity (Lopez, 1974). Brazilian fermentations appear to be insufficiently aerated and under these conditions there is an accumulation of acetic and lactic acids (Lopez, 1982a). Attempts at reducing acidity by increasing the number of "turns" during fermentation have been partially successful; however, these reductions in acidity induced by increased aeration have often been accompanied by undesirable flavors (Chong et al., 1978).

It would appear that these alterations in the aeration induce changes in the microbial flora which reduce acidity but stimulate the production of compounds responsible for off-flavors.

Little is known about the microbiology of Brazilian fermentations and this study was undertaken to examine the microbial composition and the changes induced by changing aeration regimes.

MATERIALS & METHODS

THE INVESTIGATIONS were carried out at the laboratories of the Cacao Research Centre of CEPLAC (Comissão Executiva do Plano da Lavoura Cacauerira) at Ilhéus, Bahia, Brazil.

Fermentations treatments

The method for fermenting cacao beans recomended by CEPLAC (FT) was used as a standard against which treatments (FAR and FSA) varying in the degree of aeration of the fermenting cacao beans were compared. In the prescribed method, seeds from ripe harvested fruits were extracted and put to ferment on the same day in wooden boxes. The boxes (sweat-boxes) are about four to six times longer than wide and are divided by removable wooden partitions into compartments measuring 1.0 x 1.2 and 1.0m deep. The wooden floor has 1 cm diameter holes drilled 15 cm apart for drainage of the juices from the pulp surrounding the seeds and for aeration. The seeds were left to ferment in these boxes for 5-7 days during which time they were periodically mixed by "turning" into an adjacent empty box to homogenize and aerate the mass. This was performed about 24, 48, 96 and 120 hr from the start of the process. The cacao beans to be fermented were not deliberately inoculated with specific microorganisms. The inoculum came from the hands of the operators, unwashed baskets used for transporting the seeds, and dried muscilage left on the walls of the boxes from the previous fermentation. After fermentation, the beans were dried and bagged.

In this investigation it was assumed that the aeration of the mass was related to the number of turns during fermentation and to the construction of the sweat-box. In one treatment which was designed to retard aeration (FAR), turning was carried out daily, beginning 68 hr after the start of the fermentation. In the second, super aerated treatment, (FSA), cacao was fermented in a box with a slatted floor which allowed better drainage of the juices and aeration. Besides, the ferementing mass was turned twice daily beginning 20 hr after the beans were put to ferment. The second turn in the day was given 8 hr after the first. The three fermentations were carried out simultaneously using the same variety of cacao.

Sampling

Each fermentation was sampled at 8 hr intervals, each sample being taken from an undisturbed location, approximately 30 cm from the walls and 45 cm below the surface of the mass. Samples of 20 random beans were transferred using a pair of sterile forceps, into a sterilized flask containing sterile peptone water and sand, and teken immediately to the laboratory for analysis.

Temperature variations in the fermenting mass were recorded at 8 hr intervals using a recorder (Grant Instruments, Berks, U.K.) with a sensor positioned at a point similar to a sample location.

Authors Passos and Lopez are affiliate with the Div. of Agricultural Technology & Engineering (DITEC) CEPEC, CEPLAC, Itabuna, Bahia, Brazil. Author Silva is affiliated with the Dept. of General Biology, Federal Univ. of Viçosa, Minas Gerais, Brazil.

Microbial counts and isolation

The samples were extracted by stirring the contents of each flask for 5 min using a magnetic stirrer. Several dilutions between 1 x 10^{-3} and 10^{-5} were made from the extracts and aliquots of these (0.1 mL) were inoculated into each of 8 plates containing the same medium used by Ostovar and Keeney (1973). These were incubated for 72 hr under aerobic and semi-anaerobic (Kunkee, 1967) conditions at temperatures similar to those of the fermenting mass at the time at which the sample was taken. Samples taken from the sweatbox at temperature below 40°C were incubated at 37°C and those above 40°C, at 45°C. Plates with between 30 and 300 colonies were selected and a number of clones equal to the square root count were transferred to Ostovar medium slants, and after 24 hr incubation were submitted to the catalase and Gram color tests. Gram positive and catalase negative bacilli were classified as lactic bacteria of the family *Lactobacillus* and *Streptococeaceae* (Bredd et al., 1978). Yeasts were identified by their morphological characters observed under the microscope. The number of yeasts, lactic and nonlactic acid bacteria colonies were noted.

RESULTS

THE TEMPERATURE PROFILE of each test is shown in Fig. 1. Over the first 16 hr, the three fermentations maintained a mean temperature of about 26°C after which the temperatures rose at different rates in each treatment. In FT, after the first turn there was a steep rise in temperature up to 48 hr, followed by a slower rise to a peak of 52°C which held more or less constant until the end of the fermentation process. In FAR, with a delay in turning, there was a slower rise in temperature, (about 9°C in 3 days), during the period when the beans were left unturned. Immediately after the first turn at 63 hr, the rate of temperature rise increased such that the mass reached 53°C in the following 20 hr. The over-aerated fermentation showed the highest rate of temperature rise, reaching a peak of 53°C after 48 hr. These differences in temperature rise were evidently due to the degree and time of aeration of the beans which stimulated the exothermic reactions of the aerobic microflora. They are also indicative of the rate of fermentation of each treatment; the more aerated ones fermented faster producing more heat than the less aerated fermentations. These variations in rates of fermentation were also apparent from the differences in color of the fermenting beans. The appearence of a red/brown color, typical of well fermenting cacao, was evident after 96 hr in FSA, which was the most aerated fermentation. The same stage was reached after 136 hr in FT and much later, 152-160 hr, in FAR.

Microbial counts

In general, the curves presented in Fig. 1, allow the fermentations to be regarded as having two temperature phases: a low temperature phase which occurred during a relatively anaerobic period and during which the temperature of the fermenting mass was between 26° and 37°C, and a high temperature phase during an aerobic period when temperatures were above 50°C. The dependence of temperature on aeration is well documented (Forsyth and Quesnel, 1963; Quesnel, 1968; Lopez, 1982b.) and is confirmed by these results.

During the high temperature phase, an oscillation between 50 and 53°C was observed in each treatment. These cyclic variations in temperature, which were not in phase with diurnal ambient temperature variations, may be due to activation and inactivation of the microflora by heat (Quesnel and White, 1968). This suggests that the heat developed by the microflora itself inhibited their growth until the resulting fall in temperature approached the optimum, reinitiating activity and thus repeating the cycle (Kenton and Powell, 1960). These high temperatures, common in Bahia fermentation, are infrequently encountered in fermentation in other countries (Chong et al., 1978; Lopez, 1979; Ostovar and Keeney 1973). It is probable that the microflora of Bahia fermentation has, over the decades, developed a tolerance to the higher temperatures. The microbial counts were lower in this study than those encountered by Ostovar and Keeney (1973), Rombouts (1953), and Camargo et al. (1963) but on the other hand the temperatures registered by those authors were much lower, which may account for the larger population.

Bar charts in Fig. 2, 3, and 4 show the percent distribution of yeast, lactic acid and other bacteria encountered in these treatments. In none of them was there a well defined yeast phase as described eleswhere (Ostovar and Keeney 1973; Roelofsen, 1958; Rombouts, 1953). The initial yeast phase did not absolutely dominate the fermentation but occurred concomitantly with lactic acid bacteria which subsequently outgrew the former. It was followed, in each of the three fermentations, by a phase in which bacteria other than *Lactobacillaceae* and *Streptococcaceae* were present. This differed in each treatment. Fig. 2 shows the microbial succession in FT. The yeast phase, which lasted up to 48 hr, was shared chiefly by the lactic acid bacteria whose numbers increased during 16-48 hr. Other bacteria dominated the fermentation after 72 hr but there was also a brief reappearance of lactic acid bacteria during this period.

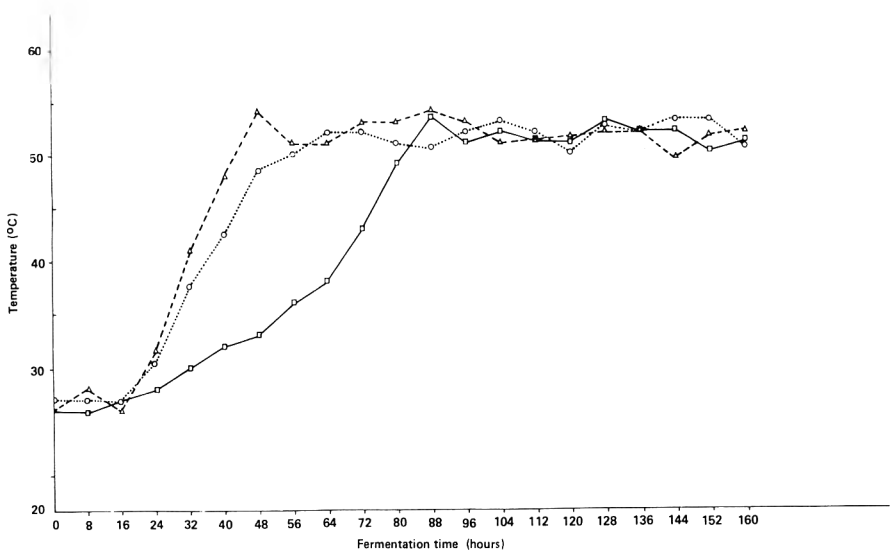

Fig. 1—Variation in temperature (°C) during a traditional fermentation (FT ○ . . . ○), a fermentation with delayed aeration (FAR □———□) and a super-aerated fermentation (FSA △ - - - - △).

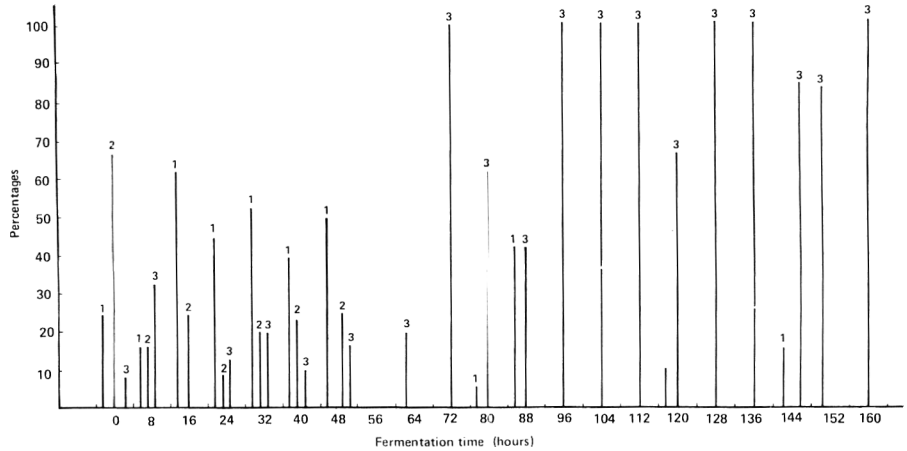

Fig. 2—Percent distribution of lactic acid bacteria (1), yeast (2), and nonlactic acid bacteria (3), isolated from cocoa fermented by the traditional method (FT).

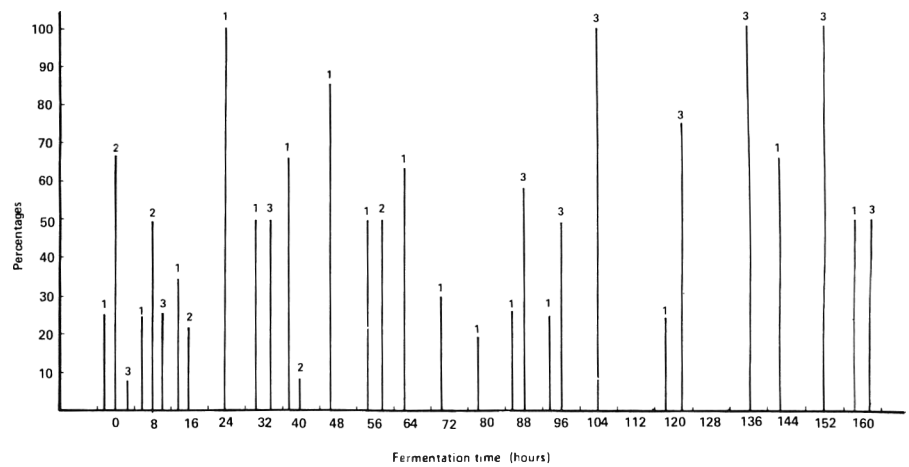

Fig. 3—Percent distribution of lactic acid bacteria (1), yeasts (2), and nonlactic acid bacteria (3), isolated from cacao fermented with delayed aeration (FAR).

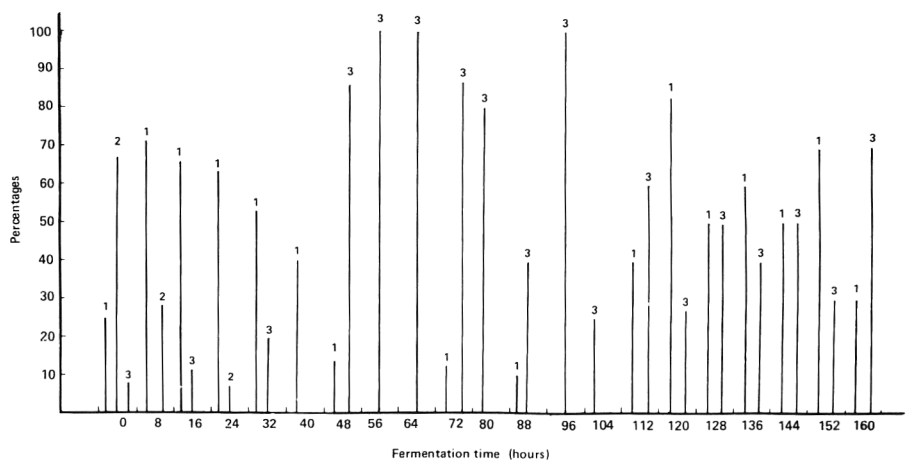

Fig. 4—Percent distribution of lactic acid bacteria (1), yeasts (2), and nonlactic acid bacteria (3), isolated from cacao fermented with excessive aeration (FSA).

The first turn did not seem to influence the microbial trend but after the second turn there was a surge in the growth of the other bacteria which dominated the fermentation until the end. A brief reappearance of lactic acid bacteria occurred between 48 and 96 hr, i.e., during the period that cacao was left unturned. The yeast phase was similar in the less aerated ferementation FAR, but an accentuated lactic acid phase between 16 and 72 hr was noted. The appearance of other bacteria was delayed until after the second turn at 96 hr (Fig. 3).

In the better aerated fermentation FSA, there was a short yeast phase in the first 24 hr and a pronounced lactic acid phase up to 48 hr. The excessive aeration appeared to inhibit the lactic acid bacteria and favor the growth of other bacteria which appeared between 48 and 84 hr; earlier than in the other tests. After this, a mixed population of lactic and other bacteria (thermophyllic bacilli) was present during the rest of the fermentation. Probably the excessive aeration at this point encouraged the growth of lactic acid bacteria and other organisms tolerant of aerobic conditions and high temperatures (Weissberger et al., 1971).

DISCUSSION

THE DISTRIBUTION of microrganisms presented in Fig. 2, 3 and 4 is different from that of a Trinidad fermentation (Ostovar and Keeney, 1973). Yeast did not dominate the

early phase in any of them, but on the other hand, there was a very marked growth of lactic bacteria during the anaerobic and early aerobic phases.

Emphasis has been placed on the lactic acid bacteria because of their versatility in the production of both acetic and lactic acid and the importance of these in context of the acidity problem related to Brazilian cacao.

The lactic acid bacteria were recorded in greater numbers than previously reported (Chatt, 1953; Rombouts, 1952) and their dominance in the fermentation suggest that they may play an important role in influencing the flavor of the product (Fig. 2, 3, 4). They were superceded by other bacteria later, when the aeration increased and the temperatures were high.

If the heat produced during fermentation is due mainly to the oxidation of alcohol to acetic acid and further oxidation of the latter to carbon dioxide and water, then it is most likely that the unidentified bacteria were mainly acetic acid bacteria (Chatt, 1953; Knapp, 1937; Rombout, 1952) of the genus *Gluconobacter* and *Acetobacter.* It is surprising that these bacteria [whose growth limit lies between 45 and 48°C (Bredd et al., 1979)] can multiply at temperatures about 52°C. The same applies to the lactic acid bacteria which, although reported to be more thermotolerant, were not expected to thrive at the high temperatures of the sweat-box. Various reasons have been given for this (Roelofsen, 1958; Rombouts, 1952) but it is also possible that successive generations subjected to these unfavorable conditions over decades have developed a protective adaption to the high temperatures (Ingram, 1966; Weissberger et al., 1971). Although the production of heat during fermentation has been attributed solely to the acetic acid bacteria, there is evidence that other microorganisms such as thermogenic fungi (*Mucor pusillus* and *Aspergillus fumigatus* sp) encountered in cacao (Cooney and Emerson, 1964) may also be contributing. Bridgeland and Friend (1958) reported temperatures of 55-65°C in aerated fermentations in New Guinea and associated these with the presence of rod form bacteria. Fungi, also, have often been reported in the overaerated regions of the fermenting mass and it is quite likely that some thermotolerant strains may be responsible for the high temperature and off-flavors associated with these types of fermentations.

A strong odor of acetic acid, which was evident in all three fermentations, appeared to decrease progressively towards the end. However, the only case in which this reduction was marked was in FSA where, after 128 hr, an odor resembling that of malt was detected. At this time the beans had taken on a dark brown external color. From the analyses of the microflora of FSA, it was observed that at this stage in the fermentation lactic acid bacteria were frequently present in the form of cocci. It is interesting to note that *Streptococcus lactis* var. maltigenes in dairy products has been reported to produce malt odors (Jackson and Morgan, 1954).

Up to the present it had been assumed that the lactic acid bacteria do not play a prominent role in the fermentation of cacao beans. However, the data presented here point to the contrary as far as Brazilian fermentations are concerned and also account for the presence of excessive amounts of lactic acid in Brazilian beans. In general the species *Lactobacillaceae* were more frequently found during the early phase of fermentation and the *Streptococcaceae* during the more aerobic period. This is probably because the former grow better at the low pH of the bean pulp while the latter prefer the less acid conditions found in well aerated locations.

Greater emphasis has been placed on attempting to control acidity by manipulation of the acetic acid bacteria through modifications in fermentation methods (Chong et al., 1978; Seiki, 1977).

The role of the lactic acid bacteria in relation to the acidity of the beans is more important than hitherto considered, because, not only are they able to metabolize all the sugars of the cacao bean pulp, but the heterofermentative species can also metabolize citric acid. Besides producing lactic acid, they can also produce acetic acid. It is quite likely that the acetic and lactic acids found early in fermentation (Lopez, 1982c) is due to this.

Although the relatively lower yeast activity and the higher lactic acid bacteria activity would deprive the acetic acid bacteria of ethyl alcohol, which they normally metabolize, the fermentation will still be acid because of the acetic acid and lactic acid produced by the lactic acid bacteria. It is possible that a proportion of the lactic acid produced during fermentation is used as substrate by the microflora. It has been shown, however, that the permeability of the bean testa was drastically reduced after the death of the seed which generally occurred between 48 and 72 hr of fermentation (Lopez et al., 1982). This implies that acid produced before this period could easily enter the bean and would be trapped inside after the death of the bean. In these fermentations with a pronounced lactic acid activity at the very start of fermentation the chances of acid entrapment are greater than in cases with marked yeast phases.

The reason for the absence of a pronounced yeast phase in these fermentations is not immediately evident. It seems possible that the conditions of fermentation that favor good lactic acid growth during the anaerobic phase in fermentation are less suitable for yeast multiplication or that the starting yeast inoculum which is contributed by the mucilage left on the walls of the sweat-box from a previous fermentation may be inactivated by the persistant high temperatures of the fermentation being unable therefore to guarantee a good start.

CONCLUSIONS

ALTHOUGH LACTIC ACID is present in Brazilian cacao (Lopez, 1982a; 1983; Weissberger et al., 1971) its occurrence in normal processes is not so prominent as to over shadow acetic acid, which remains the chief agent responsible for acidity (Lopez, 1983). However, the results presented here suggest that whatever modifications in fermentation methods are planned to influence the acetic acid producing, microbial population, should also take into account the effect of such measures on the lactic acid bacteria, as it is quite possible that conditions which suppress acetic acid bacteria might stimulate other acid-producing microorganisms. This in fact was the case with FSA where an excess of aeration reduced acetic acid (judging by the odor of the beans) but provoked a second lactic acid bacteria phase.

Traditional Brazilian fermentations are reported to have higher than desired acid levels (Lopez, 1982; 1983). The results presented here are not sufficient to define the conditions required for reducing this acidity. If lactic acid is the undesirable element in Brazilian fermentation, one of the means of reducing it may be to stimulate the growth of yeast at the beginning of fermentation by an early aeration. The same measure would reduce lactic acid bacteria growth as indicated by the results presented. A certain amount of acid is essential for the curing process but just how much can be determined only by experimentation. Acetic acid has been shown to contribute to the flavor of chocolate (Griffiths, 1960; Quesnel, 1958) but lactic acid is believed to depress flavor. The prerequisite to resolving the flavor problem of Brazilian cacao, however, is the identification and characterization of the aerobic microflora of these fermentations. This phase of the research is in fact being undertaken at present and the results will form a separate report.

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Comparison Between Sensory Quality of Freshly Prepared Spaghetti with Meat Sauce Before and After Hot Holding on a Cafeteria Counter

H. M. AL-OBAIDY, M. A. KHAN, and B. P. KLEIN

ABSTRACT

Sensory quality of freshly prepared spaghetti with meat sauce entree was compared to that held hot on a cafeteria counter. A study of the time and temperature relationship indicated a steady decline in temperatures during the first 30 min of steam-table holding, although within safe limits. An experienced taste panel consisting of 8-10 members rated the samples for various sensory attributes. The freshly cooked product was rated significantly higher compared to the hot-held product for moistness, appearance of spaghetti, intensity of spice flavor in the sauce, spaghetti texture, meat texture, and general acceptability.

INTRODUCTION

CURRENT CHANGES in foodservice systems have been aimed primarily at either increasing productivity or decreasing food costs. Sensory quality and safety of foods often have not received adequate emphasis during these developments (Unklesbay et al., 1977). Within each foodservice operation, identification of time-temperature relationships or critical control points involved in the handling of food products is necessary for adequate managerial controls of food quality and safety.

Observations of time-temperature relationships for equipment and food prepared in airline catering operations were reported by Bryan et al. (1978). Many of the prepared foods were exposed to temperatures within the 45-140°F (7.2-60°C) range for several hours. Some of the equipment used could not maintain food temperatures above 140°F (60°C) or below 45°F (7.2°C) because of capacity, insufficient refrigerating medium or poor conditions.

Studies have been conducted to determine the effects of time and temperature on sensory and microbiological quality of food in "ready-prepared" systems (Cremer and Chipley, 1979, 1980; Jakobsson and Bengtsson, 1972; Kossovitsas et al., 1973). A commissary foodservice system transporting heated food to service locations was examined by Cremer and Chipley (1979) to determine time and temperature conditions and microbiological and sensory quality of meat loaf. The number of microorganisms was below those levels considered to be dangerous to the health of consumers; nevertheless, these results, coupled with findings of variable time and temperature, point to the potential for public health hazards if food is mishandled in such systems.

Bobeng and David (1978) assessed sensory quality of beef loaf related to preparation in conventional, cook-chill, and cook-freeze foodservice systems. Scores for general acceptability and flavor were significantly higher for meat in the conventional system, and scores for the same characteristics were higher in the cook-chill than in the cook-freeze system. Zallen et al. (1975) found significantly higher scores for sensory quality of freshly prepared beef loaf than for loaves stored at 0.0 or 5.5°C for various time intervals up to 9 days and heated in an electric deck oven. Sensory quality of spaghetti was determined for a freshly prepared sample and after heating in institutional microwave and convection ovens following holding 1 hr chilled; 24 hr chilled; 24 hr frozen; 24 hr frozen, 24 hr chilled (Cremer, 1983). Differences attributable to holding treatment occurred in moistness; in clumping, firmness and chewiness of pasta; and in overall ratings. Samples held 1 or 24 hr chilled had better scores than those held 24 hr frozen or 24 hr frozen, 24 hr chilled. Spaghetti heated in the microwave compared to the convection oven had greater fat separation, clumping, greasiness, firmness, and chewiness.

Determination of optimal holding conditions and equipment for use in foodservice systems is important in the public interest for efficiency and high microbial and sensory quality in food. The purpose of this study was to compare the sensory quality of freshly prepared spaghetti with meat sauce and after hot holding on a cafeteria counter.

Authors Al-Obaidy, Khan, and Klein are with the Foods & Nutrition Dept., Univ. of Illinois, 274 Bevier Hall, 905 South Goodwin, Urbana, IL 61801.

MATERIALS & METHODS

Procurement and preparation

The product was prepared according to a formula which was being used in The North Central Regional (NC-120) Committee's research (1981). For pasta, 600g spaghett, 10g salt, and 9 kg water; for sauce, 1200g ground beef (not less than 80% lean), 1 No. 10 can of commercially prepared tomato sauce, 4g garlic powder, 15g dehydrated chopped onion, 5g leaf oregano, 5g granulated sugar, 3g whole basil, and 0.5g black pepper were used.

Ground beef and tomato sauce were purchased from a local supermarket, using the same label and trade name each time. Spaghetti and all other ingredients were obtained from a commercial food purveyor. Spaghetti was cooked in a Market Forge 10 qt steam-jacketed kettle. Water with salt was brought to a boil. Spaghetti was broken into 12-14 cm lengths, dropped into boiling salt water, and cooked (for 14 min) until the white "thread" of starch in spaghetti strands disappeared. Spaghetti was drained for 2 min in a collander, and held until meat sauce was prepared. Ground beef was cooked until brown (for 4 min) in the same steam-jacketed kettle stirring frequently. All of the remaining sauce ingredients were added to the meat and the mixture was cooked until boiling (about 4 min). The spaghetti was poured over the cooked meat sauce, thoroughly combined, and the mixture cooked until the internal temperature reached 74°C as measured by a thermometer Model No. 2261 (Weston).

For each replication, 40 servings were prepared. All data collected, including time and temperature recordings, and sensory evaluations were replicated four times. For time and temperature recording throughout the study, beginning and end-points for each step of product flow were precisely defined.

Counter-holding

Immediately after preparation, the product was placed in a pan (25x56x6.4 cm) on a preheated counter-table Model No. APQ-537 (Alex Janous & Co.) for 90 min at approximately 66°C. Ninety minutes of holding as the basis for study was selected after observing serving times in various university cafeterias. Normally, foods are served for 1 hr, and 30 min are needed for setup and removal of the food from the hot counter. Although food may not be on the counter for 90 min, the impact of the maximum possible exposure was selected for study.

Thermocouples (copper-constantan) were placed at nine different locations (diagonally to form an "X" shape) in the serving

pan to monitor changes in temperature during holding. Temperatures were recorded at 10 min intervals by multipoint digital thermometers Model No. 2166A (Omega) and Model No. 4002 (YSI). The mean of the temperatures at the nine locations were calculated.

Sensory evaluation

Sensory evaluations for freshly prepared spaghetti and meat sauce and after 90 min holding were conducted by an 8 to 10 member experienced and trained taste panel consisting of advanced-level graduate students in a food related area. The same panelists were involved in all replications in this study. The panelists were familiarized with the product and scoring instrument during three preliminary training sessions. Each serving (approximately 150g) was placed in a small bowl in order to insure uniform appearance and to obtain a representative sample. Temperature of samples was between 60-65°C. All evaluations were made in partitioned booths under incandescent lights, with use of water for rinsing after each test.

A score sheet which was prepared by the North Central Regional (NC-120) Committee (1981) using an unstructured category scale (10 cm long) was used with modifications for sensory evaluation and was interpreted by measuring the response of the panelists. Descriptive terms for each characteristic were supplied to aid in judging. Characteristics evaluated were appearance (color of tomato sauce, moistness, fat separation and spaghetti), flavor (intensity of spice flavors in the sauce, blended flavor in the sauce, intensity of spice flavors in the meat, intensity of beefy flavor in the meat, and intensity of off-flavor), mouthfeel (dryness and greasiness), spaghetti texture (firmness and chewiness), meat texture (chewiness), and general acceptability (sauce, spaghetti, and spaghetti with meat sauce). Sensory evaluation scores were statistically analyzed according to the paired t test (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

Time and temperature

Time and internal temperatures of spaghetti and meat sauce in various phases of product flow were recorded. Soon after preparation, the mean internal temperature of the finished product was 74°C which is within the safe limit. To comply with foodservice code requirements (Illinois Dept. of Public Health, 1983), cold foods should be at 7°C (45°F) or below, and hot foods should be at 60°C (140°F) or above. Internal temperatures of the product (mean temperature) during 90 min steam-table holding are shown in Fig. 1. A steady decline in temperature was noted for the first 30 min of holding, from 72°C to 65°C. On an average, a 10% decrease was noted in the internal temperatures upon 90 min steam-table holding.

Sensory evaluation

Mean palatability scores for sensory attributes evaluated by the taste panels are presented in Table 1. The mean scores for moistness for freshly cooked products were significantly higher ($p < 0.05$) compared to those after 90 min of steam-table holding. The moisture loss was further confirmed by the results of moisture determinations which showed some difference (from 72.2 to 70.9%) between the freshly cooked products and after 90 min holding.

The scores for appearance of spaghetti were found to be significantly lower ($p < 0.05$) for hot-held than freshly

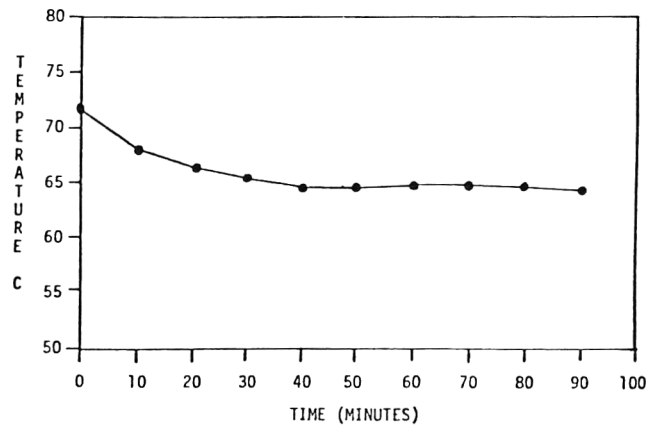

Fig. 1—Time-temperature relationships during hot-holding.

Table 1—Mean scores for sensory characteristics for freshly cooked and hot-held spaghetti with meat sauce[a]

Characteristics		Lower range — Higher range	Freshly cooked	After 90 min of steam-table holding
Appearance	1. Color of tomato sauce	red-orange — brownigh-red	3.99 ± 2.58	3.26 ± 2.28
	2. Moistness	dry — moist, juicy	8.45 ± 1.54*	4.15 ± 2.86
	3. Fat separation	little — much	2.83 ± 2.02	2.51 ± 1.80
	4. Spaghetti	clumped together — individual strands	7.76 ± 2.87*	4.31 ± 3.54
Flavor	1. Intensity of spice flavor in the sauce	weak — strong	6.00 ± 2.10*	4.33 ± 2.41
	2. Blended flavor in the sauce	unblended — well-blended	7.53 ± 1.90	6.83 ± 2.45
	3. Intensity of spice flavors in the meat	weak — strong	4.09 ± 2.26	3.64 ± 2.58
	4. Intensity of beefy flavor in the meat	weak — strong	6.04 ± 2.47	5.78 ± 2.66
	5. Intensity of off-flavor	weak — strong	0.92 ± 1.62*	2.12 ± 2.48
Mouthfeel	1. Dryness	very wet — very dry	3.17 ± 1.72*	5.59 ± 2.97
	2. Greasiness	not greasy — very greasy	3.52 ± 2.48*	4.59 ± 2.42
Spaghetti texture	1. Firmness	extremely soft — extremely firm	4.75 ± 2.12*	1.62 ± 1.74
	2. Chewiness	extremely tender — extremely chewy	4.94 ± 2.18*	2.04 ± 2.34
Meat texture	1. Chewiness	little resistance to breakdown on mastication — much resistance to breakdown on mastication	5.02 ± 1.90*	3.58 ± 1.93
General acceptability	1. Sauce	low quality — high quality	6.33 ± 2.01*	4.62 ± 2.71
	2. Spaghetti	low quality — high quality	6.36 ± 2.58*	2.61 ± 2.44
	3. Spaghetti and meat sauce	dislike extremely — like extremely	6.44 ± 2.10*	4.17 ± 2.22

[a] Mean ± standard deviation of scores of 8 to 10 taste panelists (4 replication). Maximum possible score was 10.
* Means in the same row are significantly different at the 5% level.

cooked products. No significant differences were noted for color of tomato sauce and fat separation between hot-held and freshly cooked products.

The results also indicated that the scores for intensity of spice flavor in the sauce for the freshly cooked product were significantly higher ($p < 0.05$) than the scores for the hot-held product. This difference may be attributed to the loss of moisture upon holding, resulting in relatively more acidity, which may mask the spice flavor. The changes in acidity were confirmed by the differences in pH which decreased from 4.8 to 4.5 after holding. Also, the evaporation of aromatic compounds from the products upon holding may partially contribute to the significant decrease in the intensity of spice flavor in the sauce. Significant differences in the scores of greasiness of product, spaghetti texture, and meat texture between freshly cooked and held products were also noted.

The general acceptability scores for sauce, spaghetti, and spaghetti with meat sauce were found significantly higher ($p < 0.05$) for the freshly cooked product than for the hot-held product. Therefore, the freshly cooked spaghetti with meat sauce was scored better for moistness, spaghetti appearance, and intensity of spice flavor in the sauce. It had less dry and greasy mouthfeel and the pasta was firmer and chewier than those held for 90 min on a steam table. This indicates that a freshly cooked product should be used whenever possible and that it must not be held for a long time on a steam table. This result is consistent with findings of other researchers (Bobeng and David, 1978; Zallen et al., 1975; Cremer, 1983) that serving feshly prepared products may be the best option in a foodservice systems operation provided the food can be served quickly. In conclusion, it is advisable to use freshly cooked products rather than hot-held products and to minimize the hot-holding time whenever possible.

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Water Vapor Permeability of Edible Bilayer Films

S. L. KAMPER and O. FENNEMA

ABSTRACT

Edible films composed of a water soluble, carbohydrate layer (hydroxypropyl methylcellulose) and various kinds of lipid layers were tested for resistance to water vapor permeability. Films were tested at 25°C and a relative humidity differential of 85%. Films containing solid lipids, such as beeswax, paraffin, hydrogenated palm oil or stearic acid yielded permeabilities of 0.2 g · mil · day^{-1} · $mmHg^{-1}$ or less which is a smaller value than that for low density polyethylene.

INTRODUCTION

THE WATER PROPERTIES of processed foods must be controlled to ensure that they remain safe, stable and appetizing. The dominant mechanism for moisture transfer is water vapor diffusion caused by a vapor pressure (a_w) gradient (or concentration gradient) existing between the food product and its environment and/or between internal components of a food product (Matz, 1965; Oswin, 1976; Salwin and Slawson, 1959; Stewart, 1975; Szulmayer, 1973). Transfer of moisture between the food product and its environment can be controlled by using a moisture impermeable packaging material (Brody, 1970; Cairns et al., 1974; Charlton and Delong, 1956; Heiss, 1958; Karel, 1967, 1973; Karel et al., 1959; Matz, 1965; Oswin, 1976; Quast and Teixeira Neto, 1976), but control of moisture exchange among components of a food is more difficult.

Control of moisture migration within heterogeneous foods can be achieved: (1) by eliminating or reducing the vapor pressure (a_w) gradient, i.e., by bringing the various components to the same water activity, or by (2) separating those components with different water activities by an edible, moisture impermeable barrier.

In general, the rate of moisture transfer within a food will increase as the a_w gradient is increased, the particle size is decreased (closer contact between components), and the temperature is increased (King, 1968; Matz, 1965; Stewart, 1975). Control of these factors will reduce the rate of moisture transfer; however, moisture migration will continue as long as a vapor pressure gradient (or concentration gradient) exists in the food.

Use of edible food coatings to retard moisture transfer is certainly not new. Enrobing foods with fat, a form of "larding," was practiced in England in the 16th century (Labuza and Contreras-Medellin, 1981) and food coating processes using wax and gelatin were patented in the 1800's (Allen et al., 1963b).

Information on edible food coatings, although not scarce, is of limited value because it is found mostly in the patent literature and in other publications where experimental detail and quantitative results are often lacking. Films prepared from acetostearin, acetylated glycerols, algin, amylose fatty esters, carrageenan, casein, corn syrup, dextrin, ethyl cellulose, gelatin, lard, lecithin, oils (natural or hydrogenated vegetable or animal oils), pectin, soy protein, starch, tallow, waxes, and zein have been investigated as barriers to water movement in foods (Alfin-Slater et al., 1958; Allen et al., 1963a,b; Bauer et al., 1968; Cole, 1969; Cosler, 1957; D'Atri et al., 1980; Earle, 1968; Earle and McKee, 1976; Earle and Snyder, 1966; Feuge, 1955; Fox, 1958; Hamdy and White, 1969; Landmann et al., 1960; Lazarus et al., 1976; Lovegren and Feuge, 1954; McKee, 1978; Newman, 1962; Shaw et al., 1980; Shea, 1970; Silva et al., 1981; Stemmler and Stemmler, 1974; Ukai et al., 1976; Watters and Brekke, 1959, 1961; Werbin et al., 1970; Williams et al., 1978).

Several studies have also been conducted on the permeability characteristics of cell membranes and their analogues and these studies have relevance to the problem of moisture transfer in foods (Bull, 1964; De Gier et al., 1968; Demel et al., 1968; Gould and Measures, 1977; Jain, 1972; McElhaney et al., 1970; Sweet and Zull, 1969; Tien, 1974; Van Deenan, 1969).

About all that can be concluded from all of the aforementioned studies is that water permeability through films varies greatly with film composition and with orientation of molecules in the film and that lipids seem to be the most effective barrier to the movement of water.

The availability of an edible coating which could maintain initial water activity gradients among major food components would be commercially useful in several situations, for example, in products such as frozen pizzas and pies where it is highly desirable to maintain a dry crust and a moist filling.

The objective of this study is to evaluate the water vapor permeability characteristics of laminated edible films, one component being a lipid (moisture barrier) and the other being a polysaccharide (structural matrix) and to investigate the effect of lipid type on moisture transmission through these films.

MATERIALS & METHODS

Materials

The following materials were used to prepare edible films of various compositions: beeswax, white, refined (composition unknown); corn oil, Mazola, (Best Foods, CPC International Inc., Englewood Cliffs, NJ); hydrogenated vegetable oil, consisting of soybean and palm oils with mono- and diacyglycerols, Crisco (Procter and Gamble, Cincinnati, OH); hydrogenated vegetable oil, palm, Durkee 27 (Durkee SCM Corp., Cleveland, OH); hydrogenated vegetable oil, cottonseed and soybean, Durkex 500 (Durkee SCM Corp., Cleveland, OH); hydroxypropyl methylcellulose, Methocel E 50 Premium (Dow Chemical Co., Midland, MI); lauric acid, 97%+ purity (Eastman Organic Chemicals, Rochester, NY); oleic acid, USP (Allied Chemical, Morristown, NJ); paraffin wax, Paraseal (W & F Mfg. Co., Rochester, NY); polyethylene glycol 400 (J.T. Baker Chemical Co., Phillipsburg, NJ); polyoxyethylene (20) sorbitan monooleate, Tween 80 (J.T. Baker Chemical Co., Phillipsburg, NJ); stearic acid, 97%+ purity (Eastman Kodak Co., Rochester, NY); stearic acid, blend 50.4:47.5 stearic acid-palmitic acid, NF (Mallinckrodt, Inc., Paris, KY).

Author Tennema is affiliated with the Dept. of Food Science, Univ. of Wisconsin-Madison, Madison, WI 53706. Author Kamper, formerly with the Univ. of Wisconsin, is now affiliated with General Mills, Inc., 9000 Plymouth Ave., North, Minneapolis, MN 55427.

Preparation of the polysaccharide, film-forming component (Kamper, 1983).

The film forming solution was prepared using 9g hydroxypropyl methylcellulose (HPMC), 1g polyethylene glycol 400 (PEG) dissolved in 100 mL distilled water and 200 mL 95% ethanol. Distilled water (approximately 90°C) was added to wet the HPMC. Once the HPMC was completely dissolved, the 95% ethanol was added. The solution was thoroughly mixed before addition of PEG. Air bubbles in the solution were removed by reducing the pressure over the solution.

The composition of the film forming solution was derived after numerous attempts with many compounds. With the equipment available, prime consideration was given to films which could be easily removed from glass plates on which they were formed, and to durability (for example, gum arabic films could not be removed without tearing the film and starch films were excessively brittle).

The concentration of HPMC used (3 parts HPMC to 100 parts solvent) produced a desirable viscosity for the plating technique employed. Reduction of the water to alcohol ratio below 1:2 led to grainy films, an indication of undissolved HPMC. The PEG was initially added to the film forming solution to aid in removal of the dried films from the glass surface but was later found to produce improved films when the emulsion technique was used. Levels of PEG greater than 9:1 HPMC:PEG led to splitting of the films during drying.

Preparation of the bilayer film (Kamper, 1983)

Two techniques were used to manufacture the edible bilayer films' (1) casting a lipid layer onto a dried water-soluble edible film (coating technique), and (2) adding lipid to the film-forming solution (emulsion technique), distributing the solution in a thin layer and drying.

For the coating technique, the film forming solution (100 mL) was added to the spreader used to prepare thin-layer chromatography (TLC) plates and the solution was plated onto three 8" X 8" TLC glass plates at a thickness of 0.75 mm. The plates were dried in an oven at approximately 90°C, cooled to room temperature and the films removed. The dried water-soluble (HPMC:PEG) film was used as a support for the lipid layer not as a water vapor barrier. The film was cut into disks of the desired size and weighed to the nearest 0.0001g. The appropriate lipid material was painted onto the surface of the film, and the film reweighed. The amount of lipid deposited was determined and expressed as mg lipid/cm^2 film. Total film thickness was measured with a micrometer.

For the emulsion technique, the lipid was added directly to the film forming solution and the solution was warmed, if necessary, to melt lipids with high melting points. The TLC spreader was heated to prevent solidification of film components during application. The mixed solution (100 mL) was added to the TLC spreader and plated onto three 8" X 8" glass TLC plates at a thickness of 0.75 mm. The coated plates were then dried in an oven at approximately 90°C (drying time approximately 15 min). After drying, the plates were cooled and the films were peeled from the plates. Film samples were cut into uniform disks of the desired size and weighed to the nearest 0.0001g. Portions of the film were analyzed for lipid content. Film thickness was measured with a micrometer. The amount of triacylglycerol (expressed as fatty acid) present in the dried emulsion film was determined by hydrolyzing the lipid and then using an AOAC procedure for acid value of fats (AOAC, 1975). This enabled calculation of mg lipid/cm^2 film.

Water vapor transmission tests

These tests were conducted using the ASTM (1983) procedure. A glass cup containing desiccant, sealed with the test film, was exposed to a controlled humidity. The water vapor transferred through the film and absorbed by the desiccant was determined from the weight gain of the cup.

The edible films used were of known lipid content (mg/cm^2), thickness (mils), area (cm^2) and weight (mg). The glass cups used were 9.90 cm (o.d.) by 9.05 cm (i.d.) by 1.50 cm (deep) with an exposed film area of 64.33 cm^2. The films were sealed to the cups with a blend of molten microcrystalline wax and paraffin wax.

The cups were stored in laboratory desiccators each maintained at a constant relative humidity with a saturated salt solution (Rockland, 1960; Stoloff, 1978; Wink and Sears, 1950). The relative humidity inside the cup was always lower than that existing outside so water vapor transmission was determined from weight gain of the cup. Cup weight, to the nearest 0.0001g, was recorded at various times. All tests were conducted in duplicate.

The water vapor transmission rate (WVTR) and permeability (P) of the film was determined as follows (Heiss, 1958; Karel et al., 1959; Labuza and Contreras-Medellin, 1981):

$$WVTR = \frac{\Delta W}{A\ \Delta t} = \frac{(g)}{(m^2)\ (day)}$$

where: ΔW is the weight of water absorbed in the cup, Δt is the time for weight change, A is the area of film,

$$P = \frac{\Delta W\ \Delta X}{A\ \Delta t\ (P_2 - P_1)} = \frac{(g)\ (mil)}{(m^2)\ (day)\ (mm\ Hg)}$$

ΔX is the film thickness (1 mil = 0.001 inch) and $P_2 - P_1$ is the vapor pressure differential across the film.

RESULTS & DISCUSSION

Films prepared by the coating technique

In this study, a vapor pressure gradient (20 mm Hg at 25°C) was established across the film using anhydrous calcium chloride (0% RH) inside the test cup and a saturated solution of potassium chloride (85% RH) to control the relative humidity in the desiccator. The lipid side of the film was exposed to the high humidity condition.

The water vapor transmission rate (WVTR) of the water-soluble film was 480 g/m^2 day (Fig. 1). Lipid layers applied to the surface of the HPMC:PEG film showed a trend of decreased permeability with decreased fluidity of the lipid layer. Fluid lipid layers consisting of: (1) polyunsaturated corn oil with 5% polyoxyethylene (20) sorbitan monooleate, and (2) hydrogenated cottonseed-soybean oils, had WVTR values of 340 g/m^2 day and 290 g/m^2 day, respec-

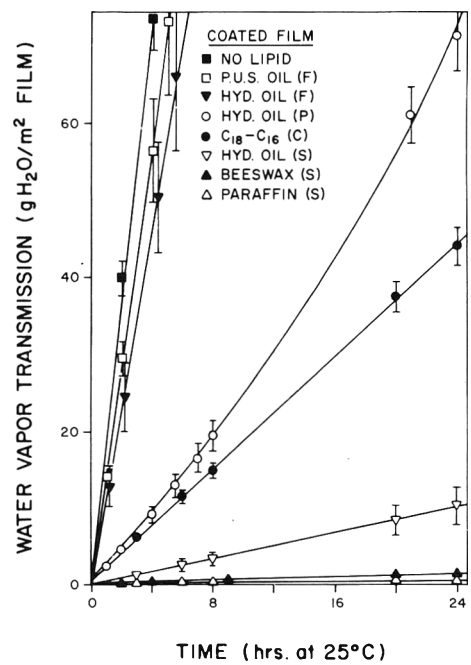

Fig. 1—Water vapor transmission through edible bilayer films prepared by the coating technique (25°C, 85% RH). Lipid coatings (9 mg/cm^2 film, film thickness 3.9 - 5.9 mils): ■ HPMC:PEG water-soluble film without lipid layer; □ polyunsaturated corn oil with 5% polyoxyethylene (20) sorbitan monooleate, fluid (F); ▼ hydrogenated cottonseed and soybean oils, fluid (F); ○ hydrogenated soybean and palm oils with mono- and diacyglycerols, plastic (P); ● stearic acid-palmitic acid blend, crystalline (C); ▽ hydrogenated palm oil, solid (S); ▲ beeswax, solid (S); and paraffin wax, solid (S). Variability between the two replicates is indicated only when the variability exceeded the size of the symbol.

tively. In comparison, the solid lipid layers, beeswax and paraffin wax, had WVTR values of 1.4 g/m^2 day and 0.73 g/m^2 day, respectively. No significance should be attached to small differences among the slopes of the curves since the coating thickness varied somewhat among the films (see values in legend of Fig. 1).

The large difference in permeability between liquid and solid lipids apparently can be explained in terms of the solubility of water vapor in the lipid film and/or the molecular organization of the lipid film (Charlton and Delong, 1956).

Polyunsaturated corn oil would not form a continuous layer on the surface of the HPMC:PEG film without the addition of an emulsifier to reduce the repellency of the two materials. Exposure of both liquid lipid films (19:1 polyunsaturated corn oil:polyoxyethylene (20) sorbitan monooleate and hydrogenated cottonseed-soybean oils) to the high humidity (85% RH) eventually caused the oil layers to pull away from the underlying film, thus exposing the HPMC:PEG film to the high humidity. The presence of emulsifiers (increased solubility of water vapor inthe film) and/or the molecular mobility of the liquid films apparently resulted in their large permeability to water vapor.

Hydrogenated soybean and palm oils with mono- and diacylglycerols retained a continuous plastic lipid layer throughout the test period. The reduction of fluidity in this film (increased solids at 25°C) led to improved barrier properties; however, the WVTR increased with time (55 g/m^2 day from 0 - 4 hr to 80 g/m^2 day from 20 - 24 hr) suggesting a reorientation of lipid molecules during storage, perhaps allowing the hydrophilic groups of the emulsifiers to more readily absorb and transmit water vapor. In comparison, the WVTR of the film coated with hydrogenated palm oil (solid, without emulsifiers) remained constant and provided a more impermeable barrier to moisture transfer (WVTR 10.3 g/m^2 day) than the film coated with hydrogenated soybean and palm oil (plastic, with emulsifiers).

The film coated with stearic acid-palmitic acid (crystalline) was a relatively good barrier to water vapor (43.9 g/m^2 day) but was extremely brittle. Moisture transfer through this film could occur by hydration of polar carboxyl groups and/or from the presence of cracks or flaws in the crystalline layer.

The films coated with solid beeswax or pariffin wax were very effective barriers to the transfer of water vapor. Unfortunately these films were very brittle. Thus, avoidance of cracks during application of these lipids to foods and during subsequent handling of the foods would be difficult if not impossible. It is likely that use of these coated films on food products would require a compromise between durability and water permeability, for example the plastic coating (hydrogenated soybean and palm oils without emulsifiers) or the solid coatings with a moderately low degree of hydrogenation might be useful in some situations.

The results in Fig. 1 are in general agreement with the studies of Landmann et al. (1960) and Watters and Erekke (1961) who reported increased water vapor permeability of lipid coatings with increasing proportions of liquid oil in the coating. Although many of the lipids used in this study were mentioned in the patent literature cited earlier, the film forming technique used here was quite different. The lipid films used in this study were formed prior to food application, therefore lipid concentration, molecular organization, film thickness and film continuity were controlled to a greater extent than in previous studies.

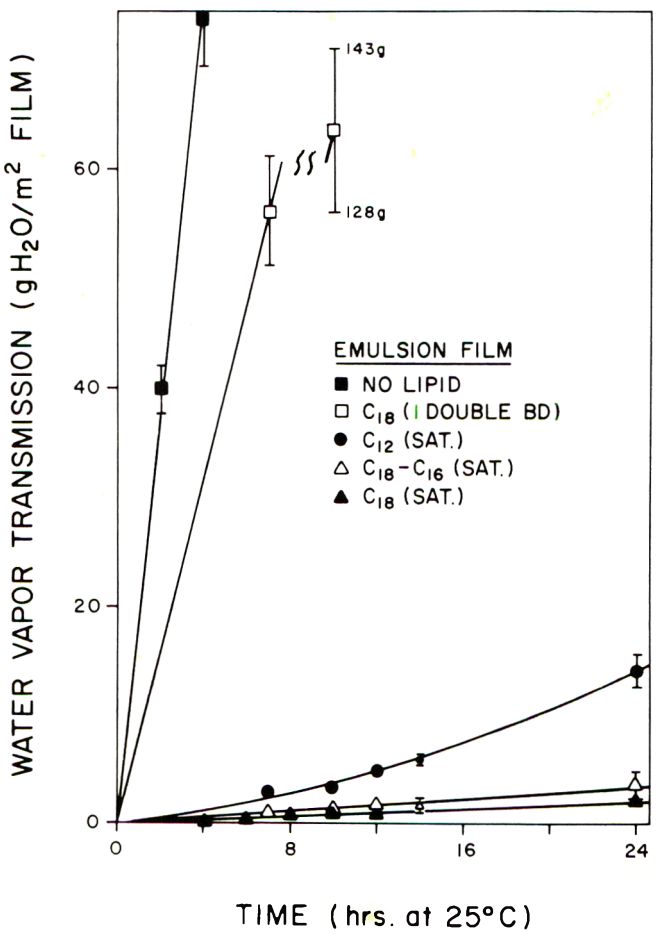

Fig. 2—Water vapor transmission through edible bilayer films prepared by the emulsion technique (25°C, 85% RH). Lipid films (0.8 mg lipid/cm^2 film, film thickness 1.4 to 1.8 mils): ■ HPMC:PEG water soluble film without lipid; □ water-soluble film with oleic acid ($C_{18:1}$); ● water soluble film with lauric acid ($C_{12:0}$); △ water soluble film with stearic acid-palmitic acid blend ($C_{18:0}$–$C_{16:0}$); ▲ stearic acid ($C_{18:0}$); Variability between the two replicates is indicated only when the variability exceeded the size of the symbol.

Films prepared by the emulsion technique

A vapor pressure gradient of 20 mm Hg at 25°C was established across the emulsion films using the technique and conditions described earlier. The lipid concentration in these films (0.8 mg/cm^2) was 10-fold less than that used for the layered films.

Water vapor transmission through the emulsion films was highly dependent on chain length and degree of saturation of the fatty acids (Fig. 2). Introduction of the one double bond to the fatty acid hydrocarbon chain increased the WVTR from 2.2 g/m^2 day (stearic acid, $C_{18:0}$, solid at 25°C) to over 190 g/m^2 day (oleic acid, $C_{18:1}$, liquid at 25°C). The permeability of the oleic acid emulsion film also increased with time; for example, WVTR increased from 190 g/m^2 day (slope of curve from 0 - 7 hr) to 260 g/m^2 day (slope of curve from 0 - 10 hr). The double bond (cis configuration), in the oleic acid hydrocarbon chain, presumably changes the packing of the lipid molecules at the air-water (film forming solution) interface from a very dense layer (stearic acid) to a more expanded layer with greater molecular mobility. The surface area occupied by one molecule of oleic acid in an oleci acid monolayer (surface pressure 5 dyne/cm) is 48.0 A^2, whereas the molecular area of a stearic acid molecule under comparable circumstances is 23.5 A^2 (Jain, 1972). This difference no doubt accounts largely for the difference in resistance to water vapor transmission.

Decreasing the chain length of the saturated fatty acid also increased the permeability of the emulsion film to water vapor. The emulsion films of stearic acid (C_{18}),

Table 1—Water vapor transmission rate and permeability of edible and nonedible films

	Temp (°C)	Thickness (mil)	Relative humidity (% RH)	Vapor pressure gradient (mm Hg)	WTVR (g) (m^2) (day)	Permeability g mil (m^2) (day) (mm Hg)
Plain cellophane[b]	37.8	–	95	46.7	310-1550[a]	6.64-33.2
Polystyrene (oriented)[c]	37.8	1.0	90	44.3	100+	2.26+
Vinyl (PVC & plasticized)[c]	37.8	1.0	90	44.3	25+	0.564+
Polyethylene (low density)[c]	37.8	1.0	90	44.3	18	0.406
C_{18}-C_{16} E-Film[d]	25.0	1.6	85	20.2	3.05	0.2
Waxed paper[b]	37.8	–	95	46.7	3.1-233[a]	0.0664-4.99
Saran (polyvinylidene chloride)[c]	37.8	1.0	90	44.3	1.5-5.0	0.0334-0.113

[a] WVTR expressed as (g) (mil)/(m^2) (day)
[b] Karel (1975)
[c] Anon. (1971)
[d] Stearic acid-palmitic acid emulsion film, edible (0.8 mg lipid/cm^2 film)

stearic acid-palmitic acid (C_{18}-C_{16}), and laric acid (C_{12}) had WVTR values of 2.2 g/m^2 day, 3.1 g/m^2 day and over 9.6 g/m^2 day (based on slope of curve from 0 - 10 hr), respectively. Even though these fatty acids are solid at 25°C, the fatty acids with shorter chain lengths have greater chain mobility and are consequently more permeable to water vapor. Although fatty acids previously have been proposed for use in edible coatings, the ability to control water vapor permeability through alterations in chain length and degree of saturation of the fatty acid has not been reported.

The findings of this study are in general agreement with those of studies involving synthetic membranes. De Gier et al. (1968) used liposomes, synthetic lipid vesicles, to study the effect of lipid composition on membrane permeability. Liposomes prepared from synthetic lecithins with variations in the length and number of double bonds of the acyl chain exhibited the following characteristics: (1) penetration of nonelectrolytes (glycerol and erythritol) into the liposome increased greatly as the temperature was increased; (2) permeability of the liposomes decreased as the length of the acyl chain was increased; (3) permeability of the liposomes increased as the number of double bonds in the acyl chains was increased; (4) at low temperatures, lecithins with asymmetric chain lengths were more permeable to non-electrolyte penetration than lecithins having equal chain lengths, both having the same total number of carbon atoms; and (5) liposome permeability decreased when cholesterol was added to the liposome. In addition, McElhaney et al. (1970) found that liposomes containing *cis* fatty acids were more permeable to solute penetration than liposomes containing *trans* fatty acids of equal chain length.

The stearic acid-palmitic acid blend was used in films prepared by both the coating technique and the emulsion technique (equal vapor pressure gradient and temperature). The C_{18}-C_{16} coated film had a WVTR of 43.9 g/m^2 day as compared to a WVTR of 3.1 g/m^2 day for the emulsion film, even though the lipid layer in the emulsion film was about one-tenth as thick as that of the coated film (9 mg lipid/cm^2 film vs. 0.8 gm lipid/cm^2 film). This difference in permeability is apparently due to orientation of the fatty acid molecules. In the coating technique, the fatty acid was simply deposited onto the surface of the dried HPMC:PEG film; however, in the emulsion technique, the fatty acid was allowed to orient at the air-water (film forming solution) interface before allowing the lipid to solidify at the HPMC:PEG surface. Aside from the superior barrier properties of the dried emulsion films, these films were also very flexible and quite resistant to mechanical damage.

The data in Table 1 provide a perspective of how the barrier properties of the C_{18}-C_{16} emulsion film compare to those of some commercial, nonedible packaging films. The water vapor permeability of the edible C_{18}-C_{16} E-Film (25°C) is less than that of plain cellophane, polystyrene (orientated), vinyl (PVC and plasticized) and polyethylene (low density, within the range of waxed paper, but greater than that of Saran (polyvinylidene chloride) and aluminum foil. At elevated temperatures, this relationship would be expected to change. It should also be noted that the C_{18}-C_{16} emulsion film was tested using an 85% relative humidity gradient and that the other films were presumably tested using a 100% gradient. Although this difference is compensated for during calculation of the permeability value, it should be realized that the C_{18}-C_{16} emulsion film will not function effectively at relative humidities much above 85%.

Edible bilayer films of the kind investigated here may be useful in some kinds of food products, such as frozen pizzas and pies, to retard moisture migration from areas of high water activity to areas of low water activity. Latter articles will provide information relevant to these kinds of applications.

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Water Vapor Permeability of an Edible, Fatty Acid, Bilayer Film

S. L. KAMPER and O. FENNEMA

ABSTRACT

A bilayer film consisting of stearic and palmitic acids as one layer and hydroxypropyl methylcellulose as the other was prepared. The permeability of this film to water vapor was tested under a variety of conditions involving film composition, temperature and relative humidity. This film exhibited permeability values (g · mil · m^{-2} · day^{-1} · mm Hg^{-1}) of 0.5 at 40°C, 0.3 at 25°C, 1.7 at 5°C, and 6.0 at −19°C. This film would be expected to perform well at relative humidities below 90% and temperatures within the range of 40°C to −19°C.

INTRODUCTION

STABILIZATION of moisture gradients among components of fabricated foods is desirable in many situations. For example, maintenance of a dry crust and a moist topping or filling is desirable in frozen pizzas and frozen pies. An edible film with good moisture barrier properties, if placed between components of differing water activity, would help retard moisture equilibration in heterogeneous food products.

In a previous study (Kamper and Fennema, 1984) several bilayer lipid-based edible films were evaluated for their effectiveness as moisture barriers. Edible films containing either stearic acid or a blend of stearic acid and palmitic acid were found to be excellent barriers to water vapor transmission at 25°C. It is the objective of this study to determine the moisture barrier properties of these films under various conditions of lipid concentration, relative humidity and temperature.

MATERIALS & METHODS

Materials

The following materials were used to prepare edible films: hydroxypropyl methylcellulose (HPMC) Methocel E 50 Premium (Dow Chemical Co., Midland, MI); polyethylene glycol (PEG) 400 (J.T. Baker Chemical Co., Phillipsburg, NJ); stearic acid, 97%+ purity (Eastman Kodak Co., Rochester, NY) and a 50.4:47.5 stearic acid-palmitic acid blend, NF (Mallinckrodt, Inc., Paris, KY).

Methods

The bilayer edible films were prepared using the emulsion technique described by Kamper and Fennema (1984). The fatty acids (stearic acid or stearic acid-palmitic acid blend) were added directly to the film forming solution (200 parts 95% ethanol:100 parts distilled water:9 parts hydroxypropyl methylcellulose:1 part polyethylene glycol 400) and heated to dissolve the fatty acids. It was shown in a previous study by Kamper and Fennema (1984) that films prepared from stearic acid and a stearic-palmitic blend exhibited essentially the same permeability to water vapor. The mixed solution (100 ml) was added to a spreader for thin layer chromatography (TLC) and plated onto three 8" x 8" glass TLC plates at a thickness of 0.75 mm. The coated plates were placed in an oven at approximately 90°C. After drying for approximately 15 min, the plates were cooled and the films were peeled from the plates.

The amount of lipid (fatty acid) present in the dried film was determined using a modified AOAC procedure for acid value of fat (AOAC, 1975). The fatty acid concentration in the film was exprssed as mg lipid/cm^2 film. Film thickness was measured with a micrometer.

The moisture barrier properties of the edible films were evaluated using a modified ASTM method (1983). A glass cup containing either desiccant or a saturated salt solution and sealed with the test film was placed in an environment of controlled humidity and temperature. The relative humidity inside the cup was always lower than that existing outside the cup so water vapor transmission was determined from the weight gain of the cup.

The edible films used were of known lipid content (mg/cm^2), thickness (mils), area (cm^2) and weight (mg). The glass cups were of two sizes: (1) 9.9 cm o.d., 9.0 cm i.d. and 1.5 cm deep with an exposed film area of 64.3 cm^2, and (2) 6.3 cm o.d., 5.0 cm i.d. and 2.0 cm deep with an exposed film area of 19.6 cm^2. The deeper cups were used when saturated salt solutions were placed in the cup to establish various fixed, intermediate, relative humidities on the interior side of the film. The films were sealed to the cups with a

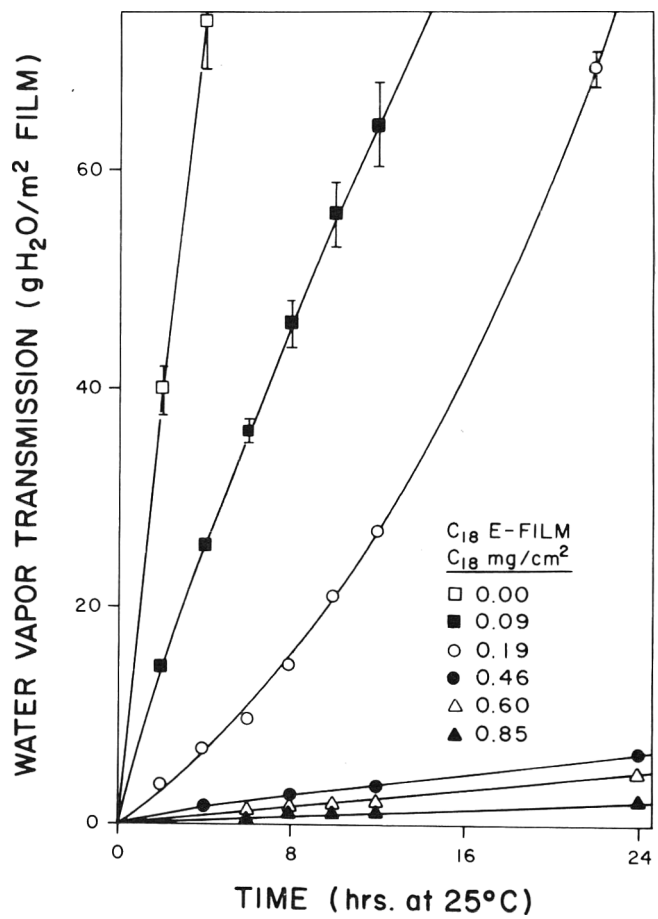

Fig. 1—Water vapor transmission through edible (emulsion technique) films at 25°C and 85% RH as a function of stearic acid concentration. Variability between the two treatment replicates is indicated only when the variability exceeds the size of the symbol.

Author Fennema is affiliated with the Dept. of Food Science, Univ. of Wisconsin, Madison, WI 53706. Author Kamper, formerly with the Univ. of Wisconsin, is now with General Mills, Inc., 9000 Plymouth Ave., North, Minneapolis, MN 55427.

blend of molten microcrystalline wax and paraffin wax, applied so as to expose the film areas previously mentioned.

The cups were stored in glass laboratory desiccators each maintained at a constant relative humidity. The following saturated salt solutions were used to control relative humidity: $MgCl_2$ (33% RH at 25°C); K_2CO_3 (43% RH at 25°C); $Mg(NO_3)_2$ (53% RH at 25°C); NaBr (58% RH at 25°C); $NaNO_2$ (65% RH at 25°C); NaCl (75% RH at 25°C); KCl (83% RH at 40°C); KBr (83% RH at 25°C); $CdCl_2$ (83% RH at 5°C); KCl (85% RH at 25°C); $BaCl_2$ (90% RH at 25°C) and K_2SO_4 (97% RH at 25°C) (Rockland, 1960; Stoloff, 1978; Wink and Sears, 1950). In addition, anhydrous calcium chloride (0% RH) and ice 83% RH at −19°C) (Fennema and Berny, 1974) were used to control relative humidity. Cup weight, to the nearest 0.0001 g, was recorded at various times. All tests were conducted in duplicate.

The water vapor transmission rates (WVTR) and permeability (P) values of the films were determined as described in the previous article by Kamper and Fennema (1984) and by Heiss (1958), Karel et al. (1959) and Labuza and Contreras-Medellin (1981).

RESULTS & DISCUSSION

Lipid concentration

The amount of lipid added to the film forming solution influenced the WVTR. Increasing the stearic acid concentration (mg/cm^2) in the stearic acid emulsion film (C_{18} E-Film) decreased the WVTR at 25°C and 85% RH as indicated in Fig. 1. Increasing the stearic acid concentration up to 0.46 mg/cm^2 greatly decreased film permeability, whereas further increases in stearic acid above 0.46 mg/cm^2 resulted in less dramatic decreases in permeability. When the stearic acid concentration was increased to very high levels (above 1.2 mg/cm^2; data not shown) the WVTR often increased because in certain areas of the film stearic acid penetrated the full depth of the film resulting in adherence to the glass plates during the process of film formation and damage to the film during removal from the plate. Thus, the optimum concentration of stearic acid (minimum water vapor permeability) using the fabrication procedure developed here appears to be about 0.85 mg/cm^2.

Because the WVTR of the C_{18} and the C_{18}-C_{16} E-Films was essentially the same (Kamper and Fennema, 1984) and the C_{18}-C_{16} E-Film was easier to prepare in a laboratory situation (lower melting point) and had a more consistent appearance the C_{18}-C_{16} E-Film was used in most of the following studies.

Relative humidity

Increasing the relative humidity gradient at a constant temperature increased the moisture transfer through the C_{18}-C_{16} E-Film (Fig. 2), with the greatest difference occurring as the relative humidity was increased above 90%. Quantitatively, the WVTR increased from 4.2 g/m^2 day at 90% RH to 25.5 g/m^2 day at 97% RH.

The C_{18}-C_{16} E-Film was also exposed, at constant temperature, to a constant relative humidity gradient (7.6 mm Hg, 32% RH), with the gradient situated at various locations in the relative humidity spectrum (Fig. 3). The film

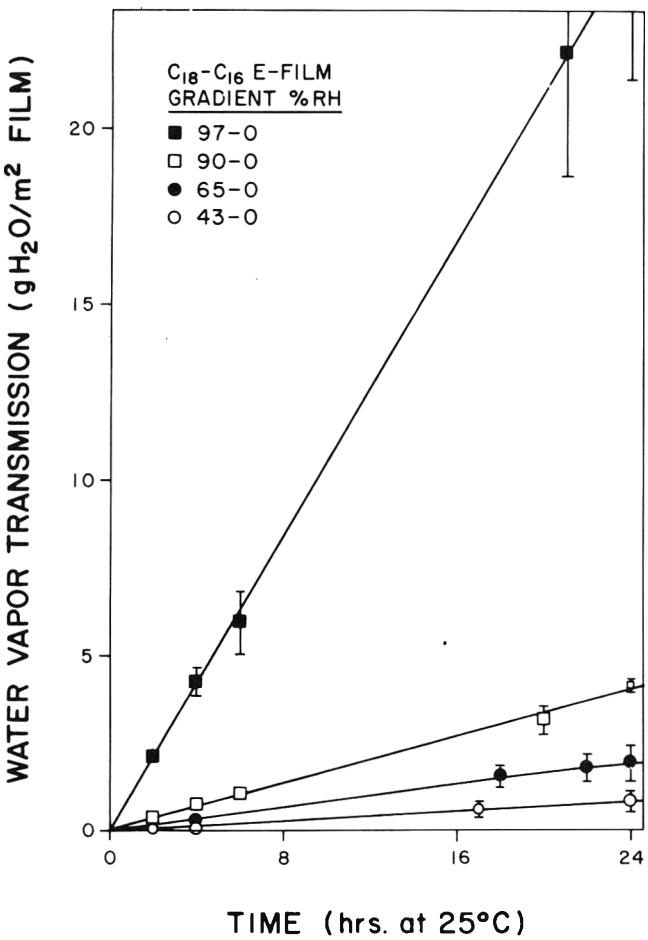

Fig. 2—Water vapor transmission through edible (emulsion technique) films at 25°C as a function of the relative humidity gradient. C_{18}-C_{16} E-Film: 0.84 mg lipid/cm^2 film, thickness 1.4-1.8 mils. Variability between the two treatment replicates is indicated only when the variability exceeds the size of the symbol.

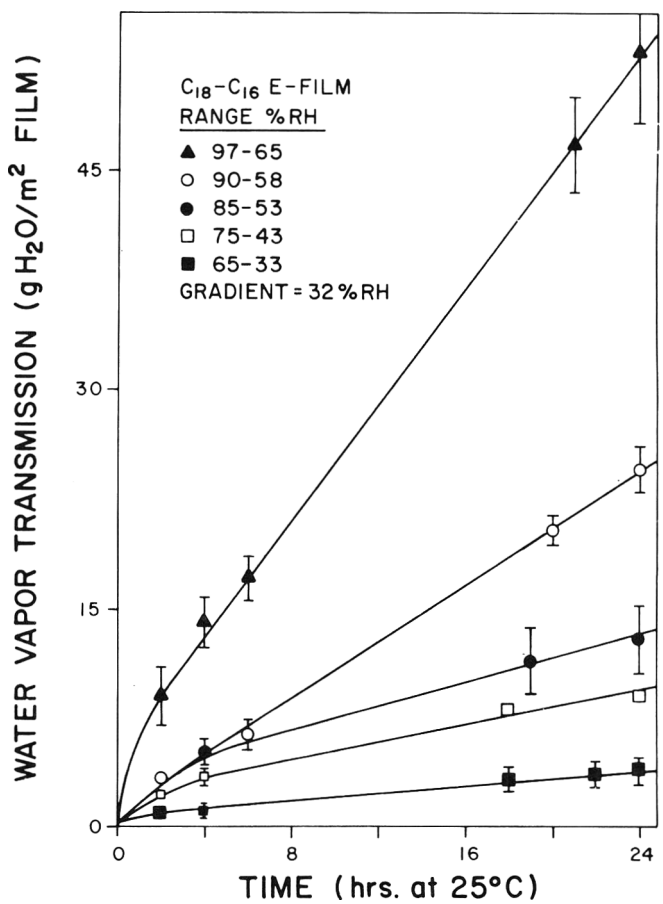

Fig. 3—Water vapor transmission through edible (emulsion technique) films at 25°C as a function of a constant relative humidity gradient (32% RH) situated at various locations in the RH spectrum. C_{18}-C_{16} E-Film: 0.76 mg lipid/cm^2 film, thickness 1.4-1.8 mils. Variability between the two treatment replicates is indicated only when the variability exceeds the size of the symbol.

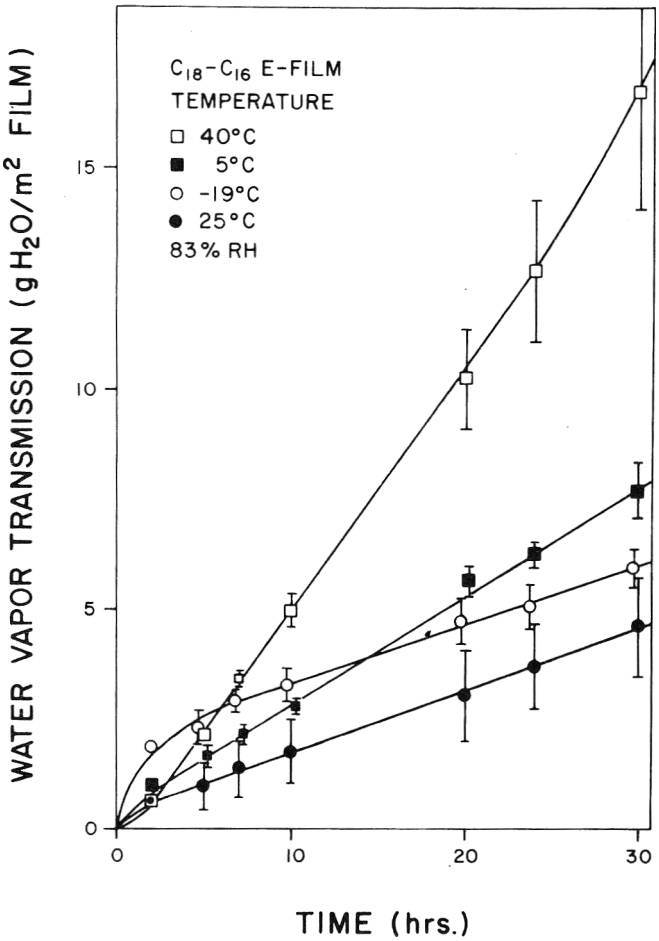

Fig. 4—Water vapor transmission through edible (emulsion technique) films at 83% RH (0% RH inside dish) as a function of temperature. C_{18}-C_{16} E-Film: 0.75 mg lipid/cm^2 film, thickness 1.4-1.8 mils. Variability between the two treatment replicates is indicated only when the variability exceeds the size of the symbol.

exhibited greater permeability as the RH gradient was moved upward in the RH spectrum. The permeability value of the C_{18}-C_{16} E-Film increased from 0.69 (g)(mil)/(m^2)(day)(mm Hg) for the 65-33% RH range to 10.1 (g)(mil)/(m^2)(day)(mm Hg) for the 97-65% RH Range.

Increased permeability of the film at high humidities presumably results from increased hydration of the film components, the hydrophilic side of the film being especially sensitive to high humidities. The films stored at 97% RH were softer and more elastic than films stored at the lower relative humidities.

This large increase in permeability of the C_{18}-C_{16} E-Film above 90% RH indicates that if the film is to serve as an effective barrier to moisture transfer between food components, the water activity of the high a_w component should be below 0.90, and the water activity of the low a_w component should be as low as possible.

Temperature

The C_{18}-C_{16} E-Film required approximately 5 hr at a given storage temperature and relative humidity to attain constant permeability characteristics (Fig. 4). In general, the amount of water vapor transferred through the C_{18}-C_{16} E-Films increased as the temperature increased. This conclusion, however, is misleading since the pressure differential across the film changed with changes in temperature. Thus, the 83% RH differential which was used throughout the experiment, translates to a pressure differential of 45.9 mm Hg at 40°C, 19.7 at 25°C, 5.4 at 5°C and 1.02 at

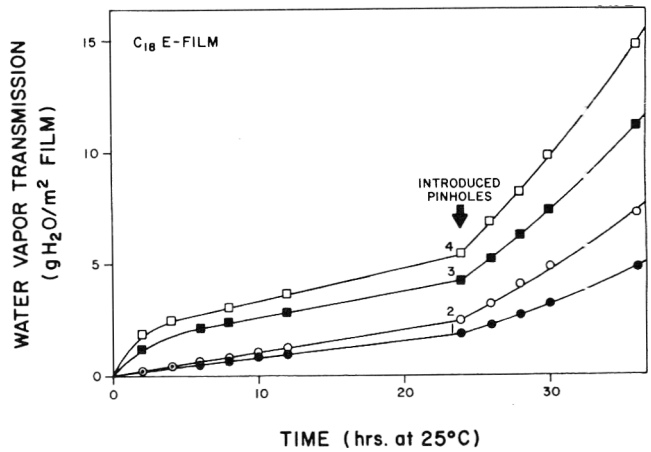

Fig. 5—The effect of intentional pinholes on the water vapor transmission through edible (emulsion technique) films at 25°C and 85% RH. Each pinhole (1-4 pinholes introduced) represents 0.008% of the exposed film area. C_{18} E-Film: ● 1 pinholes, 0.85 mg stearic acid/cm^2 film; ○ 2 pinholes, 0.85 mg stearic acid/cm^2 film; ■ 3 pinholes, 0.60 mg stearic acid/cm^2 film; and □ 4 pinholes, 0.60 mg stearic acid/cm^2 film.

−19°C. Presenting the results in terms of permeability values [P=(g)(mil)/(m^2)(day)(mm Hg)] compensates for these differences in pressure differential and is clearly a better approach. The resulting P values derived from the linear portions of the curves are 0.5 at 40°C, 0.3 at 25°C, 1.7 at 5°C and 6.0 at −19°C. Replication of the experiment reported in Fig. 4 gave consistently similar results, indicating that the P value for the C_{18}-C_{16} E-Film increased as the temperature was raised or lowered from 25°C. The increase in P as the temperature was lowered below 25°C was not anticipated, but is perhaps explainable on the basis of increased film hydration at lower temperatures, which would favor increased permeability; and the possibility of low temperature fracturing of the film as a result of lipid rigidity coupled with lipid contraction. Noguchi (1981) reported that hydration around polar groups increased with decreasing temperature and that hydration around biopolymers (such as polysaccharides) near 0°C should be twice that at room temperature. Labuza and Contreras-Medellin (1981) have shown that certain packaging films (such as polyethylene) are more permeable to water vapor at −30°C than at 35°C.

A comparison of the 25°C P value from Fig. 4 with those from Fig. 3 proves interesting. The 25°C P value from Fig. 4 is 0.3 and it was obtained using an RH gradient of 0-83. This value is smaller than the P values (0.6-10.1) from Fig. 3, the latter values being obtained using constant RH gradients of 32% located at various points in the RH spectrum. It should be noted that the hydrophilic side of the film in Fig. 4 was always exposed to a RH of 0%, whereas the hydrophilic sides of the films in Fig. 3 were exposed to RH's ranging from 33-65%. Thus, the hydrophilic sides of the films in Fig. 3 clearly would have absorbed water from the atmosphere, apparently resulting in alteration of the lipid layer and diminished resistance to the transfer of water vapor.

Pinholes

The presence of pinholes in the edible film greatly affected the moisture barrier properties of the film. Introduction of one intentional pinhole, about 0.008% of the exposed film area, increased the WVTR (ratio of slopes before and after pinhole introduced) through the C_{18} E-Film (0.85 mg stearic acid/cm^2 film) 2.7 fold (Fig. 5). It

is apparent from these results that small disruptions in film integrity will significantly lessen the ability of this film to retard the passage of water vapor.

The C_{18}-C_{16} E-Film tested here, provides good barrier properties to the transfer of water vapor provided the hydrophilic side of the film is maintained in a dry condition. This film might be useful as a means of retarding the transfer of water vapor between the high and low moisture layers of foods such as frozen pizzas or frozen pies. This application will be assessed in a future article.

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Unsaturated Solutions of Sodium Chloride as Reference Sources of Water Activity at Various Temperatures

JORGE CHIRIFE and SILVIA L. RESNIK

ABSTRACT

In view of the uncertainty about the exact water activity (a_w) of some solutions (saturated and unsaturated) used as references sources, the use of unsaturated NaCl solutions as isopiestic standards for calibration of hygrometers is proposed. It is shown that there is an excellent agreement on the exact value of NaCl solutions between various literature compilations and theoretical models. Further, it is also shown that in the important range (for food related applications) of 15–50°C the a_w of NaCl solutions is almost invariant.

INTRODUCTION

IT IS WELL KNOWN that water activity (a_w) is one of the single most important factors affecting the microbial stability of foods (Troller and Christian, 1978). Most methods frequently used for measuring a_w, involve the need of reference standard sources of relative vapor pressures in the range of interest. Hygrometers (electric or fiber-dimensional type) are widely used to measure a_w in food-related applications (Stoloff, 1978; Gál, 1981; Favetto et al., 1983) and the reference sources are needed for calibration of these devices.

Saturated salt solutions have been recommended by numerous workers as a convenient, easy and accurate way to provide solutions of known a_w. They are reproducible reference standards because no measurement of concentration is needed and if the salts are properly chosen no interfering vapors are present (Stoloff, 1978). Unfortunately, most reports in the literature do not agree on the exact value of each saturated salt solution and so it is difficult to calibrate instruments to measure a_w; thus, the a_w measurements performed by different workers are not always comparable. The review of Greenspan (1977) reflects very well the uncertainties in the literature values for saturated salt solutions. Recently Resnik et al. (1984) reported the results of a world survey of a_w values at 25°C of selected saturated salt solutions used as standards. The salts covered the range of $a_w \cong 0.57$–0.97. Their results indicated various discrepancies between laboratories regarding the correct a_w value to be assigned to each salt. The survey was limited to 25°C but it was expected that discrepancies would be even larger if other temperatures were considered. It is obvious that the uncertainty about the exact value of certain reference saturated salt solutions is a problem in the study of foods preserved by control of their a_w.

The thermodynamic properties of NaCl solutions have been exhaustively determined over the past 50 years. Dozens of workers in the physical chemistry area determined the activity of water in solutions of NaCl and reported their data on osmotic coefficients (ϕ) for the whole range of concentration and at various temperatures. It is the purpose of the present paper to propose the use of unsaturated solutions of NaCl as isopiestic standards for calibration of a_w measuring devices in a range of a_w and temperature of most interest to food microbiology applications.

The authors are affiliated with Proipa (CONICET-FCEyN), Depto. de Industrias, Facultad de Ciencias Exactas y Naturales, Univ. de Buenos Aires, 1428 Buenos Aires, Argentina. Author Resnik is also a member of Comisión de Investigaciones Cientificas de la Provincia de Buenos Aires.

RESULTS & DISCUSSION

Experimental data on the a_w of sodium chloride solutions

The literature references are so numerous that they can not be mentioned here. Experimental determinations were based on precise and accurate methods (i.e. isopiestic, vapor pressure measurement). The accuracy of water activity measurements of NaCl solutions using these methods has been reported to be 0.0001–0.0002 a_w. Scatchard et al., 1938; Olynyk and Gordon, 1943; Platford, 1979). This accuracy largely exceeds the needs for food microbiology research where a_w values are usually measured to ±0.005 or at the best at ±0.002 (Troller and Crhistian, 1978; Favetto et al., 1983). From time to time well known researchers critically evaluated the literature data on measurement of the osmotic coefficient of NaCl solutions and published "best" values of ϕ for rounded molalities. These compilations are internally consistent and in general accurate to about 0.001 in ϕ (Platford, 1979; Gibbard et al., 1974). The osmotic coefficient is defined as

$$\phi = \frac{-55.51 \ln a_w}{v_i m_i} \quad (1)$$

where v_i is the number of particles into which each solute of molality m_i dissociates.

For our study we have selected the following compilations of ϕ in NaCl solutions: (a) the tables in the classical book by Robinson and Stokes (1965), for NaCl solutions at 25°C and 60°C; (b) the compilation of Hamer and Wu (1972) for univalent electrolytes at 25°C; (c) the measurements and compilation of Gibbard et al. (1974) for NaCl solutions at 25°C and 50°C; (d) the compilation of Platford (1979) for NaCl solutions at 15 and 37°C.

The values of osmotic coefficient were transformed to a_w using Eq. (1) and rounded to the fourth decimal value.

Prediction of a_w of NaCl solutions at 25°C

We are interested in using an equation which reproduces measured properties of NaCl solutions within experimental accuracy and which is also convenient in the sense that only a few parameters need to be tabulated. It is also desirable that those parameters have physical meaning as far as possible.

Pitzer (1973) developed a system of equations for the thermodynamic properties of electrolytes on the basis of an improved analysis of the classical Debye-Hückel model. By modifying the usual second virial coefficient to include the recognition of an ionic strength dependence of the effect of short range forces in binary interactions, he obtained a system of equations which yield agreement within experimental error up to concentrations of several mol/kg. The equations represent experimental data substantially within the experimental error from dilute solutions up to an ionic strength varying from case to case typically 6 molal. The fit was particularly good for 1-1 electrolytes, which is the one of present concern (NaCl).

Table 1—Comparison of various literature compilations and predicted a_w values in NaCl solutions at 25°C

Molality (m)	Literature compilation[a] (1)	(2)	(3)	Predicted by Pitzer's model Eq. (2)
0.1	0.9967	0.9966	0.9966	0.9966
0.2	0.9934	0.9934	0.9934	0.9934
0.3	0.9901	0.9901	0.9901	0.9901
0.4	0.9868	0.9868	0.9868	0.9868
0.5	0.9836	0.9835	0.9836	0.9835
0.6	0.9803	0.9802	0.9803	0.9803
0.7	0.9769	0.9769	0.9769	0.9769
0.8	0.9736	0.9736	0.9736	0.9736
0.9	0.9702	0.9702	0.9702	0.9702
1.0	0.9669	0.9668	0.9668	0.9669
1.2	0.9601	0.9600	0.9600	0.9600
1.4	0.9532	0.9531	0.9531	0.9531
1.5	0.9497	0.9496	–	0.9496
1.8	0.9389	0.9389	0.9389	0.9388
2.0	0.9316	0.9316	0.9316	0.9315
2.5	0.9127	0.9128	0.9128	0.9128
3.0	0.8932	0.8932	0.8932	0.8932
3.5	0.8727	0.8728	0.8727	0.8727
4.0	0.8515	0.8517	0.8514	0.8515
4.5	0.8295	0.8298	0.8295	0.8295
5.0	0.8068	0.8071	0.8069	0.8068
5.5	0.7836	0.7839	0.7835	0.7834
6.0	0.7598	0.7601	0.7599	0.7594

[a] (1) Robinson and Stokes (1965); (2) Gibbard et al., (1974); (3) Hamer and Wu (1972).

The osmotic coefficient, ϕ, is given by the equations developed by Pitzer (1973) as:

$$\phi - 1 = |Z_M Z_X| f + m\left(\frac{2\nu_M\nu_X}{\nu}\right)B_{MX} + m^2\, 2\frac{(\nu_M\nu_X)^{3/2}}{\nu} C_{MX} \quad (2)$$

where, ν_M and ν_X are the number of M and X ions in the formula and Z_M and Z_X give their respective charges in electronic units; also, $\nu = \nu_M + \nu_X$. The other quantities have the form:

$$f = -A\left[\frac{I^{1/2}}{1 + b\, I^{1/2}}\right]$$

$$B_{MX} = \beta^{(0)}_{MX} + \beta^{(1)}_{MX} \exp(-\alpha I^{1/2})$$

where, $\beta^{(0)}_{MX}$ and $\beta^{(1)}_{MX}$ define the second virial coefficient and C_{MX} defines the third virial coefficient, and I (ionic strength) = $\frac{1}{2} \Sigma\, m_i Z_i^2$. A is the Debye-Hückel coefficient for the osmotic function and has a value of 0.392 at 25°C. Constant b was taken equal to 1.2 for all solutes and also the value of $\alpha = 2$ was found by Pitzer and Mayorga (1973) to be satisfactory. Pitzer and Mayorga (1973) evaluated the best values of $\beta^{(0)}$, $\beta^{(1)}$ and C for NaCl at 25°C from the tables of osmotic coefficients in the book of Robinson and Stokes (1965). Values found were 0.0765, 0.2664, and 0.00127 for $\beta^{(0)}$, $\beta^{(1)}$ and C, respectively, and the maximum molality for which agreement was attained to 0.01 in ϕ was 6m; the standard deviation of the fit was 0.001.

Water activity of NaCl solutions at 25°C

The data on ϕ of NaCl solutions at 25°C reported in the various literature compilations were transformed to a_w using Eq. (1) and compared to values predicted using the model of Pitzer (1973). This is shown on Table 1. It can be seen that the agreement between the various literature compilations and predicted values is excellent for the whole range of molalities (up to 6m). At the lowest a_w (≅0.76) the largest absolute difference between the literature compilations themselves is only 0.003 a_w, and the discrepancies with the values predicted by Pitzer's (1973) model are below 0.0007 a_w. It may be concluded that there is excellent agreement on the exact values to be assigned to NaCl solutions at 25°C.

Table 2—Comparison of a_w of NaCl solutions at different temperatures

Molality (m)	15°C (3)	25°C Literature compilation[a] (1)	37°C (3)	50°C (2)	60°C (1)
0.1	0.9966	0.9967	0.9966	0.9967	0.9967
0.2	0.9934	0.9934	0.9934	0.9934	0.9934
0.3	0.9901	0.9901	0.9901	0.9901	0.9901
0.4	0.9869	0.9868	0.9868	0.9868	0.9868
0.5	0.9836	0.9836	0.9835	0.9835	0.9835
0.6	0.9803	0.9803	0.9802	0.9802	0.9802
0.7	0.9771	0.9769	0.9768	0.9768	0.9768
0.8	0.9738	0.9736	0.9735	0.9735	0.9734
0.9	0.9705	0.9702	0.9701	0.9701	–
1.0	0.9671	0.9669	0.9667	0.9666	0.9666
1.2	–	0.9601	–	0.9597	–
1.4	–	0.9532	–	0.9527	–
1.5	0.9501	0.9497	0.9492	0.9491	0.9490
1.6	–	0.9461	–	0.9455	–
1.8	–	0.9389	–	0.9382	–
2.0	0.9322	0.9316	0.9310	0.9308	0.9305
2.5	0.9137	0.9127	0.9121	0.9118	0.9113
3.0	0.8945	0.8932	0.8925	0.8921	0.8917
3.5	0.8743	0.8727	0.8720	0.8716	0.8714
4.0	0.8532	0.8515	0.8508	0.8505	0.8497
4.5	0.8314	0.8295	0.8290	0.8288	–
5.0	0.8088	0.8068	0.8066	0.8066	–
5.5	0.7857	0.7836	0.7834	0.7840	–
6.0	0.7621	0.7598	0.7596	0.7611	–

[a] (1) Robinson and Stokes (1965); (2) Gibbard et al. (1974); (3) Platford (1979).

Table 3—a_w of NaCl solutions: values proposed to be used as standards in the range 15-50°C

Conc. (% w/w)	a_w	Conc. (% w/w)	a_w	Conc. (% w/w)	a_w	Conc. (% w/w)	a_w
0.5	0.997	7.0	0.957	13.5	0.906	20.0	0.839
1.0	0.994	7.5	0.954	14.0	0.902	20.5	0.833
1.5	0.991	8.0	0.950	14.5	0.897	21.0	0.827
2.0	0.989	8.5	0.946	15.0	0.892	21.5	0.821
2.5	0.986	9.0	0.943	15.5	0.888	22.0	0.815
3.0	0.983	9.5	0.939	16.0	0.883	22.5	0.808
3.5	0.980	10.0	0.935	16.5	0.878	23.0	0.802
4.0	0.977	10.5	0.931	17.0	0.873	23.5	0.795
4.5	0.973	11.0	0.927	17.5	0.867	24.0	0.788
5.0	0.970	11.5	0.923	18.0	0.862	24.5	0.781
5.5	0.967	12.0	0.919	18.5	0.857	25.0	0.774
6.0	0.964	12.5	0.915	19.0	0.851	25.5	0.766
6.5	0.960	13.0	0.911	19.5	0.845	26.0	0.759

Table 2 compares the a_w of NaCl at various temperatures (obtained from different literature compilations) with the value at 25°C (the compilation by Robinson and Stokes (1965) in this case). It can be seen that the a_w of NaCl solutions varies very little with temperature in the range examined. In fact, the a_w of NaCl solutions (up to 6m) at 15, 37, and 50°C agrees to 0.002 with that at 25°C.

Proposal of NaCl as iospiestic standard for calibration of a_w measuring devices

On the basis of present results we propose the use of unsaturated NaCl solutions as auxiliary standards for the calibration of a_w-measuring devices and for a_w values above ≅0.76. They may be used specially for cases where there is not general agreement on the value to be assigned to certain saturated salt solutions (Resnik et al., 1984). The use of NaCl solutions displays two unique features which are not always found with saturated salt solutions. They are the

following: (1) there is a "universal" agreement on the a_w values of experimental and predicted values of NaCl solutions; the data at 25°C from different sources agree at the 0.0007 a_w level, a value which far exceeds the actual needs for a_w measuring in food microbiology research; (2) the a_w of NaCl solutions in the important range of 15–50°C may be considered almost equal (maximum error is around 0.002 a_w) to that at 25°C. Of course, NaCl solutions are useful as standards only for values of a_w above ≅0.76; however, this is an important range for food microbiology since it contains the a_w limits for growth of almost all bacteria and most yeasts and molds. For this purpose Eq. (2) was programmed to give the a_w of NaCl solutions at 0.5% weight intervals and the results (rounded to the nearest third decimal figure) are shown in Table 3; these values are thus recommended as reference standards sources at any temperature between 15 and 50°C. This is the most useful range for food-microbiology related applications.

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Structural Stability of Fresh and Frozen-Thawed 'Valencia' (*C. sinensis*) Orange Juice

UZI MERIN and ILAN SHOMER

ABSTRACT

Valencia orange juice was subjected to different freezing rates and different storage times to study the effects of those treatments on the structural and dispersion properties of the insoluble particles. Examination with an electron microscope revealed that the juice contained plastids, mitochondria, oil droplets and cell walls. Fragments consisting mainly of vesicular membranes were present both before and after freezing and thawing. A slow freezing rate resulted in plastids with various degrees of disintegration. Separation of insoluble particles from the juice serum increased with increasing frozen storage time and was especially apparent at slower primary freezing rates. A decrease in color intensity and deterioration in appearance is related to longer storage time and to slower primary freezing rates.

INTRODUCTION

JUICE-SACS of citrus fruits differentiate from the epidermis and sub-epidermis of the endocarp. When mature, juice-sacs completely fill the carpal locules (Esau, 1977; Schneider, 1968). Chromoplasts from juice-sacs exhibit several shapes as observed by light microscopy (Matlack, 1931; Yuasa et al., 1957). The central zone of the juice-sac contains chromoplasts which produce and secrete essential oils into the cytoplasm. These cells are constructed of very thin and undulating walls. Squeezing the juice-sacs results in a liquid homogenate which contains insoluble components, mainly the intracellular content of the sacs.

The most important factor affecting the appearance of the juice are opacity and color (Stewart, 1980). The insoluble color in citrus fruit and in many other plant species is due to osmiophilic bodies and membranes present in the chromplasts (Kirk, 1970; Shomer and Fahn, 1976; Thomson et al., 1967).

Natural and concentrated orange juice is usually exposed to heat treatment and to vacuum heat evaporation before freeze preservation. Avoiding heat and vacuum treatment could lead to better quality products. Freeze preservation is known to keep degradative processes to a minimum during long periods of storage (Olson, 1968). The quality of natural juice preserved by freezing without heat treatment and consumed immediately after thawing, is the closest to that of freshly squeezed orange juice.

Although marketing fresh frozen juice may not be economically feasible, it is important to understand the structural changes which occur in frozen thawed insoluble particles in relation to their stability in the reconstituted juice.

Studies of freezing have indicated that secondary crystallization destroyed protoplasmic structures and damaged cell viability even in plants which withstand natural freezing by avoiding the growth of intracellular ice crystals (Burke et al., 1076; Levitt, 1978; Mazur, 1969). Insoluble particles of juice which are expelled from the protoplasm of the juice-sac cells, are exposed to ice crystals in the frozen homogenate. The size and number of ice crystals formed within a solution is a function of freezing and thawing rates, time and temperature of the storage, and type and concentration of solutes (Luyet, 1968).

Since the dispersion and color of the insoluble fragments after freezing and thawing affect the juice's appearance, it is important to study the structural and dispersion properties of those fragments under different freezing rates and storage times, with emphasis on bodies which produce and accumulate the pigments in the juice-sac.

Authors Merin and Shomer are affiliated with the Dept. of Food Technology, Agricultural Research Organization, P.O. Box 6, Bet Dagan 50250, Israel.

MATERIALS & METHODS

Orange Juice

Fresh Valencia orange juice was obtained from a local processing plant prior to treatment and after a 0.76-mm pore size screening.

Freezing methods

Juice was packed in 400-mL polyamid bags, which were heat sealed. Temperature recording during freezing was done with a copper-constantan thermocouple placed in the middle of each juice bag (Fig. 1).

Liquid nitrogen freezing (N). Liquid N_2 was poured into a foam polystyrene container, and the bags of juice were immersed in the N_2 for 10 min to achieve complete freezing and to ensure lowering of the temperature of the whole sample to −60°C (Fig. 1, N).

Blast freezing (B). A pilot size blast freezer (Frigoscandia Ltd., Sweden) was used at −40°C, with 3.5 m/sec air flow velocity. The bags were placed ontrays and left at −40°C for 3 hr to ensure complete freezing (Fig. 1, B).

Chamber freezing (C). A freezer chamber at −18°C served for slow freezing. The samples were placed in the freezer for 24 hr (Fig. 1, C).

All samples were stored at −18°C until examined. Thawing was done by immersion in a warm-water bath (45°C), with continuous stirring in order to achieve equal and rapid thawing of the samples to 25°C.

Color measurements

Standard USDA color tubes were used to rate the color appearance of the juice, with grades from 1 to 6, with 1 = yellow-orange and 6 = yellow.

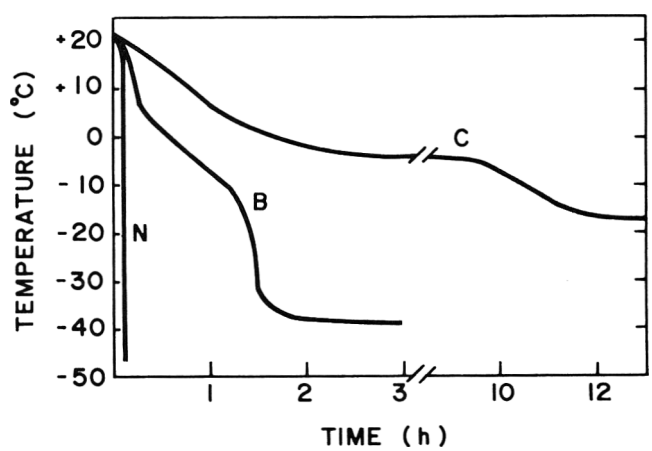

Fig. 1—Freezing rates of the applied primary freezing. (N) nitrogen freezing. (B) blast freezing. (C) freezer chamber.

A modified procedure, as suggested by Shibata et al. (1954) was used for the color measurements of the whole juice. Juice was diluted in equal volumes of water and 96% glycerol. the cuvettes were covered with an opaque screen in order to collect the scattered light. The color was scanned at between 400 and 500 nm, while the instrument was calibrated according to the Operating Manual. The spectrum was recorded using a double beam spectrophotometer. Measurements were carried out on fresh and thawed orange juice, and on petroleum ether extract in 1 cm glass cuvettes.

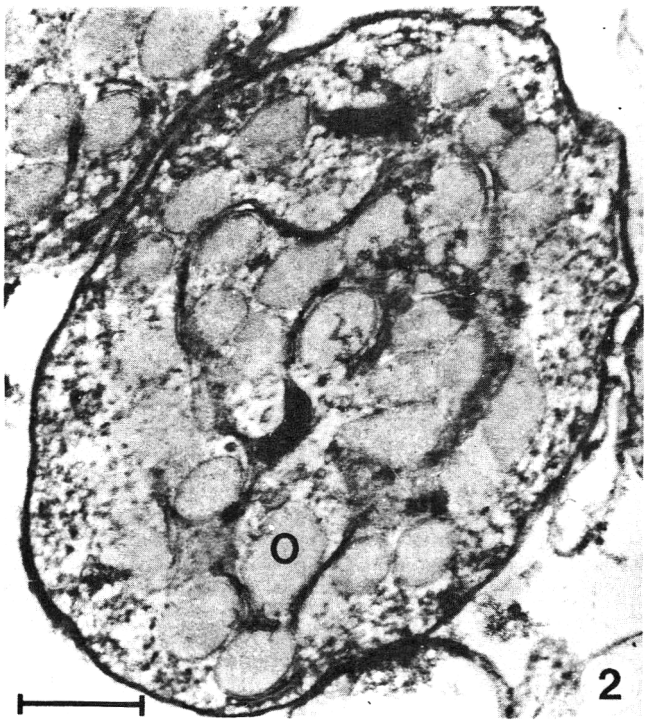

Fig. 2—Chromoplast of juice from mature Valencia orange. (O) oil droplet. Magnification bar = 0.3 μm.

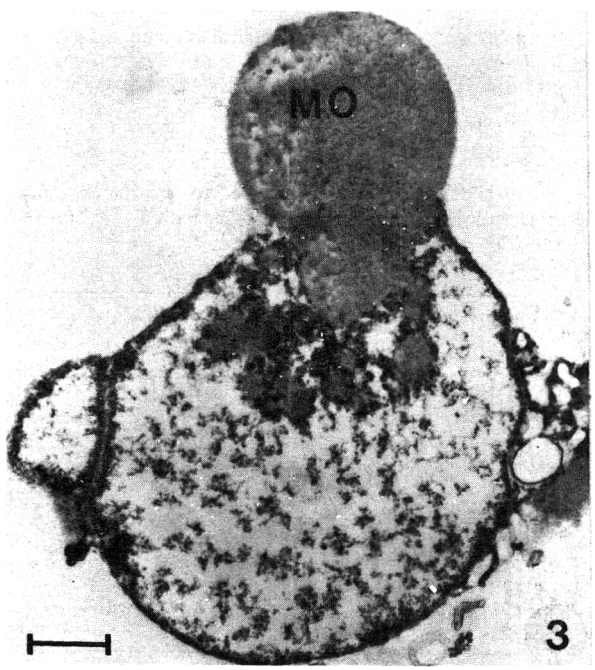

Fig. 3—Chromoplasts of juice from ripe Valencia orange with a large oil droplet in migration stage through the chromoplast envelope. (MO) oil droplet at migration stage from chromoplast. Magnification bar = 0.6 μm.

Electron microscopy

Fresh juice was placed in 10 mL centrifuge tubes and centrifuged for 5 min at 4000 rpm. The supernatant was decanted and the pellet immediately fixed in 3.5% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7 for 4 hr at 4°C. After a rinse with the same buffer, the pellet was postfixed for 2 hr in 2% OsO_4 in the same buffer at room temperature. The material was then dehydrated in a graded ethanol series and embedded in low-viscosity epoxy resin (Spurr, 1969). Ultrathin sections were stained with uranyl acetate followed by lead citrate, and examined with a Philips 300 electron microscope.

RESULTS & DISCUSSION

DIFFERENTIATED CELLS in mature juice-sacs were found to have chromoplasts with two distinct ultrastructural appearances: (1) those of the peripheral concentric zone of the juice-sac tissue, which is characterized by a central lamelar body surrounded by distinct osmiophilic droplets, and (2) chromoplasts of cells from the central zone of the juice-sac which were found to include many droplets of essential oil that in many cases were fused and had migrated into the cytoplasm (Shomer and Fahn, 1976). Similar chromplasts from either the peripheral or central zone of the juice-sac are found in the juice of ripe fruit as shown in Fig. 2 through 4. Large oil droplets were found in migration stages from the chromoplasts and free in the juice (Fig. 3 and 4). Some of the chromoplasts were observed to have starch grains in addition to the osmiophilic droplets (Fig. 5 and 7).

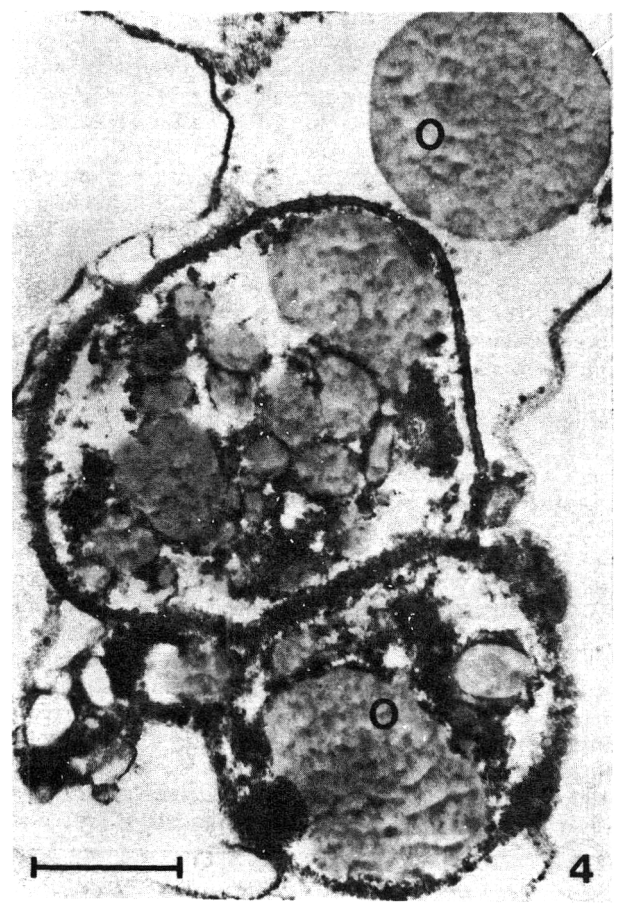

Fig. 4—Insoluble components of juice after freezing with liquid nitrogen and thawing. (O) oil droplet. Magnification bar = 0.5 μm.

After cryogenic freezing with liquid nitrogen (Fig. 6), osmiophilic substances appeared as a cluster of droplets and the plastid envelope was similar to that before freezing (Fig. 2 and 4). Since a rapid freezing rate results in many nucleations and small ice crystals, no obvious damage could be observed after nitrogen freezing. Shomer and Fahn (1976), described five different concentric zones of the juice-sac tissue in grapefruit and the central zone was characterized by thin and undulating walls. The present study showed many wall fragments from cells of the central zone of the juice-sac in the extracted screened juice. The wall fragments were found to be associated with the other cytoplasmic material (Fig. 7). The thawed walls after liquid nitrogen freezing (Fig. 7) were not injured, although some torn sites were observed. This may indicate that these delicate walls could be easily extracted with the liquid content of the juice-sac by the squeezing process. It is suggested that, at the same time, thicker walls of the peripheral concentric zones of the juice-sac remain as the main component of the squeezed and screened pulp, in addition to other cytoplasmic material.

The blast freezing (Fig. 8) resulted in chromoplasts where the internal volume was disintegrated while the chromoplast envelope seemed undamaged, and the osmiophilic droplets appeared in a close compact configuration.

Mechanical disintegration of protoplasmic components is known to result in the formation of vesicular fragments in the tissue homogenate. Mechanical freeze damage of juice-sac protoplasm was found to result in many vesicular fragments in cells of the whole juice-sac tissue (Shomer, unpublished). Since vesicular fragments are already present in the juice before freezing, it is impossible to determine whether their formation is increased by freezing injury in the thawed juice. After slow freezing, various degrees of structural degradation stages were observed in chromoplasts and some of them were almost disintegrated (Fig. 9). The envelope was ruptured, probably due to ice crystal growth which pierced the chromoplast membrane, as suggested by Asahina (1975) for freeze damage in cell walls.

Measuring the rate of separation of insoluble particles from clear serum, provides an indication to physical changes which occur in the juice during frozen storage. It was found that the settling rate advanced with the frozen storage time

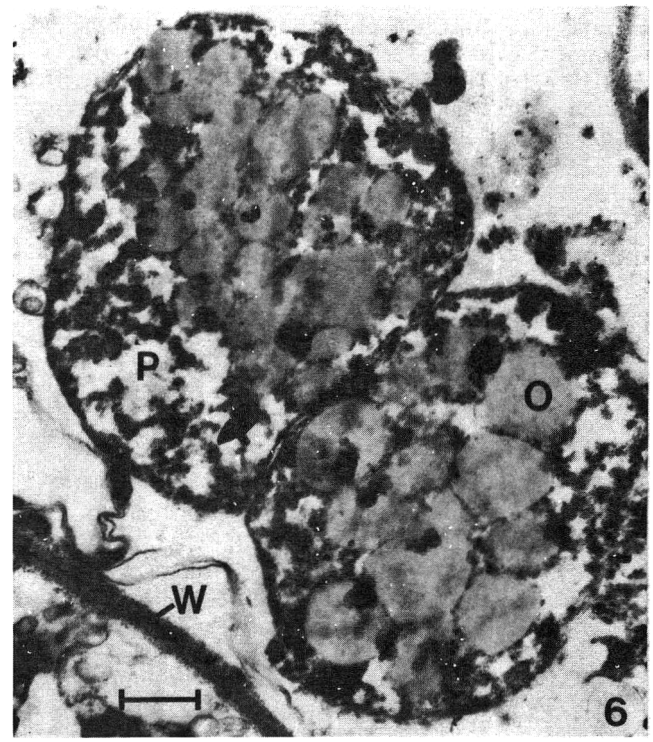

Fig. 6—Insoluble components of juice after freezing with liquid nitrogen and thawing. (O) oil droplet. (P) chromoplast; (W) cell wall. Magnification bar = 0.5 μm.

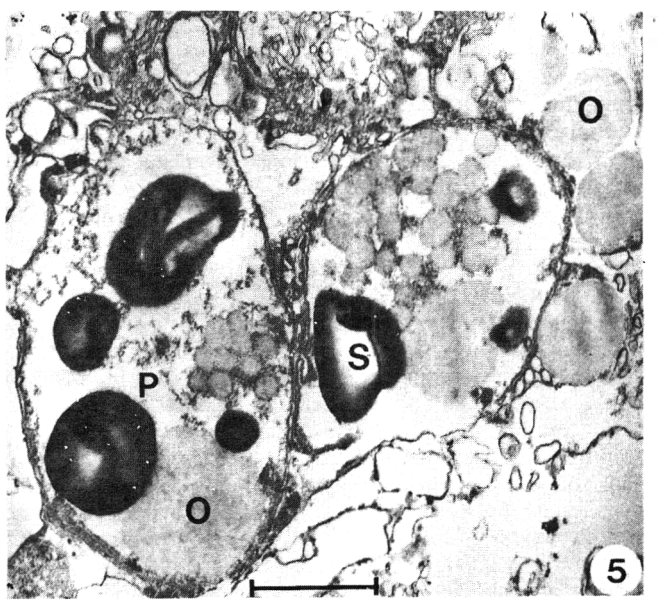

Fig. 5—Insoluble fragments and chromoplasts with distinct starch grain. (O) oil droplet; (P) chromoplast. (S) starch. Magnification bar = 0.5 μm.

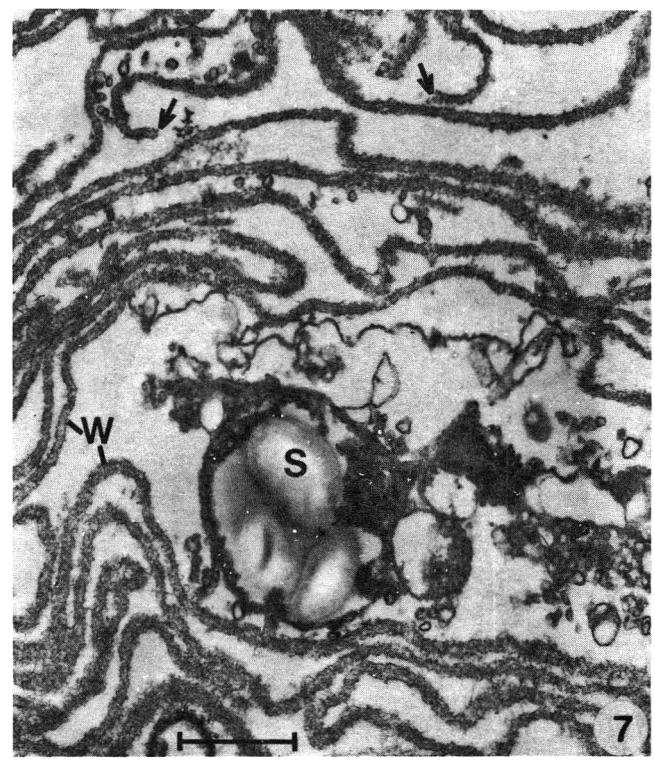

Fig. 7—Insoluble wall fragments of juice after freezing with liquid nitrogen and thawing. (O) oil droplet. (P) chromoplast. (S) starch. (W) cell wall; (arrows indicate torn cell walls.) Magnification bar = 1.0 μm.

and in accordance with the sequence of the primary freezing rate (Fig. 10). Quick primary freezing delayed the separation of thawed juice over a relatively long storage period (Fig. 10 A-D). The significant difference between the effect of primary freezing rates on separation during the frozen storage period is illustrated in Fig. 11.

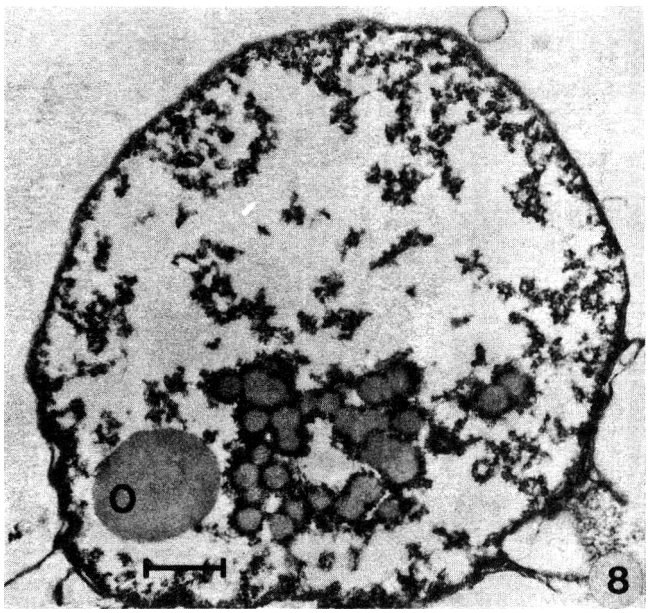

Fig. 8—Chromoplast after freezing with blast freezer and thawing. (O) oil droplet. Magnification bar = 0.5 μm.

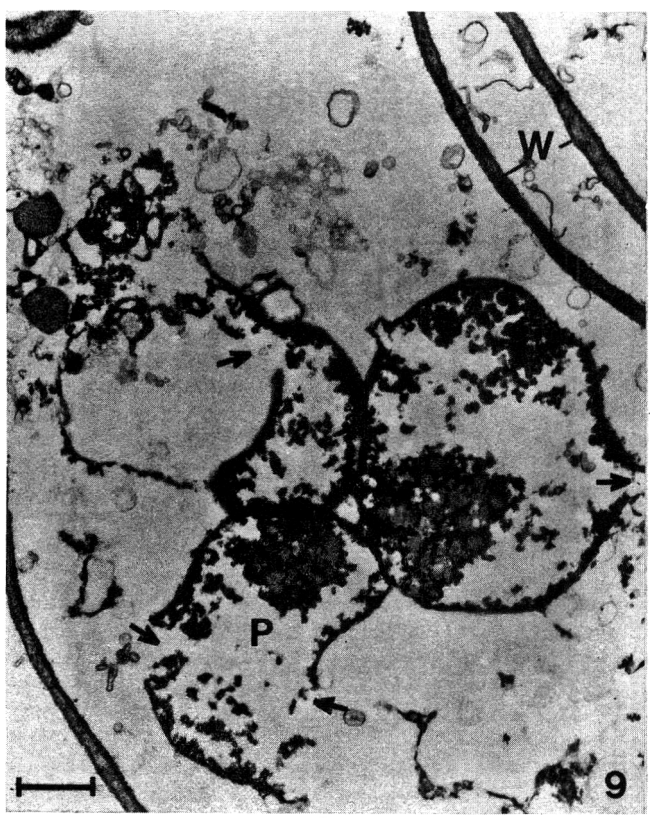

Fig. 9—Chromoplast after freezing with chamber freezer and thawing. (O) oil droplets; (P) chromoplast; (W) cell wall (arrows indicate ruptures in plastid envelope.) Magnification bar = 1.0 μm.

A comparison of the juice color with USDA standard tubes showed that there was a steady decrease in color in the three samples. It should be noted that this measurement is the closest to that of the consumer's eye, since it is based on matching color to aknown reference.

Spectra intensities of petroleum ether extraction were characterized by usual maxima of 420, 430 and 465 nm and did not differ between the treatments during storage. Spectrophotometric color measurements of the whole juice with equal parts of water and glycerol, using an opaque screen, are shown in Fig. 12. The spectra of the whole juice were characterized by a shift of the maxima toward a longer wavelength in all treatments. This shift could be explained by the presence of suspended particles in the whole juice, which scattered the light (Shibata et al., 1954; Butler and Noriss, 1960). However, spectrum maxima remained at the same wavelength for all treatments, and decreased in intensity with length of storage. Quick primary freezing retained the color intensity of thawed juice for at least 60 days of frozen storage (Fig. 12C), while slow primary freezing resulted in immediate depression of the intensity (Fig. 12A). After 90 days of frozen storage, the intensity dropped markedly in juices from all freezing rates (Fig. 12D). Since insoluble particles scatter the light in suspension and prevent spectrum measurements, the method described by Shibata et al., (1954) was used. An opaque screen enabled the collection of the transmitted scattered light of the suspension and the measurement of its spectrum.

It is suggested that the "apparent" loss of color which was recorded for the whole juice with the spectrophotometer and the standard USDA color tubes was not due to real loss of color, but rather to increased aggregation which resulted in increased transmittance and lowered color intensity. Aggregation upon thawing after frozen storage caused the appearance of the juice to change, while the extracted color was not altered by the flocculation of insoluble particles. The increased flocculation observed with the slower freezing rate, might have been due to compaction of the insolubles in the juice caused by the ice crystals, which, upon thawing were not redispersed in the juice.

Active pectic enzymes lead to catalyzed flocculation, mainly as a result of demethoxylation in the presence of Ca^{++} ions (Bruemer, 1980). This process tends to form pectic gel and is known to be enhanced by activated pectin esterase in the extracted juice (Veldhuis, 1971). Frozen stored juice became separated after a relatively short time, and the tendency to separation was greater after slow freezing (Fig. 10 and 11).

It is suggested that preservation by freezing affected the activity of the pectic enzymes (which was enhanced by squeezing) and kept it to a minimum; it was equal in all samples. This led to the conclusion that the significant differences in flocculation between the samples were due to the rate of primary freezing. Slow freezing, which resulted in large ice crystals having a small surface area, was in effect, causing concentration by freezing. This concentration increased during the crystal growth period and could be extended to the eutectic level of the frozen solution (Shomer et al., 1973). When the cooling was done simultaneously from the surface in a concentric direction, the soluble and insoluble components were forced to accumulate between the forming ice crystals. The increase in volume of ice caused compression of insoluble particles between ice crystals. Release of the pressure by thawing after a relatively short frozen storage period did not lead to complete redispersion of the aggregated particles and they remained in clusters and tended to separate from the serum. In addition, the close physical association of the insoluble particles caused by pressure during freezing may

lead to the formation of calcium bridges between the carboxyl groups of the pectin with time. The release of these bridges will eventually result in the redispersion of the compacted insolubles (Shomer and Merin, unpublished).

When quick freezing was carried out, the ice nucleations and crystals were numerous and small in the frozen mass. This situation permitted the insoluble particles to remain dispersed as before primary freezing, therefore, upon thawing the redispersion was close to that of fresh juice.

It is a known phenomenon that ice crystals of pure water or of dilute solution have a lower vapor pressure than ice crystals of more concentrated solutes. Differences in the vapor pressure lead to fluctuation in the size of the ice crystals, by transfer of water molecules from regions of relatively high concentration of solutes to those of lower concentration. Hence, it is suggested that frozen storage of the samples at higher temperature (−18°C) than the primary freezing temperature resulted in secondary crystallization. The secondary crystallization, which takes place with frozen storage time, may lead to compression of the insolubles, as it is during slow primary freezing rate. Hence, aggregation appears in thawed juice for all treatments after a long period of frozen storage.

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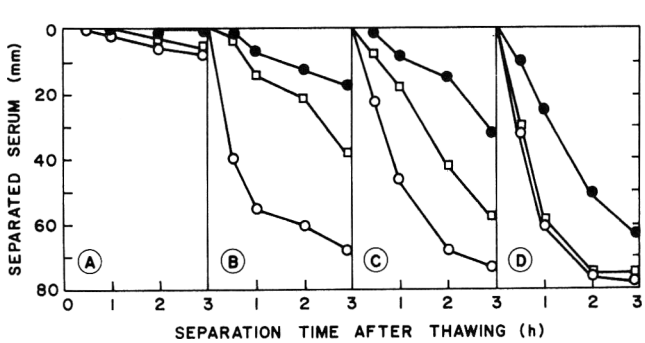

Fig. 10—Separation of clear serum from suspension of frozen natural Valencia orange juice after thawing. (A—D) after frozen storage of 0, 30, 60 and 90 days, respectively; ● — ● liquid nitrogen freezing; □ — □ blast freezing; ○ — ○ chamber freezing.

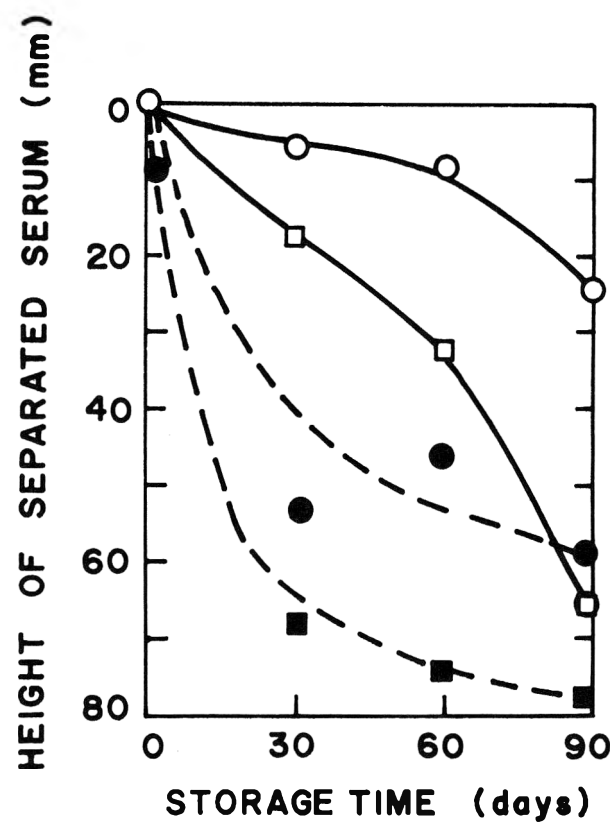

Fig. 11—Effect of primary freezing rate on separation of clear serum from suspension of frozen thawed natural Valencia orange juice (1 hr ○ — ○ and 3 hr □ — □ after thawing of liquid nitrogen frozen juice; 1 hr ● - - - ● and 3 hr ■ - - - ■ after thawing of chamber freezing frozen juice.)

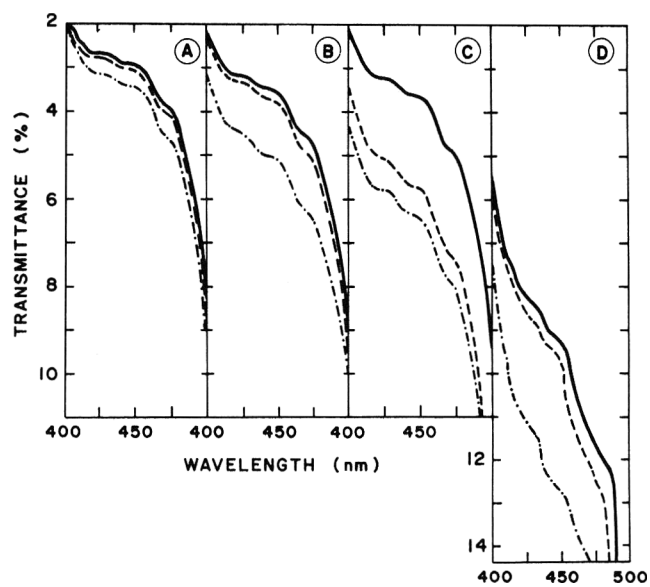

Fig. 12—Spectrum of thawed juice (obtained through opaque screen) after different primary freezing rates and frozen storage. (A—D) after frozen storage of 0, 30, 60 and 90 days respectively. — nitrogen freezing; - - - - blast freezing; · · · · · chamber freezing.

Partial Characterization of a Crude Enzyme Extract from the Freshwater Prawn, *Macrobrachium rosenbergii*

E. S. BARANOWSKI, W. K. NIP, and J. H. MOY

ABSTRACT

The activity of a crude enzyme preparation extracted from hepatopancreas of the freshwater prawn, *Macrobrachium rosenbergii*, was assayed for collagenolytic, trypsinolytic, α-chymotrypsinolytic, and pepsinolytic activities against collagen, lyophilized prawn tissue, and artificial substrates. At optimum pH for each activity, the enzyme preparation had collagenolytic activitiy, slight trypsinolytic and α-chymotrypsinolytic activities; and no pepsinolytic activity. Of the commercial enzymes tested, only collagenase significantly degraded lyophilized prawn tissue. These results suggest that the prawn enzyme preparation may contain a collagenolytic portion which might affect the texture of the prawn.

INTRODUCTION

PRODUCTION of the freshwater prawn, *Macrobrachium rosenbergii*, has been designated as a high priority by Hawaii's aquaculture development plan (DPED, 1978). However, the shelf life of prawns held at ice-chilled and refrigerator temperatures is only 2-3 days (Hanson and Goodwin, 1977), too short a time to allow competitive marketing as a nonfrozen seafood from the islands. After this period tissues begin to breakdown resulting in a mushy texture after cooking (DPED, 1978; Hanson and Goodwin, 1977; Miyajima and Cobb, 1977). This is most pronounced in the first section of the abdomen (tail) of the prawn, the section adjacent to the hepatopancreas. Such autolysis of muscle adjacent to the hepatopancreas has also been demonstrated in the brown shrimp, *Penaeus aztecus* (Lightner, 1973). Thus it is suspected that a release of digestive enzymes from this organ occurs upon death of the prawn, and that these enzymes migrate into the abdomen and catalyze degradation. Digestive enzymes have been isolated from prawn hepatopancreases (Lee et al., 1980) as well as from similar species (Eisen et al., 1973; Eisen and Jeffrey, 1969); these enzymes were found to exhibit collagenolytic, trypsinolytic, α-chymotrypsinolytic, pepsinolytic, amylytic, and lipolytic activities. It has not, however, been determined what effect these enzymes may have on the freshwater prawn tissue itself.

The objective of this work was to investigate the nature of the prawn hepatopancreatic proteases, and to determine the action of suspected enzymes on collagen, a major structural component of prawn tissue, and on prawn tissue itself. It is hoped that a better understanding of this degradative process will lead to the development of methods to control it, and thus allow a more economic means for exporting high quality prawns from Hawaii.

MATERIALS & METHODS

Extraction of enzyme preparation

Live freshwater prawns (*Macrobrachium rosenbergii*), grown at Kahuku, Hawaii, were obtained from a local market. The prawns were purged in running tap water overnight at room temperature, and dispatched by placement into an ice-water slurry. The remainder of the extraction procedure was done at 3°C, by methods based on those of Eisen and Jeffrey (1969). About 50g of hepatopancreases were removed from about one kg of prawns of mixed sexes. The hepatopancreases were blended with three volumes of cold 0.05M Tris-HCl, pH 7.4, containing 5.0 mM $CaCl_2$, for 5 min at low speed. This homogenate was centrifuged at 2000 x *g* for 30 min at 0°C, and the supernatant filtered through glass wool. The residue was re-extracted as before, combined with the original supernate, centrifuged at 12,000 x *g* for 60 min at 0°C and filtered through a 0.45 μm sterile filter. Protein content of the crude extract was determined by use of the Bio-Rad dye-binding protein assay (Bio-Rad Laboratories, Richmond, CA). The crude extract was stored under nitrogen at −20°C.

Analysis of enzyme activities

The activity of the crude prawn enzyme preparation was evaluated in comparison to the activities of commercial pepsin (Sigma No. P-7012 Sigma Chemical Co., St. Louis, MO), trypsin (Sigma No. T-1005), and α-chymotrypsin (Sigma No. C-3142) on their artificial substrates (N-acetyl-L-phenylalanyl- L-3, 5-di-iodotyrosine, benzoylarginine ethyl ester, and benzoyl-L- tyrosine ethyl ester), respectively, all from Sigma. Procedures used were based upon standard enzyme assays (Decker, 1977; Rick and Fritch, 1974; Wirnt, 1965) except that the concentrations of enzymes used were altered. For the pepsin assay, pepsin was 9 μg/mL, and crude prawn enzyme preparation 8 μg/mL. The α-chymotrypsin assay used α-chymotrypsin at 4 μg/mL and crude prawn enzyme preparation at 60 μg/mL, while the trypsin assay used trypsin at 3 μg/mL and crude prawn enzyme preparation at 60 μg/mL. Samples were replicated 12 times. Specific activities were calculated and those for the crude enzyme preparation compared to those of the purchased enzymes.

The activities of commercial trypsin, α-chymotrypsin, and collagenase (Sigma No. C-0773) against insoluble bovine collagen (Sigma Chemical Co., St. Louis, MO) and lyophilized raw prawn tissue pre-sterilized by propylene oxide were measured by endpoint assays based on methods for collagenase (Decker, 1977) with minor modifications. Incubation times were lengthened to 15 hr for all enzymes and the concentrations of trypsin and α-chymotrypsin were increased to 0.04 mg/mL. Freeze-dried prawn tissue and bovine collagen were used as substrate at a final concentration of 5 mg/mL. Enzyme action was measured as a function of release of hydroxyproline, using the method of Woessner (1961). Samples were tested in triplicate, with each test repeated four times. For comparative purposes, activities were expressed as mg hydroxyproline produced per μg enzyme, in 15 hr.

The action of the crude prawn enzyme preparation on bovine collagen and sterilized lyophilized prawn tissues was compared to that of commercial collagenase by use of the assay detailed in the previous paragraph, but with incubation times shortened to 6 hr. Protein concentrations of crude prawn enzyme preparation ranged between 4 and 200 μg/mL. Enzymatic action was measured and compared as above.

Data were statistically analyzed by the Analysis of Variance and Duncan's Multiple Range Test as detailed by Steel and Torrie (1960).

RESULTS

THE EXTRACTION of the crude enzyme preparation from prawn hepatopancreases produced a solution of 9.3 mg protein per mL. Yield was approximately 38 mg protein per g of hepatopancreas.

The comparison of the activity of the crude prawn enzyme preparation to the activities of commercial pepsin,

Authors Baranowski, Nip and Moy are affiliated with the Dept. of Food Science & Human Nutrition, Univ. of Hawaii at Manoa, 1920 Edmondson Road, Honolulu, HI 96822.

trypsin, and α-chymotrypsin showed that the prawn enzyme preparation had little or no activity. The prawn enzyme preparation was found, at the concentration used, to have no pepsinolytic activity against the artificial substrate; this concentration could not be increased due to interference from the high absorbance of the enzyme preparation. The trypsinolytic and α-chymotrypsinolytic activities from the prawn enzyme preparation were found to be a factor of 10^2 less than those of the purchased trypsin and α-chymotrypsin. Statistical analyses showed that these differences were highly significant ($p \leq 0.005$).

Commercial trypsin and α-chymotrypsin were found to produce 0.09 mg hydroxyproline per μg enzyme (in 15 hr) when insoluble bovine collagen was the substrate, and 0.18 and 0.19 mg hydroxyproline (in 15 hr), respectively, per μg enzyme with freeze-dried prawn tissue as the substrate. Commercial collagenase produced 30 mg and 11 mg hydroxyproline per μg enzyme in 15 hr when acting on bovine collagen and freeze-dried prawn tissue, respectively. Statistical analyses (see Table 1) showed that the actions of commercial trypsin and α-chymotrypsin on bovine collagen or lyophilized prawn tissue were not significantly different ($p \leq 0.01$), nor were these treatments significantly different from the controls of bovine collagen alone or lyophilized prawn tissue alone. The action of purchased collagenase on lyophilized prawn tissue was significantly different from (about 80 times greater than) all other treatments. This was also true for the action of commercial collagenase on collagen (about 200 times greater than the trypsin and α-chymotrypsin activities).

The crude prawn enzyme preparation showed activity on both lyophilized prawn tissue and bovine collagen, producing 8.5×10^{-5} and 1.5×10^{-4} mg hydroxyproline per μg enzyme preparation in 6 hr, respectively. Commercial collagenase produced 1.3×10^{-4} and 5.8×10^{-4} mg hydroxyproline per μg enzyme in 6 hr when acting on lyophilized prawn tissue and bovine collagen, respectively. Thus, the commercial collagenase was about four times more active than the crude prawn enzyme preparation with bovine collagen as substrate, and 1.5 times more active with prawn tissue as the substrate. Statistical analyses (see Table 2) demonstrated that the actions of the crude prawn enzyme preparation on lyophilized prawn tissue or bovine collagen were not significantly different ($p \leq 0.05$), and that neither of these treatments was significantly different from that of commercial collagenase acting on lyophilized prawn tissue. All three of these treatments were significantly different fom their controls (bovine collagen or lyophilized prawn tissue alone). The activity of commercial collagenase on bovine collagen was found to be significantly different from all other treatments.

Table 1—Statistical analysis of trypsin (Tryp), α-chymotrypsin (Chymo), and collagenase (C.ase) activities on collagen and prawn tissue[a]

Kinds of enzyme and substrate	Activity, μg hydroxyproline per μg enzyme in 15 hr
Control	0 (a)
Chymo + collagen	0.09 (a)
Tryp + collagen	0.09 (a)
Tryp + prawn tissue	0.18 (a)
Chymo + prawn tissue	0.19 (a)
C.ase + prawn tissue	11 (b)
C.ase + collagen	30 (c)

[a] Values with the same letter are not significantly different ($p \leq 0.01$)

Table 2—Statistical analysis of crude prawn enzyme (C.P.E.) and collagenase (C.ase) activities on collagen and prawn tissue[a]

Kinds of enzyme and substrate	Activity, μg hydroxyproline per μg enzyme in 6 hr
Control	0 (a)
C.P.E. + prawn tissue	8.5×10^{-5} (b)
C.ase + prawn tissue	1.3×10^{-4} (b)
C.P.E. + collagen	1.5×10^{-4} (b)
C.ase + collagen	5.8×10^{-4} (c)

[a] Values with the same letter are not significantly different ($p \leq 0.05$)

DISCUSSION

BY COMPARING THE ACTIVITY of the crude prawn enzyme preparation to the activities of several commercial enzymes, information has been gained on the nature of the crude enzyme preparation. The investigation of the action of commercial pepsin, trypsin, α-chymotrypsin and collagenase on lyphilized prawn tissue has produced information on which enzymes may or may not be responsible for degradation of prawn tails during refrigerated or ice-chilled storage.

The crude prawn enzyme preparation was shown to display no pepsinolytic activity; this is as expected since the pH optimum for pepsin is around 2.0, while the pH of prawn tissue is about 7.0. Testing was done, however, since there is a report of a digestive enzyme from prawns which has pepsinolytic activity (Lee et al., 1980). Only slight trypsinolytic and α-chymotrypsinolytic activities were found. Collagenolytic activity was demonstrated, at a level not significantly different from the action of commercial collagenase on lyophilized prawn tissue. Also, the action of the crude prawn enzyme preparation against bovine collagen was not significantly different from that against lyophilized prawn tissue. These facts suggest that the crude prawn enzyme preparation may consist of a collagenase alone with smaller amounts of other enzymes, or may be an enzyme molecule containing multiple activities. This would be in agreement with results of similar work done on other shellfish species (Eisen et al., 1973).

Of the commercial enzymes tested, only collagenase was found to significantly degrade lyophilized prawn tissue. Although the collagenolytic activity noted here occurred at 37°C, it is expected that similar results would be obtained over a longer period of time in refrigerated and ice-chilled prawns, thus supporting the existence of a collagenase or an enzyme with collagenolytic activity which is responsible for the mushiness problem. The amount of hydroxyproline resulting from incubation of collagenase with collagen was much greater than that from incubation of collagenase with lyophilized prawn tissue. This is an expected result since the lyophilized prawn tissue is composed of other components as well as of collagen.

Further investigation of the crude prawn enzyme preparation will include separation into fractions by use of column chromatography, and isoelectrofocusing of the fractions. Isolation and characterization of the collagenolytic fraction will provide additional information about its action in the breakdown of prawn tissue. It is hoped that this might then enable the development of methods to control the degradation process during ice-chilled or refrigerated storage of prawns.

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Thermally Induced Gelation of Selected Comminuted Muscle Systems—Rheological Changes during Processing, Final Strengths and Microstructure

J. G. MONTEJANO, D. D. HAMANN, and T. C. LANIER

ABSTRACT

Sols were prepared from comminuted fish (surimi), beef, pork and turkey muscles. Continuous evaluation of changes in structural rigidity and energy damping during heating of the sols from 3° to 95°C was performed in a nondestructive, temperature-controlled Thermal Scanning Rigidity Monitor. Surimi presented major rigidity transitions at 40°, 48° and 65°C; beef at 43°, 56° and 69°C; pork at 44°, 53° and 69°C; and turkey at 50°, 53° and 79°C. All materials exhibited rapid decrease in energy damping (i.e. increase in elasticity) over a short temperature span. Failure testing of gels indicated differences in strength and deformability. SEM micrographs provided an insight into structural features of the gels.

INTRODUCTION

THERMAL PROCESSING of many food protein systems leads to coagulation and formation of gel-type products. The structural characteristics of heat-induced gels are typical of solid-like materials in that they possess various degrees of rigidity, elasticity, plasticity and brittleness (Kinsella, 1976). Thus, one important functional property of gel-forming proteins is to act as texture building components in a variety of heat-processed foods. This property has stimulated an increasing interest in the development of new fabricated or restructured products utilizing several protein sources, such as edible meat not suitable for marketing as fillets or portions.

The gelling properties of myofibrillar muscle proteins, either singly or in combination, have been extensively studied (Fukazawa et al., 1961; Deng et al., 1976; Ishioroshi et al., 1979; Yasui et al., 1980, 1982; Acton et al., 1981; Samejima et al., 1982). Muscle sols, however, are very complex such that separation and study of their components may not necessarily predict the characteristics of the whole system during thermal gelation.

In order to select those processing conditions which take full advantage of the structure forming properties of various proteins, information is needed on the continuous changes occurring during the sol-gel transformation. There have been very few studies employing techniques capable of continuously monitoring changes in rheological characteristics during the transition of a sol into a gel (Acton et al., 1983, Schweid and Toledo, 1981). Most of the thermal gelation studies have used discrete samples heated for differing times and temperatures to monitor the progress of gelation, an approach which may not be as sensitive in detecting transitions as continuous evaluation of the same sample during heating (Montejano et al., 1983). The development of the Thermal Scanning Rigidity Monitor or TSRM (Montejano et al., 1983), which employs nondestructive measurements, makes it possible to continuously monitor changes in the rheological properties of muscle sols during thermal processing.

It is also important to gain an understanding of how rheological changes during processing ultimately determine the textural properties of the finished product. The behavior of a food material under gross deformational and destructive forces is of prime significance in food texture determination (Jowitt, 1979). Separation of the force and deformation components in failure testing of foods and presentation of these parameters in terms of fundamental units of physics is very important for a complete rheological characterization of a food material (Hamann, 1983).

The objectives of this study were to: (1) monitor and compare changes in shear rigidity and mechanical energy damping measured non-destructively and continuously during heating of minced and salted surimi, turkey, beef and pork sols; (2) determine fundamental stress-strain conditions at structural failure of the heat-induced gels; and (3) examine the microstructure of each gel preparation by scanning electron microscopy (SEM).

MATERIALS & METHODS

Materials

Frozen surimi (Miyauchi et al., 1973) prepared from a combination of Atlantic croaker (*Micropogon undulatus*) and sand trout (*Cynoscian arenarious*) containing 4% sorbitol and 4% sucrose as cryoprotective agents was obtained from Nichibei Fisheries, Inc., Bayou LaBatre, AL. The surimi was cut in blocks of 700g, packed into plastic bags of low oxygen permeability, vacuum sealed and stored at −20°C until needed. Turkey thigh meat was obtained from House of Raeford, Raeford, NC. The hand-skinned and deboned meat was coarsely ground, packed in portions of 700g in plastic bags of low oxygen permeability, vacuum sealed and stored at −20°C. Wholesale beef loin muscles (Longissimus dorsi, psoas major and gluteus medius) and wholesale pork loin muscles (Longissimus dorsi, psoas major and gluteus medius) were obtained from a commercial processing plant in Raleigh, NC. The muscles were manually deboned and trimmed of excess fat and connective tissue. They were, then, cut into small cubes, thoroughly mixed, packed in 700-g portions in plastic bags of low oxygen permeability, vacuum sealed and stored at −20°C. The frozen storage did not exceed 1 month. All chemicals used were analytical reagent grade.

Proximate analysis

Official methods of the AOAC (1975) were used to determine moisture, fat (petroleum ether extractables) ash and protein by Kjeldahl nitrogen determination (% protein = %N X 6.25). All analyses were performed in triplicate for each material.

Pastes for rigidity and energy damping scanning

Minced pastes were prepared from the four materials in a similar manner. The frozen bagged material was allowed to temper for 1 hr at room temperature (20°C). For each replicate, 700g material, sodium chloride (2.5% of the total paste), and ice (to obtain a 5:1 moisture:protein ratio) were chopped for 5 min under vacuum, to prevent incorporation of air bubbles, in a chilled (0°C) specially constructed laboratory food processor (Lanier et al., 1982). The final temperature of the batter did not exceed 2.0°C. The raw minced paste was divided into two portions, packed into plastic bags and vacuum sealed. One portion was transferred in ice to the testing room and used for TSRM evaluations. The other portion was used to prepare heat-induced gels for mechanical failure evaluations (see below). Three replicates were prepared from each material.

Authors Montejano, Hamann, and Lanier are affiliated with the Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27695-7624.

Preparation of heat-induced gels

Heat-induced gels were prepared from the vacuum packed minced pastes in a similar way for all the materials. The raw paste was packed into stainless steel tubes (I.D. = 1.86 cm, L = 17.5 cm) with screwable brass bases and rubber stoppers on top. Some of the rubber stoppers had small holes to permit the insertion of the thin probe of a digital thermometer to monitor temperature in the center of the gels. The interior wall of the tubes was coated with a thin film of PAM (Boyle-Midway, Inc., New York, NY). The tubes were submerged vertically in a water bath at 95°C for 15 min. The final temperature in the center of the gels reached 90°C. For surimi, two additional heat treatments were used: 1) the tubes were submerged vertically in a water bath at 40°C for 1 hr. ("set" gels) and, 2) tubes were submerged in a water bath at 40°C for 1 hr. followed by heating in the water bath at 95°C for 15 min. ("set and cooked" gels). After heat treatment the tubes were immediately cooled in ice-water, the cooked gels carefully removed with a plunger and the ends trimmed. Specimens were cut randomly and shaped into the geometry required for mechanical failure testing. From each one of the replicates of each material at least 10 specimens were obtained for each type of test (i.e. a total of 30 specimens/material/test).

Evaluation of shear rigidity and energy damping during cooking

Continuous evaluation of the modulus of rigidity (G) and energy loss (energy damping) during thermal processing of the raw minced pastes was performed in the Thermal Scanning Rigidity Monitor (TSRM) developed by Montejano et al. (1983). Fig. 1 shows the TSRM which consisted of a U-shaped jacketed chamber with a hollow plaque held in the center by upper and lower removable guides. The chamber was mounted on the base of a Model 1122 Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA). The center plaque was fitted to a 50 kg compression load cell mounted on the crosshead of the machine. The TSRM was connected to a heating-cooling water bath (Haake type KT33, Berlin, West Germany). The rate of heating was manually controlled at 0.5°C/min from 3°-95°C using a variable autotransformer (Powerstat type 3PN116B, Superior Electric Co., Bristol, CT) connected to a supplemental heating coil immersed in the water bath. Water circulated through the chamber and center plaque to insure uniform temperature conditions in the pastes during cooking. Before each test, the TSRM was chilled by running cold water (approx. 1°C) for 20 min. The raw minced paste was loaded on both sides of the center plaque between the upper and lower guides. Care was taken to insure that the paste was well packed and no visible air bubbles were present. The dimensions of each sample are shown in Fig. 1. The guides were removed and all the exposed surfaces were covered with a high-vacuum grease (Dow Corning Corp. Midland, MI) to prevent dehydration and skin formation. Visual observation indicated no appreciable diffusion of the grease into the pastes. The yield strength of all the pastes was large enough to prevent any flow due to gravity effects.

At 2-min intervals a cyclic force (from the upward-downward cyclic motion of the Instron's crosshead at 1mm/min) of 1.96N to −1.96N was applied to the samples for two cycles producing a small variable cyclic deformation in the samples. The peak to peak deformation was recorded on the Instron chart and ranged from 1.5 X 10^{-3}m to 2.5 X 10^{-4}m. Shear strain values were below 2.5%. The shear strain rate was 1.3 X 10^{-3} s^{-1}. Samples were free to expand laterally during cooking to avoid any influence due to swelling. Expansion of the samples during cooking, however, was small so the dimensions of the specimens were assumed to be constant. Internal temperature was monitored by thin thermocouple probes inserted into each specimen.

Fig. 2 shows a diagram of the deformation produced in one of the specimens by the cyclic force. Since two samples were tested simultaneously, half of the applied tangential force (F) was imposed on each specimen to produce a displacement D. The shear modulus or modulus of rigidity (G) was calculated as the ratio of maximum shear stress (τ) to maximum shear strain (γ) or:

$$G = \frac{\tau}{\gamma} = \frac{(F/2)/Lw}{D/t} = \frac{Ft}{2LDw}$$

During the cyclic forces where L, w, and t are the specimen length, width, and thickness, respectively, some energy was lost as heat producing a loop in the force-deformation graph as shown in Fig. 3. The relative amount of hysteresis loss, or energy damping, is a measure of elasticity in a material. The closer the material is to being

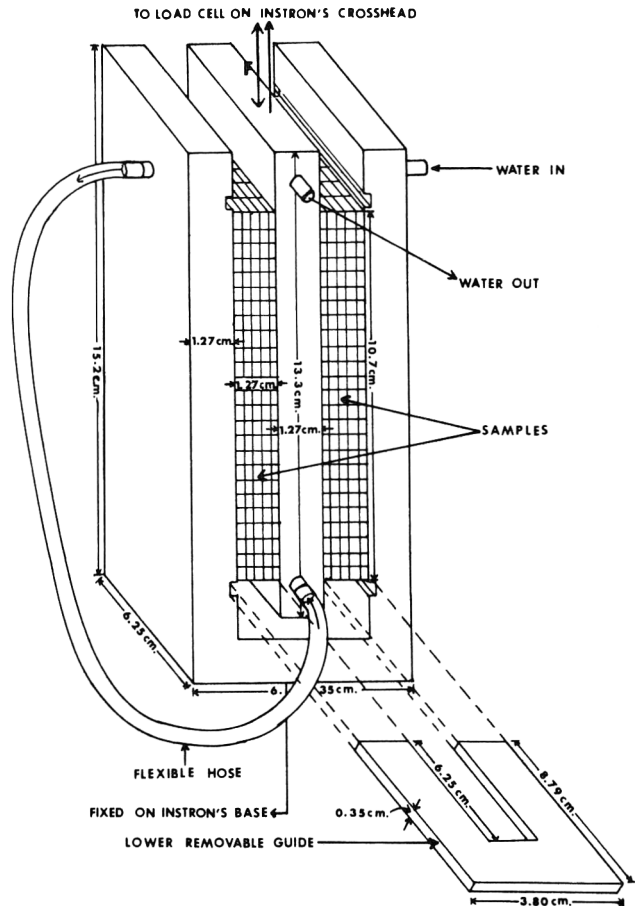

Fig. 1—Thermal Scanning Rigidity Monitor.

perfectly elastic, the smaller the energy damping is (Mohsenin 1970). Thus apparent energy loss (E.L.) was calculated as follows:

$$\% \text{ E.L.} = \frac{\text{hysteresis area}}{\text{work of deformation}} = \frac{A_2}{A_1 + A_2} \times 100$$

The term apparent was used because F = 0 at the end of the cycle would not necessarily correspond to zero deformation in the material and additional creep recovery could occur (Ferry, 1970).

Differential scanning calorimetry

Differntial scanning calorimetry (DSC) was performed on beef pastes as described by Wu et al. (1984a). Accurately weighed samples were evaluated using a Perkin-Elmer DSC II (The Perkin-Elmer Corporation, Norwalk, CT). The scanning temperature was 15-95°C with a heating rate of 10°C/min. An instrument sensitivity of 0.2 mcal/sec was selected.

Axial compression

Compression tests were carried out as described in another paper (Montejano et al., 1984b). Briefly, cylindrical specimens with a diameter = 0.98 cm and length = 1.0 cm were used for Poisson's ratio evaluation and failure testing. The flat faces of the specimens were positioned in contact with oil-lubricated horizontal plates mounted on the Instron machine. Crosshead speed was 10 cm/min and shear strain rate was 0.148 s^{-1}. Specimens were tested at room temperature. Poisson's ratio, true shear stress at failure, true shear strain at failure and initial shear modulus were calculated using appropriate equations (Hamann, 1983).

Torsion tests

Torsion failure tests were performed as described by Montejano et al. (1983). Briefly, cylindrical specimens were reduced to dumbbell shapes with length = 2.87 cm, end diameters = 1.86 cm and minimum diameter = 1.0 cm. The torsion apparatus was mounted on the Instron machine. The right end of the horizontally positioned specimen was fixed while the left end was rotated about

its cylindrical axis. Crosshead speed was 20 cm/min. (twisting speed = 1.675 rpm) and shear strain rate was 0.113 s^{-1}. Specimens were tested at room temperature (20°C). Shear stress at failure, true shear strain at failure and initial and failure shear moduli were calculated using the equations given by Hamann (1983).

Microstructure

The gel structure of each material was examined using scanning electron microscopy (SEM) according to the procedure of Montejano et al. (1984b). Specimens were observed with a Jeol JSM-T200 Scanning Electron Microscope at an acceleration voltage of 15 kV. A large number of micrographs were taken at different magnifications to select truly representative micrographs.

Statistical analysis

Data were analyzed using analyses of variance (ANOVA) and comparison of treatment differences by Scheffe's S statistic (John, 1971).

RESULTS

Proximate analysis

Proximate analyses on a wet weight basis for the raw muscles and their pastes used in the present study are given in Table 1. Surimi had the lowest protein and fat contents. The 8% carbohydrate content was due to the presence of the cryoprotective agents sucrose and sorbitol. Turkey muscles contained slightly lower protein and fat contents than the red meat muscles (beef and pork) which had very similar compositions. Values listed in Table 1 for the raw muscles were used as the basis for calculating the amount of water (ice) to be added to each material to obtain a sol paste with a 5:1 moisture:protein ratio. The proximate analysis of the adjusted pastes (Table 1) indicated that a more uniform comparative composition was attained for all the materials.

Rigidity scanning

Plots of modulus of rigidity (G) versus internal temperature for surimi, turkey, beef and pork pastes are shown in Fig. 4. Each point in the curves is the average of 3 measurements. Surimi had the highest initial rigidity value (9.3 kPa). During heating surimi presented an early increase in rigidity reaching a peak value of 18 kPa at around 40°C. In the 48-65°C temperature range there was a rapid linear increase in rigidity indicating that a stiff protein matrix was being developed. At 65°C a maximum rigidity value of 27.9 kPa was observed followed by a moderate decrease at 81°C, reaching a final value of 23.8 kPa at 93°C.

Minced beef and pork muscles presented very similar modulus of rigidity-temperature relationships (Fig. 4). Pork had the lowest initial rigidity value (2.8 kPa) while beef had an initial value twice as large (5.6 kPa). Beef and pork both showed transitions at around 13° and 25°C and a rigidity peak was observed at near 44°C. Starting at 56° and 53°C, respectively, beef and pork showed rapid linear increases in rigidity up to about 69°C where another transition in the thermogram was observed. At 69°C the rigidity value of pork (13.2 kPa) was larger than that of beef (12 kPa). Above 69°C the rigidity of beef remained approximately constant while pork still showed some increase in rigidity reaching a final value of 14 kPa at 93°C.

Turkey paste had an initial rigidity value of 4 kPa which was intermediate between those of pork and beef (Fig. 4). A slight increase in rigidity was observed during heating of turkey up to 35°C. Above 35°C a steeper increase in rigidity occurred reaching a peak value of 7.8 kPa at 50°C.

Table 1—Proximate analysis (Wet weight basis)

Material	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Raw muscle					
Surimi	73.85	15.35	1.81	0.90	8.00
Turkey	73.20	21.02	4.91	0.81	–
Beef	70.11	22.20	5.83	0.80	–
Pork	69.73	22.61	5.78	0.91	–
Paste					
Surimi	74.66	14.93	1.75	0.88	7.78
Turkey	79.68	15.93	3.71	0.63	–
Beef	78.78	15.76	4.11	0.57	–
Pork	78.85	15.77	3.98	0.63	–

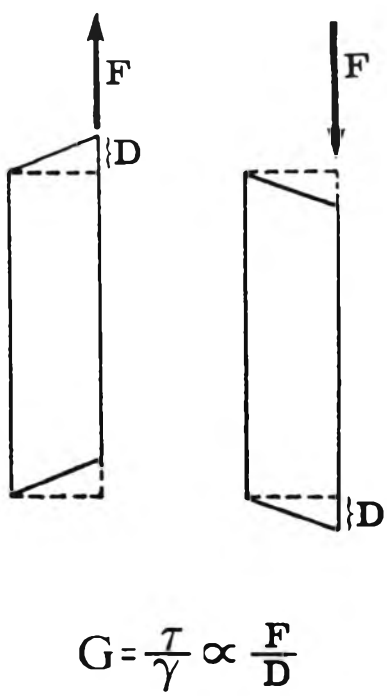

Fig. 2—Diagram of the deformation caused by the application of a cyclic force on a specimen mounted in the left side of the TSRM.

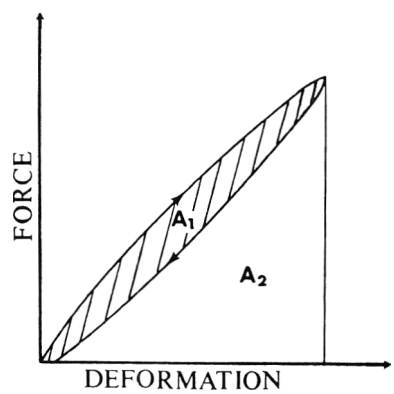

Fig. 3—Hysteresis loop produced by a viscoelastic material during a loading-unloading cycle. A_1 = hysteresis. $A_1 + A_2$ = total work of deformation.

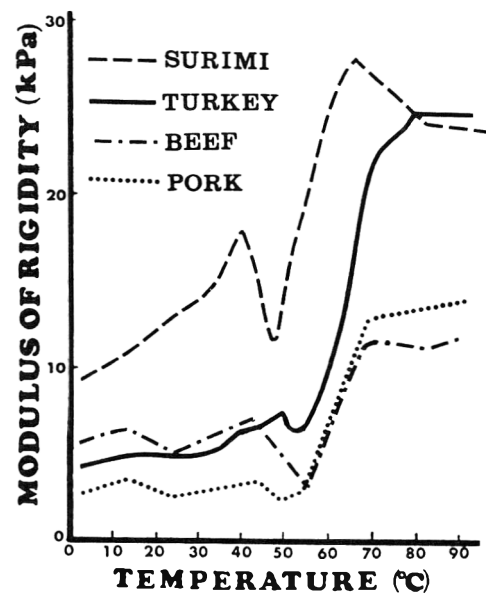

Fig. 4—Shear rigidity thermograms for surimi, turkey, beef and pork muscles. Heating rate = 0.5°C/min.

A rapid and large increase in rigidity was observed between 53° and 79°C. Constant rigidity values were observed above 79°C. Turkey had a high final rigidity value (24.7kPa) which was very close to that of surimi and almost twice as large as those for beef and pork.

Energy loss scanning

Apparent energy loss (energy damping) versus internal temperature plots for surimi, beef, pork and turkey pastes are shown in Fig. 5. Surimi had the lowest initial energy loss value (26%). During heating surimi showed small variations in energy loss with transitions occurring at 17°, 22°, and 36°C. Starting at 36°C there was a rapid decrease in energy loss which became steeper between 42° and 55°C reaching a 10% value, indicating that an elastic structure was being obtained. Above 55°C energy damping continued to decrease although at a slower rate reaching a 4% value at 80°C.

Beef had an initial energy loss value of 34.5% (Fig. 5). During the early stages of heating small variations occurred until an increase in energy damping was observed between 44° and 51°C followed by a very rapid and linear decrease in energy damping between 51° and 59°C. A final energy loss value of 6.5% was obtained at 90°C which indicated that a fairly elastic product (gel) was developed. This final energy loss value, however was the largest of the 4 materials (i.e. lowest degree of elasticity).

The highest initial energy loss value (50%) was observed in pork (Fig. 5). Approximately uniform values were observed during initial heating followed by two transitions at 21° and 44°C. Between 44° and 57°C pork presented a rapid and linear decrease in energy loss to 13.5%. A final energy loss value of 5.1% was observed in pork at 90°C. This value was very similar to that of surimi.

Minced turkey muscle had an initial energy loss value of 39% (Fig. 5). Turkey showed a fluctuating but general decrease in energy damping to about 50°C where a 28% value was reached. Between 50° and 52°C a very rapid linear decrease in energy damping to 16% was observed. From 52-68°C energy damping still decreased significantly, but at a slightly lower rate, to 3%. Further heating produced a final value of 0.8% at 90°C. This energy damping value was the lowest of all the materials indicating that the final turkey gel had a nearly-perfect elastic character.

Poisson's ratio

An average Poisson's ratio of 0.49 was obtained for each of the heat-induced gel systems tested in the present study. For a homogeneous isotropic material, as the gas volume decreases compressibility decreases and Poisson's ratio approaches a maximum of 0.5. Poisson's ratio values were used to calculate true shear stress and true shear strain at failure values from the axial compression failure tests (Hamann, 1983).

Mechanical failure

Mean values of the mechanical failure parameters from torsion and uniaxial compression tests are given in Table 2 for turkey, pork and beef. In uniaxial compression tests of surimi gels, failure did not occur even at axial strain levels of 97%. For beef and pork gels, true shear stress, true shear strain and shear modulus were not statistically different when comparing torsion and uniaxial compression failure tests. An average of 61% axial strain at failure was observed in these materials. Turkey gels, on the other hand, showed disagreement between torsion and uniaxial compression failure parameters. These gels required an average of 86% axial strain at failure which caused gross shape deformation before failure occurred.

Failure occurred in all the gel preparations tested in torsion. Table 3 lists the mean values of the torsional failure parameters. The three surimi gel preparations showed the highest values of shear stress and true shear strain at failure. These parameters were significantly different among the surimi preparations. Surimi gel B ("set gel") had a lower structural strength, as revealed by shear stress at failure, but a higher degree of ductility, as revealed by true shear strain at failure, when compared with surimi gel A ("cooked gel"). Surimi gel C ("set and cooked gel") had a shear stress at failure almost double of that of surimi gel A. The true shear strain at failure was about 22% higher when compared to gel A and about 8% higher when compared to gel B. Among the nonfish gels, turkey gels required significantly larger shear stress and true shear strain at failure than beef and pork gels (Table 3). Turkey gels, therefore, were stronger and more deformable than those from beef and pork but less than those from the surimi preparations. Beef and pork gels were not significantly different when comparing shear stresses and true shear strains at failure. Visual observation of the fractured surfaces of all the gel specimens showed the presence of a failure plane at

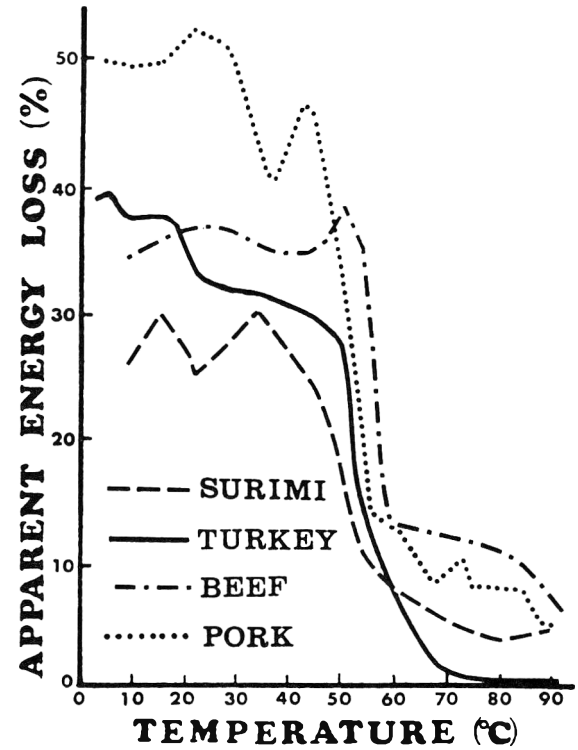

Fig. 5—Energy loss thermograms for surimi, turkey, beef and pork muscles. Heating rate = 0.5°C/min.

Table 2—Mean values of mechanical failure parameters from torsion and compression tests[a]

Material	True shear stress		True shear strain	
	Torsion (kPa)	Comp. (kPa)	Torsion	Comp.
Turkey gels	41.33[c] (5.84)[b]	50.04[d] (6.01)	1.32[c] (0.11)	1.72[d] (0.12)
Beef gels	26.80[c] (2.74)	25.41[c] (2.07)	1.18[c] (0.08)	1.22[c] (0.06)
Pork gels	27.41[c] (3.13)	25.79[c] (2.60)	1.18[c] (0.04)	1.21[c] (0.13)

[a] Means of 30 observations.
[b] Values given in parenthesis represent standard deviations.
[c,d] The same letter superscripts within each material indicate that the associated numbers in the two columns are not significantly different ($P > 0.05$).

approximately a 45° angle from the long axis of the specimen indicating a tension failure mode. A similar failure mode has been previously observed in other heat-induced protein gels (Montejano et al., 1983, 1984a, b). In torsion testing tension, compression and shear stresses of equal magnitude are created so the materials failed due to the stress for which they had the least strength (Diehl et al., 1979; Montejano et al., 1983).

Microstructure

Scanning electron micrographs of the three surimi gel preparations are shown in Fig. 6, at a magnification of 7500X. Surimi gel A ("cooked"), an opaque gel, had a smooth and very dense network structure. Some pores are observed on the gel surface in which water was probably enclosed. Surimi gel B ("set"), a translucent gel, had a very coarse structure formed by large dense aggregates. Some open pockets seem to be present in the structure, which may indicate that water was held in large amounts in localized regions. Surimi gel C ("set and cooked"), an opaque gel, exhibited a combination of features from gels A and B.

The microstructures of turkey and pork gels are shown in Fig. 7, at a magnification of 5000X. Turkey gels had a lacy network structure. A large number of pores were quite evenly distributed on the gel surface. The microstructure presented by beef and pork gels was nearly identical. A sponge-like network was observed in these gels. The presence of large pores in these gels suggested that they were able to entrap water.

Table 3—Mean values of torsional failure parameters for heat-induced muscle gels[f]

Material	Shear stress (kPa)	True shear strain
Surimi gel A (90°C/15 min)	76.61[a] (10.07)[g]	2.28[a] (0.34)
Surimi gel B (40°C/1 hr)	66.99[b] (4.37)	2.57[b] (0.21)
Surimi gel C (40°C/1 hr–90°C/15 min)	163.98[c] (23.02)	2.78[c] (0.17)
Turkey gels	41.33[d] (5.84)	1.32[d] (0.11)
Beef gels	26.80[e] (2.74)	1.18[e] (0.08)
Pork gels	27.41[e] (3.13)	1.18[e] (0.04)

[a,b,c,d,e] Means within a column with different superscripts are significantly different ($P < 0.05$).
[f] Means of 30 observations.
[g] Values given in parenthesis represent standard deviations.

DISCUSSION

Thermorheological scanning of surimi paste

Previous studies in our laboratory (Montejano et al., 1983) have indicated that surimi prepared from Alaska pollock (*Theragra chalcogramma*) presented a rigidity peak ("initial setting") in the 10-15°C range followed by a decrease between 15° and 26°C. This transition, however, was not observed in croaker-trout surimi (Fig. 4).

The rigidity peak observed at 40°C for surimi (Fig. 4) is thought to be related to the phenomenon termed "setting" or "suwari" commonly observed in a variety of minced fish muscles held between 30° and 40°C (Shimuzu et al., 1981; Montejano et al., 1983). Present evidence indicates that the increase in rigidity observed in the "setting" stage (this stage produces a translucent gel) is

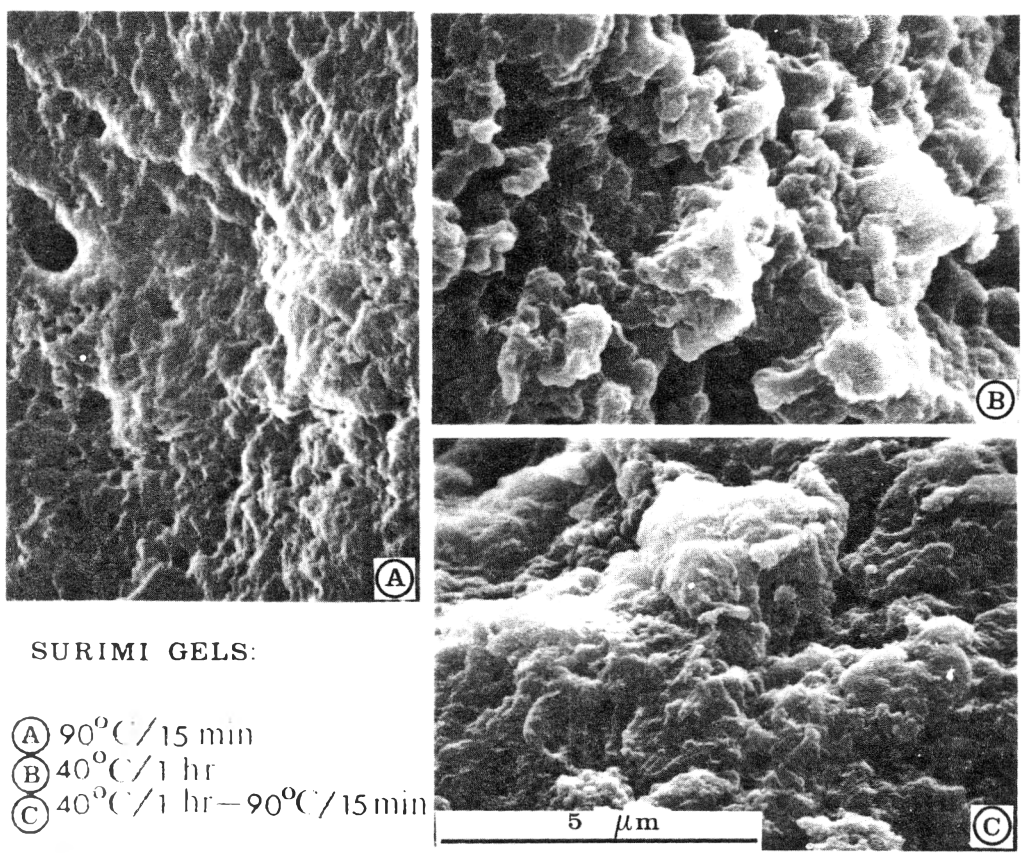

Fig. 6—SEM micrographs of cryofractured surfaces of three surimi gel preparations. Magnification = 7500X.

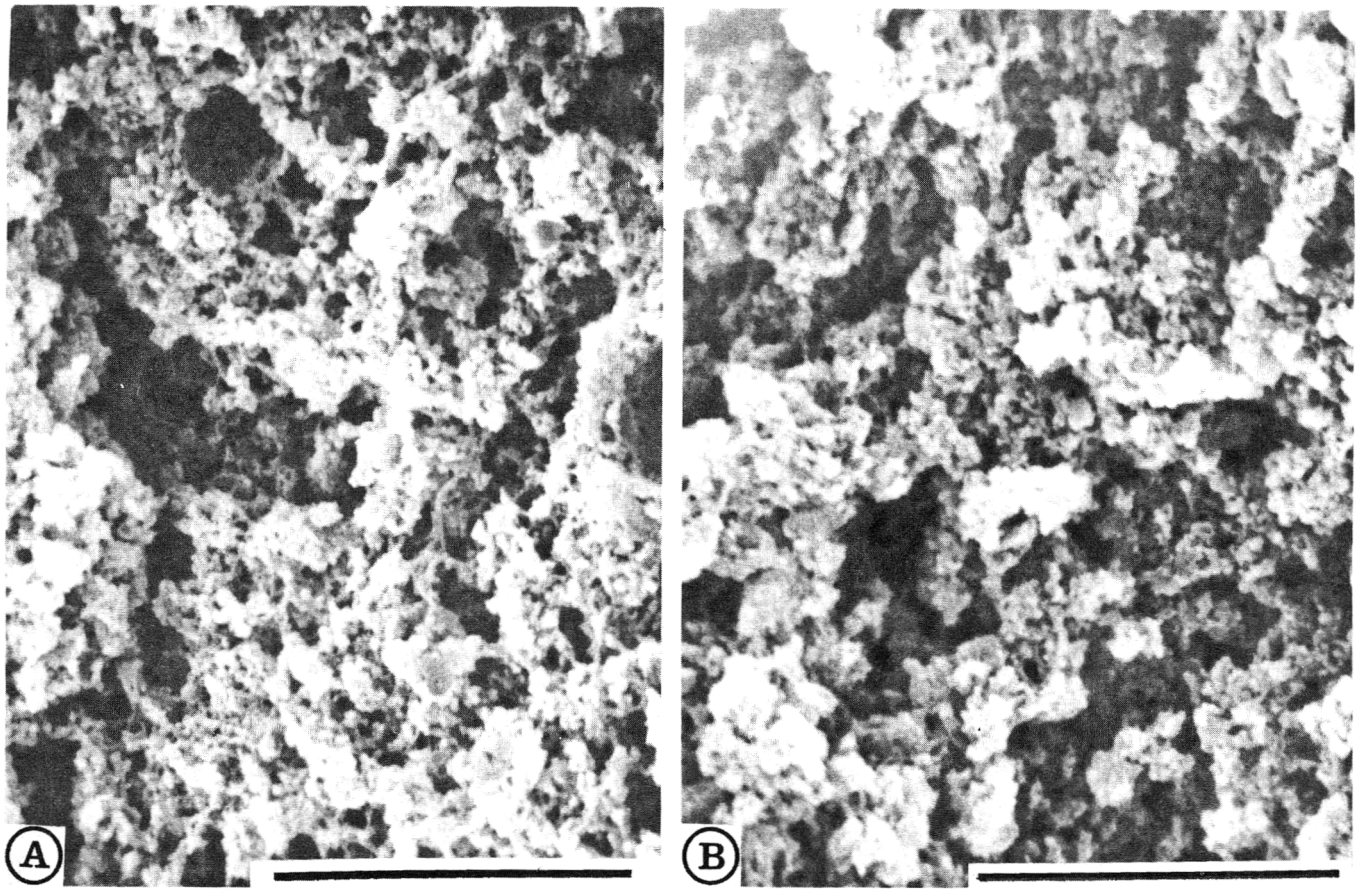

Fig. 7—SEM micrographs of cryofractured surfaces of turkey (A) and pork (B) gels. Magnification = 5000X. Bar length = 5 μ.

mainly due to hydrophobic interactions between neighboring proteins with little contribution of hydrogen or other polar bonds (Niwa 1975; Niwa et al., 1981c, d, e; 1982). Stronger and more elastic gels have been reported to be obtained by holding minced fish pastes at around 40°C prior to normal heat treatment (Watanabe et al., 1974). Inspection of Table 3 shows that surimi gel C ("set and cooked") showed a large increase in strength and a moderate increase in deformability as compared to the directly-cooked gel (A). A two-step "setting-cooking" procedure is commonly used in Japan to obtain fish gel products with good textural properties (Tanikawa, 1971). However "setting" near 40°C appears to be a distinct phenomenon from the type of gelation or coagulation which occurs at much higher temperatures. (Lanier et al., 1980; Liu et al., 1982).

The low initial energy loss value of surimi (Fig. 5) indicated that the raw paste had limited viscous character and was able to spring back to near its original shape upon the removal of a deformatory force with a modest amount of stored energy lost as heat. An ideal elastic material (complete shape recovery upon removal of a deformatory force) would have 0% energy damping. It is interesting to notice that the major decrease in energy damping in surimi (Fig. 5) began near the temperature of the initial rigidity peak (35°C). Apparently this rapid increase in elasticity of the structure depends on protein interactions responsible for the "setting" phenomenon. The rapid increase in rigidity and elasticity starting at 48°C for surimi (Fig. 4) suggests the formation of a stable structure due to continuous protein aggregation. Comparison of Fig. 4 and 5 indicates that after a fairly elastic structure has been obtained at about 55°C, further temperature increases have only a small effect on energy damping but a large effect on the rigidity of the system. A similar behavior has been observed in some globular protein systems (Montejano et al., 1984b) and Alaska pollock surimi (Montejano et al., 1983).

The decrease in rigidity observed above 65°C may be related to the phenomenon termed "modori" or "returning" by Japanese researchers. (Shimuzu et al., 1962; Tagaki, 1973; Niwa et al., 1980). The mechanism involved in "mordori" is not well understood, however; it is believed that proteolytic enzymes, termed alkaline proteases, are directly responsible for texture degradation (Lanier et al., 1981; Makinodan and Ikeda, 1971). The detrimental texture change in fish pastes held at around 60°C is irreversible and good gel texture cannot be brought back even by cooking at higher temperatures (Tanikawa, 1971). It is also possible that the decrease in rigidity was due to further protein transitions or just a temperature effect after completion of protein aggregation. In Alaska pollock surimi Montejano et al. (1983) observed that after a small plateau in the 60-64°C range there was a continuous increase in rigidity at the high temperature end.

The three distinct thermal transitions in rigidity observed in croaker-trout surimi at 40°, 48° and 65°C have also been observed in surimi from other fish species. Wu et al. (1984a) reported three endotherm peaks at 40°, 54° and 69°C from DSC studies of Atlantic croaker surimi (3% NaCl) at a heating rate of 10°C per min. Apart from species differences, the occurrence of the last two transitions at higher temperatures than in the present study may be attributed to heating rate effects. It has been reported that as heating rate increases, transitions in a system are observed at higher temperatures (Montejano et al., 1983; Donovan et al., 1975). Burgarella (1983) found transitions in rigidity of Atlantic croaker surimi at 15°, 40°, 50° and 60°C. Endotherm peaks at 36° and 50°C were observed in DSC studies of actomyosin from Atlantic croaker (3% NaCl) by Wu et al. (1984b).

Thermal scanning rigidity studies carried out by Wu et al. (1984b) on actomyosin pastes (3% NaCl) prepared from Atlantic croaker showed transition temperatures at 38°, 46° and 60°C. Montejano et al. (1983) observed transitions in rigidity of Alaska pollock surimi at 13°, 37°, 47° and 64°C.

Thermorheological scanning of beef, pork and turkey pastes

The initial small increase in rigidity observed for beef, pork and turkey (Fig. 4) may be attributed to protein-protein interactions which have been reported to occur at low temperatures (Acton et al., 1981; Deng et al., 1976). The subsequent decrease in rigidity observed in beef and pork between 13° and 25°C may be due to melting of fat which, although present in small amounts in the pastes (Table 1), could have contributed to increase the viscous character of the systems. Fig. 5 shows that the energy damping of beef and pork increased over this temperature range indicating an increase in viscous character. Townsend et al. (1968) using differential thermal analysis found that fats from both beef and pork trimmings had two melting zones. Fat from beef melted in the 3-14°C and 18-30°C ranges while fat from pork melted in the 8-14°C and 18-30°C ranges. The authors also measured the fat melting profile within a sausage batter containing beef and pork fat using fat release as a measure of stability. A significant increase in fat release was reported above 18°C. Quinn et al. (1980) reported endotherm peaks at 13° and 18.5°C for beef and pork fat, respectively.

In turkey, less sharp transitions in rigidity and energy loss (Fig. 4 and 5) were observed in the 13-25°C range as compared to beef and pork. This possibly indicates that fat melting was limited and gradual. The fat content of turkey paste was slightly lower than that of beef and pork (Table 1). Additionally, it is known that turkey fat contains a larger amount of unsaturated fatty acids than the fat of beef and pork and, therefore, has a different melting profile.

The rigidity peaks presented by beef at 43°C, pork at 44°C and turkey at 50°C (Fig. 4) resemble the initial peak observed in surimi at 40°C. The relative intensity of this peak, however, was greater in surimi. Several researchers have reported transitions in muscle proteins occurring around these temperatures (Ishioroshi et al., 1979; Yasui et al., 1980; Burke et al., 1973, Samejima et al., 1981). Beef, pork and poultry meats are classified as "non-setting" materials by several Japanese researchers (Niwa et al., 1980; Shimuzu et al., 1981); that is, muscle sols from these species do not form elastic gels at 40°C. However, evaluation of "setting" is done using batch heating and cooling of samples at predetermined temperatures for given periods of time. Continuous rigidity scanning has been observed to be more sensitive in detecting transitions in protein systems than batch techniques (Montejano et al., 1983). If a low temperature "setting" of beef, pork, and turkey does occur it is different from that of fish in at least two aspects: (1) it occurs at higher temperatures, and (2) it produces a smaller increase in rigidity. These two differences seem to be reduced by treatment of the proteins with arylating regeants (Niwa et al., 1981a, b, e). Further studies are needed to confirm that a type of "setting" does occur in beef, pork and turkey and to determine whether a two-step cooking at selected temperatures would produce a textural effect similar to that observed in fish gel products.

The increase in energy loss observed in beef and pork in the 44-51°C and 38-44°C ranges, respectively, (Fig. 5) may be due to melting of the higher melting point fractions of fat increasing the viscosity of the system. These temperature ranges closely agree with the reported melting ranges of 41-48°C for beef fat and 38-47°C for pork fat (Acton et al., 1983). It is interesting to observe that, similar to the case of surimi, a very rapid decrease in energy loss was observed in beef, pork, and turkey (Fig. 5) immediately following the occurrence of rigidity peaks (Fig. 4). These observations further suggest that the development of elasticity in the structures depended on the protein interactions that produced the rigidity peaks. As with surimi, the decrease in rigidity after the peaks for beef, pork and turkey did not seem to influence the rate of decrease in energy loss.

The uniform and rapid increase in rigidity for beef, pork and turkey that started at 56°, 53° and 52.5°C, respectively, indicated the formation of stable, stiff, and elastic matrix structures typical of heat-induced protein gels. Several researchers have reported transitions in the tail portion of the myosin molecule to occur at around 55°C which contribute to network formation (Burke et al., 1973; Samejima et al., 1981; Wright et al., 1977; Martens et al., 1982). Maximum development of rigidity and strength in myosin gels has been observed in the 55-70°C range (Ishioroshi et al., 1979).

As previously observed for surimi; beef, pork and turkey presented a rapid decrease in energy loss (Fig. 5) over a shorter temperature span than for the rapid increase in rigidity (Fig. 4) suggesting a certain independence between the development of elasticity and rigidity in the structures. The final transition in rigidity in beef and pork at 68°C and turkey at 69°C may be in part attributed to destruction of interstitial collagen which is known to occur at approximately these temperatures (Davey and Gilbert, 1974; Davey and Niederer, 1977; Stabursvik and Martens, 1980; Hamm, 1970).

In order to confirm the presence of the transitions observed in the rigidity scanning evaluation, the beef was also subjected to differential scanning calorimetric (DSC) studies. The DSC studies were conducted on a beef paste prepared as described for the scanning rigidity tests. Fig. 8 shows the thermogram obtained for beef at a heating rate of 10°C/min. Three peaks were evident with maxima at 43°, 58° and 71°C in very close agreement with the rigidity transitions observed at 43°, 56° and 69°C (Fig. 4). Some researchers have reported DSC thermograms of whole beef muscles with endotherm peaks at slightly higher temperatures than the ones given here (Wright et al., 1977; Stabursvik and Martens, 1980; Karmas and DiMarco, 1970). The difference, however, may be attributed to the addition of NaCl to the beef paste used in the present study. Salt has been found to decrease the heat stability of muscle proteins

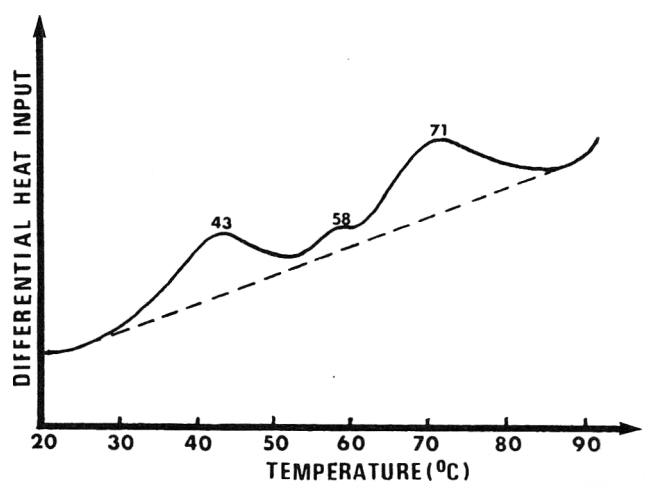

Fig. 8—DSC thermogram for comminuted beef muscles. Heating rate = 10°C/min.

shifting their transitions to lower temperatures (Wu et al., 1984a; Burgarella, 1983; Quinn et al., 1980).

Poisson's ratio and mechanical failure tests

The average Poisson's ratio value obtained for each of the heat-induced gel systems tested in the present study indicated that they behaved as nearly incompressible materials. It must be noted that a difference in compressibility exists between cooked intact muscles and cooked minced muscles, particularly if incorporation of air is prevented during mincing. Segars et al. (1977) obtained Poisson's ratio values ranging from 0.20-0.26 in various intact meat muscles cooked to an internal temperature of 63°C, indicating that they were moderately compressible.

The fundamental failure tests performed on the heat-induced gels from surimi (3 cooking methods), beef, pork and turkey should contribute to understanding their behavior under large force and deformation levels in contrast to the small levels applied by the TSRM. Mechanical failure characteristics of foods are well related to sensory texture during mastication (Wood, 1979; Hamann, 1983; Montejano and Hamann, 1984) and may provide a valuable insight into the properties of the final structure of each material.

The agreement between true compression and torsion failure parameters observed in beef and pork gels (Table 2) indicated that both tests yielded fundamental measurements nearly independent of specimen geometry or test mode. This finding plus previous observations of agreement between true compression and torsion failure parameters (Montejano et al., 1983; 1984a, b; Diehl et al., 1979) led us to believe that torsional testing yielded fundamental measurements for all the gels. In this test the specimens were subjected to a condition of pure shear that produced change in shape with negligible change in volume (Hamann, 1983). Additionally, it was possible to apply the large strain required for failure of all samples. For all the gel preparations, approximately linear torque vs. angle of twist curves were observed indicating that they exhibited nearly elastic behavior to failure. This behavior has been previously observed in other highly deformable heat-induced protein gels (Montejano et al., 1983; 1984a, b). The elastic response of these gels was also expected because final energy damping was very low in the TSRM testing (Fig. 5).

Ultimate strength in torsion and microstructure of surimi gels

Comparison of the mechanical failure parameters from torsional testing of the three surimi preparations (Table 3) indicated large differences in gel texture due to cooking method. Cooking after "setting" (gel C) produced gels with a large increase in strength and a moderate increase in deformability as compared to direct cooking (gel A). The "set" gel (gel B) presented only an increase in deformability but not in strength as compared to the cooked gel. Apparently the "setting" stage is mainly responsible for the increase in ductility (deformability) of the gels and further cooking for the increase in strength. The attractive forces involved in the "setting" stage produce relatively weak but deformable structures.

Inspection of the microstructure of the three gel preparations (Fig. 6) revealed that the cooked gel (gel A) had a random network structure. The "set" gel (Gel B) had large conglomerates. These conglomerates seem less interconnected than the smaller structural particles of gel A, which may explain the lower strength of the structure. The structure of gel B also presented larger pockets than the other structures in which water may have been held. This feature may be associated with the translucence and deformability of the gel. Montejano et al. (1984b) found large pockets in the microstructures of succinylated and oleic acid treated egg white gels, both of which were translucent and deformable materials. The "set" and cooked gel (gel C) showed some of the conglomerates formed during "setting"; however, the protein matrix was more highly interconnected than gel B and had smaller interstitial spaces. This structure presented the highest strength and deformability of the three gels.

Ultimate strength in torsion and microstructure of beef, pork and turkey gels

The greater structural strength and deformability at failure presented by turkey gels as compared to beef and pork gels (Table 3) was not surprising since the final rigidity and energy loss values from TSRM testing were also larger and smaller, respectively, in turkey than in beef and pork (Fig. 5). Although fat content was slightly lower for turkey than for beef and pork (Table 1), this does not seem to be a sufficient reason for the increased structural stability of the turkey gels. It is most likely that the overall protein quality of turkey was responsible for producing stronger and more ductile gels than those from beef or pork. It must be recalled that moisture:protein ratio was adjusted in all the materials to be the same value (5:1) to minimize the effect of compositional differences. Sensory evaluation by a trained texture profile panel (Civille and Szczesniak, 1973) of the above gels carried out by Montejano and Hamann (1984) showed that turkey gels also had significantly greater sensory firmness and springiness than either beef or pork gels.

Some structural differences were also evident between SEM micrographs of turkey and either beef or pork gels (Fig. 7). A structure with a higher content of strands and particle interconnections was evident in turkey gels as compared to beef or pork gels. However, much denser structures were observed in cooked surimi gels than in gels of turkey, beef or pork. These surimi gels also exhibited the largest values of shear stress and true shear strain at failure. Apparently, a relationship exists between the structural features of gels as shown by SEM and their textural (mechanical failure) characteristics.

Comparison of rigidity (shear) moduli

In an accompanying paper (Montejano et al., 1984b) it was observed that the initial rigidity modulus (G), determined by the initial slope of the force-deformation curve, from compressive testing of native and modified egg white gels was not a good indicator of their rheological differences. The initial and final (failure) rigidity moduli from torsional testing of these gels, however, ranked them in the same order as the final G (at 90°C) from TSRM testing. Higher G values were obtained, for all the gels, from torsional testing as compared to TSRM evaluations. To further study the relationships among the G values from different tests, the results obtained for the materials tested in the present study are listed in Table 4. The initial G from compression testing ranked the materials in a different order than any of the other measurements. The results show a particularly large discrepancy for surimi gels. The lower initial G values obtained from compression testing as compared to torsional testing indicated relatively larger deformations upon the application of small initial stresses. Calzada and Peleg (1978) noted that much of the information obtained from compressive force-deformation curves is lost when using normal ("apparent") coordinates (i.e. without correction for the change in geometry during testing) since, usually, all the apparent curves have a similar general shape. Thus, the use of the initial slope from compression testing as a measurement of rheological characteristics of heat-induced protein gels is questionable (Hermansson, 1982; Montejano et al., 1984b).

Table 4—Comparison of shear moduli from different tests for heat-induced muscle gels[d]

Material	Initial G[e] (Compression) kPa	Initial G[e] (Torsion) kPa	G at Failure[f] (Torsion) kPa	G @90°C from TSRM kPa
Surimi gel A (90°C/15 min.)	15.12[a] (4.27)	28.00[b] (4.12)	27.75[b] (4.93)	23.81[c] (1.13)
Turkey gels	26.86[a] (3.26)	29.94[b] (3.55)	29.12[b] (2.74)	24.61[c] (1.32)
Beef gels	16.30[a] (2.14)	20.88[b] (2.64)	21.40[b] (1.92)	12.20[c] (2.13)
Pork gels	16.65[a] (1.54)	21.83[b] (3.03)	21.86[b] (2.32)	14.11[c] (1.01)

[a,b,c] The same letter superscripts within each material indicated that the associated numbers are not significantly different ($P > 0.05$).
[d] Values given in parenthesis represent standard deviations.
[e] From initial slope of force-deformation curves.
[f] Ratio of shear stress/shear strain at failure.

The G values from torsional testing ranked the materials in the same order as the G values from TSRM testing. The latter, however, were always smaller. Since torsional testing evaluated cooked gels at room temperature while the TSRM evaluated gels at 90°C, it is very likely that hydrogen bonding formation during cooling contributed to the increased G values of the torsion specimens. Niwa et al. (1982) observed that the modulus of rigidity of actomyosin gels increased significantly upon cooling. Electrostatic capacity measurements, conducted by these authors, indicated that hydrogen bonding was involved in the increase in G during cooling. It is interesting to notice, however, that beef and pork gels showed relatively larger differences between G at failure from torsion and G at 90°C from TSRM testing as compared to surimi and turkey gels.

SUMMARY & CONCLUSIONS

CONTINUOUS EVALUATION of shear rigidity and energy damping during thermal processing of surimi, beef, pork and turkey pastes proved to be very sensitive in detecting structural transitions during the sol-gel transformation. The modulus of rigidity-temperature and energy loss-temperature plots for all the materials showed the same general patterns but major transitions occurred at lower temperatures in surimi as compared to pork, beef and turkey. The first rigidity peak attributed to changes in the proteins was larger in surimi than in any of the other materials, probably as a result of the type and/or degree of protein-protein interaction. The protein transitions responsible for these initial rigidity peaks in the materials seemed to be related directly to the development of the elastic characteristics of the structures. Further protein transitions at higher temperatures were required for full development of the rigidity in these materials. A steep decrease in energy loss (i.e. increase in elasticity) observed in all the pastes, occurred over a shorter temperture span than the most pronounced increase in rigidity. Thus, it appears that once an elastic protein matrix is formed, further thermal processing has only a small effect upon elasticity but a large effect on the increase of the rigidity (stiffness) of the structure. Limited DSC studies of beef pastes showed transitions at about the same temperatures as the scanning rigidity evaluation.

Fundamental structural failure testing of heat-induced gels prepared from surimi (three different heat treatments), beef, pork and turkey showed that uniaxial compression failure tests and torsion failure tests yielded results that were not significantly different when compression specimen deformation did not exceed about 80% of the original length. Torsion failure tests presented the advantage of producing failure in all the gel preparations. Torque-angle of twist curves were approximately linear to failure for all the gels tested in this study indicating that they were nearly elastic materials. Torsion failure parameters provided a quantification of both the structural strength (shear stress) and deformability (true shear strain) of the gel preparations. The initial slope of the force-deformation curve from compressive testing is not a reliable indicator of gel strength. Modulus of rigidity values from TSRM (90°C) and torsional testing (room temperature) ranked the gels in the same order of stiffness. SEM micrographs showed large differences in denseness and number of junctions among the heat-set gels evaluated in the present study.

It is expected that the methodology presented here can also be employed in the quantification of thermally-induced gelation of a large variety of materials and to study the influence of various factors, such as pH, salts, ions, protein concentration, etc. Present studies in our laboratory include the effect of starches and additive chemical compounds on fish protein systems (Wu, 1984), chemical modification of globular proteins (Montejano et al., 1984b), variable salt content in fish protein systems (Burgarella, 1983), rigidity activation energies during thermal processing of various muscle protein sols (Montejano et al., 1984c), and effects of various types of phosphates on thermally induced gelation and final strength of fish muscle protein gels (Montejano, 1984).

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Semiautomated Method for the Analysis of PSP Toxins in Shellfish

J. JONAS-DAVIES, J. J. SULLIVAN, L. L. KENTALA, J. LISTON, W. T. IWAOKA, and L. WU

ABSTRACT

The method uses an autoanalyzer continuous flow reaction system to oxidize toxin in standard acid extracts of shellfish, prepared for mouse bioassay, to derivatives which are detected by fluorescence. Oxidation is by periodic acid under alkaline (NH_4OH) conditions and is followed by acidification by acetic acid. Concentrations of 10 μg/100g toxin and above can be measured with good reproducibility and accuracy: coefficient of variation was 9.5% for samples with 60 μg/100g or greater. Correlation with the mouse bioassay was 0.82 for 204 samples (toxin from 0–2000 μg/100g). The method is proposed to screen shellfish samples for PSP toxins with only samples falling into the range 60–250 μg/100g being subject to the more tedious and expensive mouse bioassay.

INTRODUCTION

PARALYTIC SHELLFISH POISONING (PSP) is a persistent problem in many of the northern coastal areas of the world involving the sporadic occurrence of highly toxic bivalve molluscs during the summer months. Shellfish become toxic after feeding on dinoflagellates of the genus *Gonyaulax*, the primary producers of a number of potent neurotoxins (Sullivan and Iwaoka, 1983). Programs have been established in many areas affected by PSP to monitor shellfish toxicity levels and the limit at which a shellfish bed is closed is set at 80 μg of toxin per 100g of shellfish meat. Currently, testing for the presence of the PSP toxins is performed by the standard mouse bioassay (AOAC, 1980). Due to problems inherent in this assay, including time, expense and a variation of ±20% (Shimizu and Ragelis, 1979), there is a need for more rapid and accurate assay techniques.

A number of alternative assay procedures have been developed with the most sensitive being the Bates and Rapoport (1975) fluorometric technique. This assay is based on alkaline oxidation of the PSP toxins to fluorescent derivatives. The method has been adapted as a detection method in liquid column chromatography by Buckley et al. (1978) and in high performance liquid chromatography (HPLC) by Sullivan and Iwaoka (1983). A comparative study was made between the HPLC and mouse bioassay methods (Sullivan et al., 1983a) and, based on this, improvements to the system have been reported (Sullivan and Wekell, 1984).

Following the development of the HPLC assay for the PSP toxins, it became apparent that the reaction manifold apparatus used in this technique was well suited for an autoanalyzer system. This can be accomplished by replacing the HPLC pump, injector and column with an auto sampler. The fluorescence response in the autoanalyzer system is therefore representative of the total toxin content in the sample, regardless of which toxins are present. An automated system has several advantages over a bioassay, including speed, economy and reproducibility. The object of the work described in this paper was the development of the autoanalyzer system. The system was tested with a study conducted to compare the results of the autoanalyzer with the standard mouse bioassay for shellfish samples representing a variety of species.

Authors Jonas-Davies, Liston, and Wu are affiliated with the Institute for Food Science and Technology, College of Ocean and Fishery Sciences, Univ. of Washington, Seattle, WA 98195. Author Sullivan is affiliated with the Seafood Products Research Center, U.S. Food & Drug Administration, Seattle, WA 98174. Author Kentala is affiliated with the Dept. of Social & Health Services, State of Washington, Seattle, WA 98104. Author Iwaoka, formerly with the Univ. of Washington, is now with the State Dept. of Health, Laboratories Branch, Honolulu, HI 96183.

MATERIALS & METHODS

Sample preparation and mouse bioassays

Shellfish samples were collected at various locations around Puget Sound, Washington, chilled on ice, transported to the laboratory and extracted according to the methods described in the AOAC (1980). A portion of the supernate was tested for toxicity by the mouse bioassay using 19–21g Swiss Webster mice. A second aliquot of the supernate was stored frozen for periods ranging from 1 wk to 1 month and tested for total toxin content by the autoanalyzer method.

To prepare samples for the autoanalyzer, the shellfish acid homogenates were centrifuged for 5 min at 3000 rpm, a portion of the supernate mixed 1:1 with absolute methanol, chilled overnight and centrifuged for 5 min at 3000 rpm. Aliquots of the supernates were diluted 1:100 with 0.01N acetic acid, filtered through 0.45 μm filters (Millipore, Type HA) and placed in autoanalyzer cups for analysis.

Apparatus and reagents

The autoanalyzer is based on an apparatus developed for the HPLC technique described by Sullivan and Iwaoka (1983) (flow diagram is illustrated in Fig. 1). The major equipment consists of a Sampler II and proportioning pump (Technicon Industrial Systems, Tarrytown, NY), spectrofluorometer (MPF-2A, Perkin Elmer Corp., Norwalk, CT) and recorder (Omniscribe, Fisher Scientific). The reaction manifold consists of standard Technicon Autoanalyzer pump tubing with fabricated pulse dampeners, tees and a reaction coil. The pulse dampeners are 1 mL glass vials to which 1/16 inch stainless steel (ss) tubing had been fastened with epoxy cement. Low volume mixing tees were constructed from a 1/2 inch thick plexiglass block and ss tubing: the reaction coil consists of a Teflon capillary tube (0.56 mm x 4.9 m) submerged in a 75°C water bath.

Reagents for the autoanalyzer were prepared in distilled water and consisted of periodic acid (5.0 mM), ammonium hydroxide (1.5 M) and acetic acid (5.5M). The fluorescence of the oxidized toxins was measured with the spectrofluorometer set at excitation and emission wavelengths of 340 and 388 nm, respectively. Slit widths (20–30 nm) and detector settings were used which maximized sensitivity. Peak heights were measured and used in all calculations.

Standards and calculations

A group of five saxitoxin (STX) standards (2.5–40 ng/g) (diluted from STX obtained from the Food & Drug Administration, Cincinnati, OH) was run with each set of samples to prepare calibration curves. During a run, samples were analyzed in duplicate both with and without the periodic acid reagent (replaced with water). Substituting water for the periodic acid allowed determination of background fluorescence (measured as peak height) which was subtracted from the total average fluorescence (measured as peak height) for the runs with periodic acid. The toxin concentration of the extract (as STX) was multiplied by necessary dilution factors and final

toxicity was expressed in μg of saxitoxin per 100g of shellfish tissue.

Results from the mouse bioassay and autoanalyzer techniques were compared using Pearson's correlation coefficient and linear regression (Zar, 1974). The data were assigned to three groups; low (<61 μg/100g), medium (61-250 μg/100g) and high (>250 μg/100g), according to toxicities obtained from the autoanalyzer. The mean of the differences for each group was calculated by:

$$\frac{\Sigma(AA-MBA)}{n}$$

where (AA−MBA) is the difference between the autoanalyzer results (AA) and the mouse bioassay results (MBA) for a single sample and (n) is the total number of samples for that group.

To determine the precision of the autoanalyzer method, analysis of a random group of samples was repeated four times over a period of 1 wk. The samples were kept frozen until use and each day thawed and prepared as previously described. There was no indication of change in toxin content over time. The results of these repetitions were used to calculate an average coefficient of variation (the standard deviation as a percent of the mean).

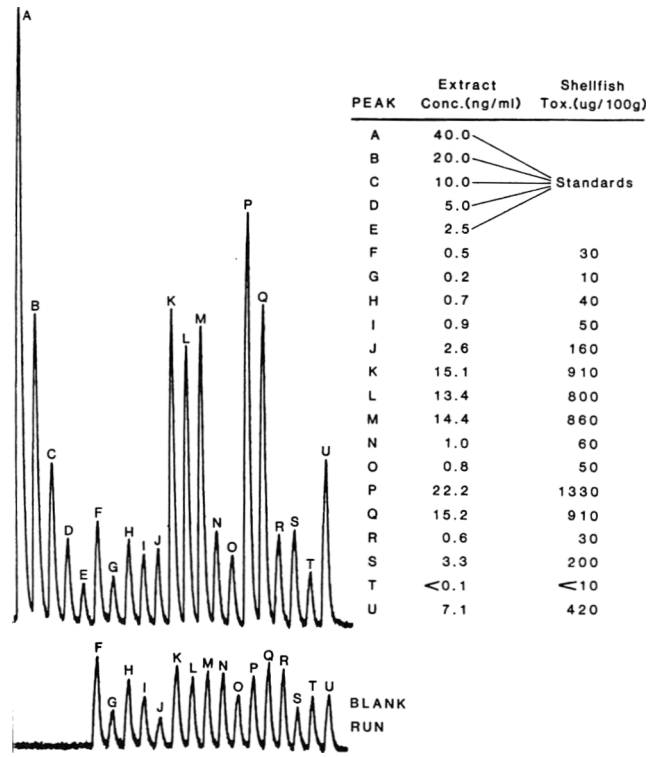
The relative intensity of fluorescence of the individual PSP toxins was determined by preparing equimolar solutions of each toxin in 0.03N HOAC and testing each toxin solution on the autoanalyzer. Resulting peak heights were compared and assigned relative values, with saxitoxin used as the basis for comparison.

RESULTS

Autoanalyzer system

A typical chart recording for a set of standards and samples is illustrated in Fig. 2. The STX standards (2.5-40ng/g) produced a linear calibration curve and the blank run (without periodic acid) exhibited no response for the standards. The background fluorescence varied slightly from sample to sample. The noise to peak height ratio gives a lower limit of toxin determination of 10 μg/100g.

Reproducibility of the autoanalyzer method was determined by calculating the Coefficient of Variation (CV) for a repeated group of randomly chosen samples. Of the 18 samples analyzed, two were repeatedly found to be NTD (no toxin detectable – under 10 μg/100g). Five samples between 10 and 60 μg/100g yielded an average CV of 29.5%, with a range of 19.4-34.1%. Five samples fell in the moderate range of 60-250 μg/100g, and showed an average CV of 14.1%, with a range of 9.8-15.8%. For the six samples over 250 μg/100g, the average CV was 5.7% and the range was 3.8-10.2%. The mean toxicities of the samples varied from NTD to 1302 μg/100g while the standard deviations ranged from 10.2 (low samples) to 55.2 (high samples). Because samples and standards were prepared fresh before each run, these measures include all potential sources of variation (storage, preparation, handling, and equipment).



PEAK	Extract Conc.(ng/ml)	Shellfish Tox.(ug/100g)
A	40.0	
B	20.0	
C	10.0	Standards
D	5.0	
E	2.5	
F	0.5	30
G	0.2	10
H	0.7	40
I	0.9	50
J	2.6	160
K	15.1	910
L	13.4	800
M	14.4	860
N	1.0	60
O	0.8	50
P	22.2	1330
Q	15.2	910
R	0.6	30
S	3.3	200
T	<0.1	<10
U	7.1	420

Fig. 2—Chart recording of saxitoxin standards and 16 shellfish samples. Upper trace: oxidized with periodic acid; lower trace: (the blank run) same samples without periodic acid. The first column of figures lists actual concentration (as STX) in final dilution and second column is calculated toxicity of shellfish tissue.

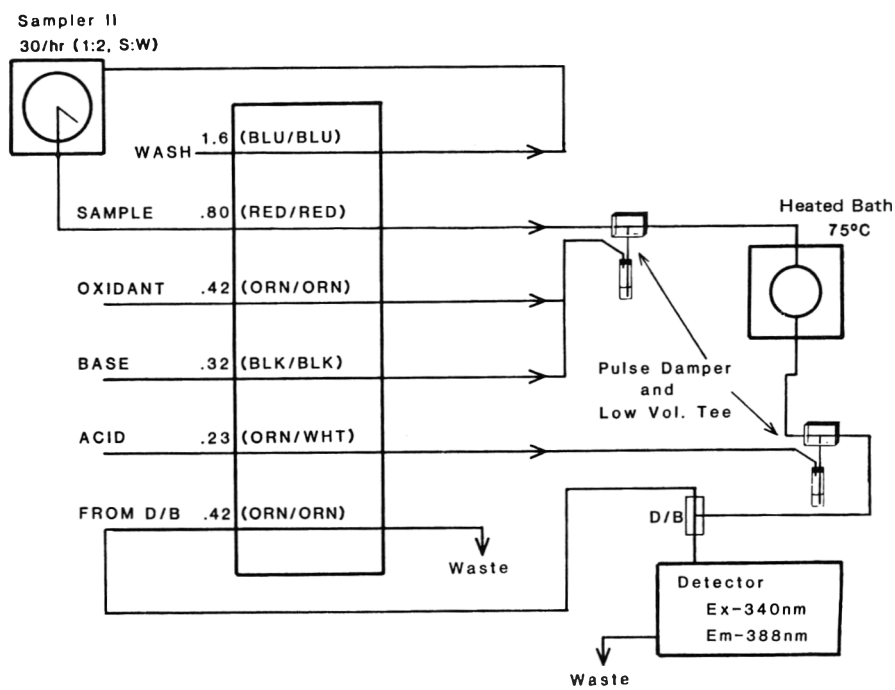


Fig. 1—Flow diagram of PSP autoanalyzer system. Flow rates are indicated, along with the corresponding color coding for Technicon autoanalyzer tubing. Details of reaction conditions are in the text (S:W-Sample:Wash ratio: D/B-Debubbler).

Comparative study

The results for the comparative study between the autoanalyzer and mouse bioassay are presented in Table 1. For purposes of statistical analysis, all values listed as NTD (no toxin detectable) for the mouse bioassay were converted to 40 μg/100g, the value considered to be the lower level of detection for the mouse bioassay. Similarly, all values from the autoanalyzer which were calculated to be below 40 μg/100g were converted to 40 μg/100g during statistical analysis. The 49 samples which were shown to be at or below 40 μg/100g by both the autoanalyzer and the mouse bioassay were omitted from Table 1, although they were included in all calculations.

In addition to Table 1, results for those samples over 40 μg/100g are presented in a scatter diagram in Fig. 3. The regression line shows a slight shift in the direction of the autoanalyzer, indicating higher than expected results from the analysis by this method. The amount and direction of the deviation of autoanalyzer results, from those of the mouse bioassay is further indicated by dividing the data into groups, based on the autoanalyzer results, and calculating the mean of the differences between the two assays (Table 2). For values under 60 μg/100g (as measured by the autoanalyzer) at the 95% confidence level there was no significant difference between the autoanalyzer and the mouse bioassay. There was a significant difference between the two methods at higher levels of toxicity and this difference increased as the toxin level increased.

Past studies have shown differences in the types of toxins present in various species of shellfish in the Puget Sound area (Sullivan, 1982). To determine whether these differing toxin profiles were affecting the comparison between the two methods, a Pearson's correlation coefficient was calculated for each individual species (Table 3). The total correlation for all samples was 0.82. When divided into species, differences in the correlation coefficient were evident, ranging from 0.77 in littleneck clams to 0.90 in butter clams.

Table 1—Toxicity of shellfish as determined by autoanalyzer (AA) and mouse bioassay (MBA) in μg STX/100g tissue

AA	MBA	AA	MBA	AA	MBA	AA	MBA
10	50	120	NTD	250	270	510	340
40	50	120	150	260	NTD	520	1200
40	50	130	80	260	130	530	440
40	50	130	40	260	450	550	230
50	NTD[a]	130	50	270	260	550	550
50	NTD	130	70	280	110	560	120
50	NTD	130	70	290	280	570	380
50	NTD	130	100	300	100	600	400
50	NTD	130	100	330	200	640	520
50	50	130	130	340	40	650	490
50	180	140	NTD	340	80	660	760
50	NTD	140	40	340	310	670	750
50	40	140	70	350	400	690	940
50	60	140	50	350	110	690	540
60	NTD	140	60	360	370	710	510
60	NTD	150	NTD	370	170	720	440
60	NTD	150	NTD	370	190	730	140
60	40	150	120	370	240	770	440
70	NTD	160	100	370	80	780	460
70	NTD	160	40	380	130	780	780
80	NTD	160	220	390	180	800	500
80	NTD	160	470	400	NTD	800	630
80	60	170	90	400	240	820	210
80	NTD	170	50	410	240	890	590
80	NTD	180	50	430	NTD	910	440
80	NTD	180	80	430	260	910	440
80	50	180	230	440	100	970	390
90	NTD	190	50	440	70	980	1050
90	NTD	190	70	450	300	1040	1530
90	40	200	120	450	440	1190	2140
90	50	200	130	450	540	1220	860
90	120	200	100	450	610	1330	970
100	40	210	260	460	210	2030	1140
100	50	220	140	460	450		
100	110	220	110	470	320		
110	80	220	130	490	430		
110	NTD	250	50	500	370		
110	60	250	260	500	400		

[a] NTD = No toxin detectable

Table 2—Mean of the differences between autoanalyzer and mouse bioassay

Group[a]	(n)	Mean	Std Dev	95% Confidence interval
Low	(71)	−0.6	17.2	−4.8 to 3.8
Medium	(54)	49.5	68.3	32.0 to 67.0
High	(73)	158.3	273.0	92.7 to 223.8
Total	(204)	69.1	177.0	44.7 to 93.4

[a] Low = <60 μg/100g; Medium = 60–250 μg/100g; High = >250 μg/100g

DISCUSSION

Autoanalyzer system

The autoanalyzer system as described here provides a rapid technique for the analysis of PSP toxins. The autoanalyzer system was developed using standard Technicon equipment with fabricated tees and reaction coil. Technicon hardware could easily be used for the tees, pulse dampeners, debubblers and fittings in this system, although it

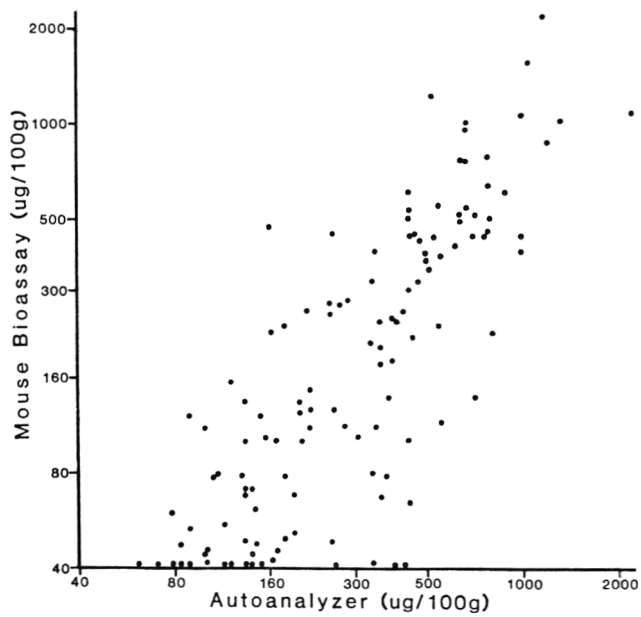


Fig. 3—Comparison of autoanalyzer and mouse bioassay results for samples over 40 μg/100g (Note log/log scale).

Table 3—Correlation of mouse bioassay and autoanalyzer results by species

Group	No. of samples	Pearson's Correlation Coefficient
Littleneck Clams	34	0.77
Mussels	64	0.78
Butter Clams	47	0.90
Oysters	29	0.87
Miscellaneous	24	0.80
Total	204	0.82

may be necessary to optimize flow rates and reaction temperatures. The fluorescence detector used throughout the study provided very good sensitivity and, because of fully adjustable emission and excitation monochromators, allowed flexibility under a variety of conditions. However, preliminary investigations in this laboratory indicate that less expensive filter fluorometric detectors may also perform adequately in this autoanalyzer system, provided that appropriate filter and lamp combinations are selected. Because of the sensitivity of the present autoanalyzer system, samples were diluted 1:100 in 0.01N acetic acid after the methanol precipitation step with the same acetic acid solution used as a wash for the autosampler. With this dilution, the toxin concentration in the majority of shellfish samples fell within the range of STX standards indicated in Fig. 2. A few samples, however, needed an additional dilution to bring the peaks on scale.

Correlation between the autoanalyzer system and the standard mouse bioassay was generally quite good and there are several factors that influence this correlation. The first of these is the differential intensities of fluorescence exhibited by the various PSP toxins. The relative fluorescence of the toxins varies by approximately a factor of 40 (Table 4). The toxin profile, therefore, will greatly affect the accuracy of estimates of overall toxicity. This point is demonstrated by the wide variation in correlation of results between species. The butter clam, *Saxidomus giganteus,* historically was noted to contain predominantly saxitoxin (Sullivan, 1982); since the autoanalyzer was calibrated with STX, this species showed the highest autoanalyzer-mouse bioassay correlation. The native littleneck clam, *Protothaca staminea*, however, has shown to contain primarily metabolites of Gonyautoxins II and III, which were almost twice as fluorescent as TTX (Sullivan et al., 1983b) and this group showed the lowest autoanalyzer-mouse bioassay correlation.

In addition to differing fluorescence, the individual PSP toxins displayed a wide range of absolute toxicities (Sullivan, 1982). In samples with a preponderance of the highly fluorescent/low toxicity toxins the autoanalyzer overestimated the toxicity, while an underestimation occurred in samples containing large amounts of the N-1 hydroxy toxins (high toxicity/low fluorescence). It is generally recognized that the types of toxins in shellfish vary depending on species and locality. It does appear, though, that in a particular area toxin profiles are failry constant, which may be due to the localized occurrence of individual strains of dinoflagellates (Hall, 1982). Applying the autoanalyzer to the analysis of shellfish is therefore a matter of determining the relationship between fluorescence and toxicity in a particular area. This can be accomplished either by determining the toxin profile or by developing the correlation between the autoanalyzer and mouse bioassay results for the shellfish from a particular locality. Preliminary findings from HPLC analyses indicated that toxin profiles in the Puget Sound area were fairly constant and, while the poorly fluorescing toxins were present, they were usually accompanied by the more highly fluorescent forms at equal or greater concentration.

Comparative study

Although most samples analyzed showed good correlation between the autoanalyzer and the mouse bioassay, some samples did not. Possible reasons for this noncorrelation, such as the wide range of toxin fluorescence and toxicities, have already been discussed. In most instances, the autoanalyzer estimate is higher than that of the bioassay. While this error might present an economic problem, due to the closure of shellfish harvest in areas where toxicities are below the closure level, it does not involve a public health risk. Only one sample was incorrectly determined to be safe by the autoanalyzer method (50 μg/100g) where the mouse bioassay indicated a closure condition (180 μg/100g). HPLC analysis of this sample indicated that the major type of toxin present was neosaxitoxin, a weakly fluorescing toxin. This was a sample of scallops taken in an area remote from the other sampling stations in Puget Sound and may indicate that there was a separate dinoflagellate population on that portion of the coast which produced primarily the weakly fluorescent N-1 hydroxytoxins, such as neosaxitoxin. This case illustrates the point that implementation of a routine autoanalyzer monitoring system must be preceded by the establishment of a correlation between fluorescence and toxicity in a particular area.

Table 4—Relative intensity of fluorescence of PSP toxins in the autoanalyzer procedure

Toxin	Relative fluorescence	Relative toxicity[a]
Saxitoxin	1.00	1.00
Neosaxitoxin	0.04	1.00
Gonyautoxin I	0.05	0.73
Gonyautoxin II	1.80	0.42
Gonyautoxin III	1.80	0.67
Gonyautoxin IV	0.05	0.27
B1	0.41	<0.05
B2	0.05	0.09
C1	0.48	0.06
C2	0.48	0.02

[a] Toxicity values are estimates based on reported toxicities in literature (Sullivan, 1982).

An additional factor contributing to the noncorrelation of certain samples is the inherent inaccuracy of the mouse bioassay itself which is often placed at ±20%. This variation in toxicity estimates should be kept in mind when comparing results for any particular sample (Table 1).

Despite these various potential sources of variability between autoanalyzer and bioassay results, the autoanalyzer has shown excellent promise as a method for shellfish toxicity monitoring in these early trials. If used as a screening method, the autoanalyzer could safely and quickly eliminate the samples found to be very high and very low in toxin content. Table 2 indicates that there was no significant difference between the two methods for samples below 60 μg/100g. Although correlation between the two methods was not as good at higher levels of toxicity, a cutoff point of 250 μg/100g would allow the autoanalyzer to eliminate as "unsafe" most of the very toxic samples. Indeed, only 3% of the samples would have been inaccurately labelled as above 80 μg/100g when they were, in fact, below the legal limit as indicated by the mouse bioassay. This "screening system" would most likely involve establishing upper and lower limits as measured by the autoanalyzer. Following the initial autoanalyzer screening, samples that fell between the upper and lower limits would be reanalyzed by bioassay, while no further analysis would be necessary for samples falling below the lower limit or above the upper limit. The choice of 60 and 250 μg/100g as lower and upper limits was based on initial examination of the data, but is supported by the statistical analysis. Using these parameters to divide the data into low, medium and high groups, a Spearman's rank correlation test of the data gives a value of 0.78. Small modifications in the lower and upper cutoff points do not alter this correlation coefficient significantly. Using the autoanalyzer as a screening test for the bioassay, with all samples between 60 and 250 μg/100g slated for retest by the bioassay, 70% of the samples analyzed in this study would have been eliminated from further testing. Records at the PSP laboratory (State of Washington, Dept. of Social & Health Services) indicate that this is a realistic estimate of

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Effects of pH and Time of Grinding on Lipid Oxidation of Fresh Ground Pork

J. J. YASOSKY, E. D. ABERLE, I. C. PENG, E. W. MILLS, and M. D. JUDGE

ABSTRACT

Experiments detected effects of pH on lipid oxidation of fresh ground pork. Pigs received antemortem epinephrine injections or postmortem carcass electrical stimulation to manipulate ultimate postmortem pH. High-pH (>6.10) samples had lower TBA values than low-pH samples. Nonsignificant difference in TBA values between high-pH prerigor- and postrigor-ground samples indicated that, at common high pH, time of grinding had no effect on lipid oxidation. Differences in TBA values between prerigor high- and low-pH samples and postrigor high- and low-pH samples indicated that, with identical grinding treatments, inhibition of oxidation occurred at high pH. Metmyoglobin was relatively high in both low- and high-pH muscle and was not catalytically active at high pH.

INTRODUCTION

SEVERAL CATALYSTS of lipid oxidation have been identified. Heme proteins catalyze lipid oxidation in linoleate model systems (Kwoh, 1971), egg yolk phospholipid dispersions (Love and Pearson, 1976), menhaden oil (Brown et al., 1963), raw meat systems (Liu, 1970) and cooked meats (Younathan and Watts, 1959). Metal catalysis is documented in model systems (Wills, 1965), raw meat systems (Tay et al., 1083), and in cooked meat (Sato and Hegarty, 1971). Igene et al. (1979) suggested that the rapid increase in thiobarbituric acid (TBA) values of meat after cooking is caused by catalytically active free iron released from heme proteins.

Prerigor-ground pork has a higher ultimate pH and is less susceptible to lipid oxidation than postrigor-ground pork (Judge and Aberle, 1980; Drerup et al., 1981; Tay et al., 1983). Whether the observed inhibition of oxidation is caused by elevated pH or a specific action of the prerigor grinding is unclear.

We conducted this study to determine the effect, if any, of induced high pH on the rate of lipid oxidation in ground pork. The experiments were designed to separate the effects of rigor state at the time of grinding from those of pH. The relationship between metmyoglobin concentrations and extent of lipid oxidation was also examined.

MATERIALS & METHODS

Experiment 1

Twenty-eight pigs weighing 95 - 120 kg were used. Control pigs had access to feed until slaughter and treated pigs were not fed during the 24 hr period before slaughter. Treated pigs were given a subcutaneous injectionof adrenalin chloride (epinephrine) solution (1:1000, Parke-Davis) 4 hr before slaughter at a dosage of 0.25 mg/kg body weight.

Within 45 min after exsanguination, the triceps brachii muscle was removed from one side of each carcass. The muscle was trimmed of all visible fat and connective tissue and ground in succession through plates with 9.5 and 4.8 mm openings. Approximately a 100-g sample was then spread into a 3 mm layer, wrapped in oxygen permeable, moisture-impermeable wrap, placed on a rack to assure maximum oxygen transmission and stored at 3°C. At 24 hr postmortem, the triceps brachii muscle was removed from the opposite side of each carcass and prepared identically to the prerigor sample.

The pH values of all samples were determined in duplicate 24 hr postmortem with a glass electrode after which each sample received a 1% addition of Neosporin powder (Polymyxin B-bacitracin neomycin) and was mixed by hand. Thiobarbituric acid (TBA) values were determined on duplicate samples at 1, 4, 8 and 12 days postmortem with the procedures of Tarladgis et al. (1960). Standard curves were prepared using tetraethoxypropane.

Log TBA values and pH values were statistically analyzed with analysis of variance for factorial design (Harvey, 1975). Significant differences among means were tested with Duncan's new multiple range test (Li, 1964). Log transformations of TBA values were made to achieve homogeneity of variance since the variance increased with time postmortem.

Experiment 2

Twenty-two pigs weighing 95 - 120 kg were used. All pigs had access to feed until slaughter. Treated carcasses were electrically stimulated approximately 30 min after exsanguination. Stimulation consisted of 2 sec pulses (480 V, 2.0 - 3.5 A) intermittent with 1 sec pauses for a total of 2 min. After a 2-min pause, the stimulation cycle was repeated until minimal muscle contraction was observed. The entire length of treatment was approximately 12 min.

Pre- and postrigor muscle samples were removed and prepared as in experiment 1. The pH measurements, antibiotic addition, TBA analyses, and statistical analyses were also done as in experiment 1.

Experiment 3

Ten pigs weighting 95 - 120 kg were used. Animal treatments, sample preparations, pH determinations, analyses of data and antibiotic additions were as in experiment 1. TBA values and metmyoglobin percentages were determined 0, 4, 8 and 12 days after grinding. An absorbance method (Krzywicki, 1982) was used to determine the relative percentages of metmyoglobin versus reduced myoglobin. Statistical analyses were performed as in experiment 1.

RESULTS & DISCUSSION

Experiment 1

Prerigor control ground pork had a higher ($P < 0.05$) ultimate pH and a lower ($P < 0.05$) rate of lipid oxidation than postrigor control ground pork (Fig. 1). These results are consistent with those of Judge and Aberle (1980) and Drerup et al. (1981). Both epinephrine-treated groups had muscle tissue with higher ($P < 0.05$) ultimate pH and lower ($P < 0.05$) rates of lipid oxidation than the control groups. The inhibition of lipid oxidation observed in these samples was probably due to the inhibitory effect of high pH on oxidation (Wills, 1965; Kwoh, 1971).

A comparison of prerigor-ground and postrigor-ground muscle tissue from epinephrine-injected pigs allows one to examine samples with different grinding times but with relatively high pH (6.53 and 6.39 respectively) values.

Author Judge is affiliated with the Dept. of Animal Science and authors Peng and Mills are affiliated with the Dept. of Food Science, Purdue Univ., West Lafayette, IN 47907. Author Yasosky, formerly with Purdue, is now with The Pillsbury Co., Research & Development Laboratories, 311 Second St. Southeast, Minneapolis, MN 55414. Author Aberle, formerly with Purdue, is now affiliated with the Dept. of Animal Science, Univ. of Nebraska, Lincoln, NE 68583.

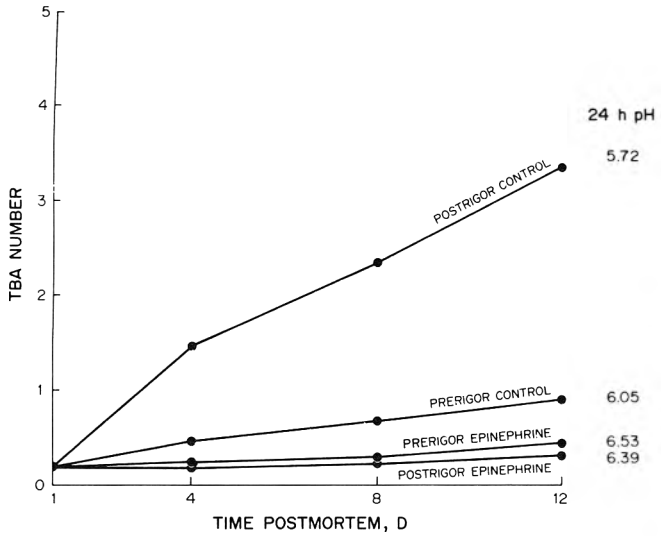

Fig. 1—TBA numbers in prerigor- and postrigor-ground pork after antemortem epinephrine (Experiment 1). SE of least squares means: Day 1 = 0.02; 4 = 0.17; 8 = 0.28; 12 = 0.46.

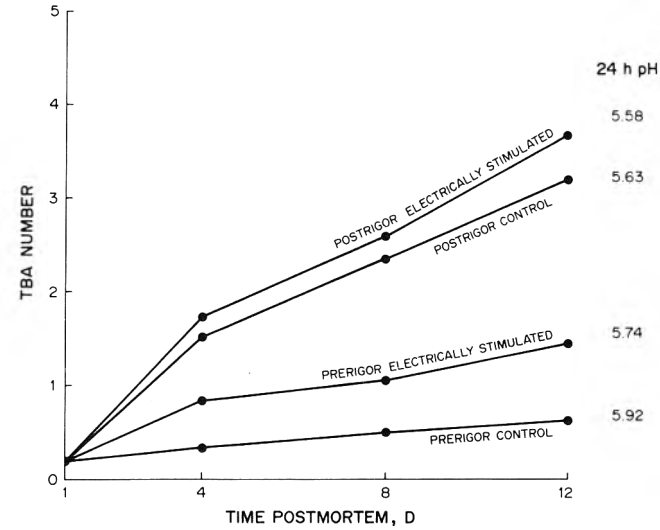

Fig. 2—TBA numbers in prerigor- and postrigor-ground pork after carcass electrical stimulation (Experiment 2). SE of least squares means: Day 1 = 0.02; 4 = 0.18; 8 = 0.27; 12 = 0.44.

Even though there was a slight difference ($P < 0.05$) in pH, the nonsignificant difference ($P > 0.05$) in TBA values indicated that, in high pH muscle tissue, time of grinding had no effect on lipid oxidation.

Postrigor-ground muscle from epinephrine-injected and control pigs can be used to compare samples with identical times of grinding but with different ($P < 0.05$) pH values (Fig. 1). Because time of grinding was identical for all samples, the lower ($P < 0.05$) TBA values observed for high pH samples indicated that, when all samples were postrigor ground, high pH inhibited lipid oxidation.

Experiment 2

Prerigor ground pork had a higher ($P < 0.05$) pH and lower ($P < 0.05$) rate of lipid oxidation when compared to comparably treated postrigor-ground samples, but we observed that carcass electrical stimulation caused elevated ($P < 0.05$) TBA values and lower ($P < 0.05$) pH in prerigor-ground samples whereas it had no significant effect on either TBA or pH values for postrigor-ground pork (Fig. 2).

Specific pairs of treatment cells were compared to determine time of grinding and pH effects. Comparison of prerigor-ground muscle tissue from the stimulated and control carcasses utilized samples with identical times of grinding but with different ($P < 0.05$) pH values (Fig. 2). The lower TBA values for the prerigor-ground muscle tissue from control carcasses indicated that, when all samples were prerigor ground, high pH inhibited lipid oxidation.

A comparison of prerigor- and postrigor-ground muscle tissue from electrically stimulated carcasses utilized samples with different grinding times and relatively low pH values. The lower ($P < 0.05$) TBA values for the prerigor ground samples suggest that time of grinding may have an effect on lipid oxidation at relatively low pH. However, the pH values of the samples, though relatively low, were significantly ($P < 0.05$) different. As shown in Fig. 3, sensitivity to oxidation increased remarkably as pH decreased from approximately 5.9. A small decrease in pH in this lower range of pH values was associated with a large increase in TBA values. Thus, the relatively low TBA values observed in prerigor-ground samples were probably a result of a slightly higher ultimate pH as compared to postrigor-ground samples and not due to time of grinding.

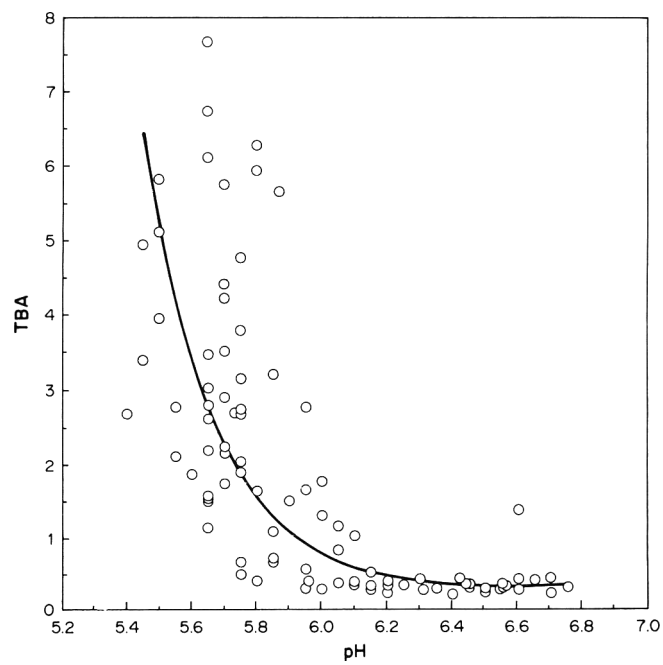

Fig. 3—TBA values after 12 d storage at 2° C vs. 24-hr pH in ground porcine muscle (Experiments 1, 2 and 3 combined). The TBA coordinates of the line were obtained by an antilog transformation of the ordinates of a best-fit line for log TBA vs pH.

Experiment 3

TBA values and metmyoglobin concentrations in muscles from control and epinephrine-injected pigs after prerigor and postrigor grinding are shown in Fig. 4. Results were similar to those of experiment 1, yet all samples had relatively high metmyoglobin concentrations. Because no relationship was observed between lipid oxidation and metmyoglobin concentrations, we conclude that metmyoglobin is not an active catalyst of lipid oxidation in a high pH raw meat system. These results may conflict with the conclusions of Liu (1970) and Kwoh (1971) because the inhibition of oxidation was apparently a direct result of high

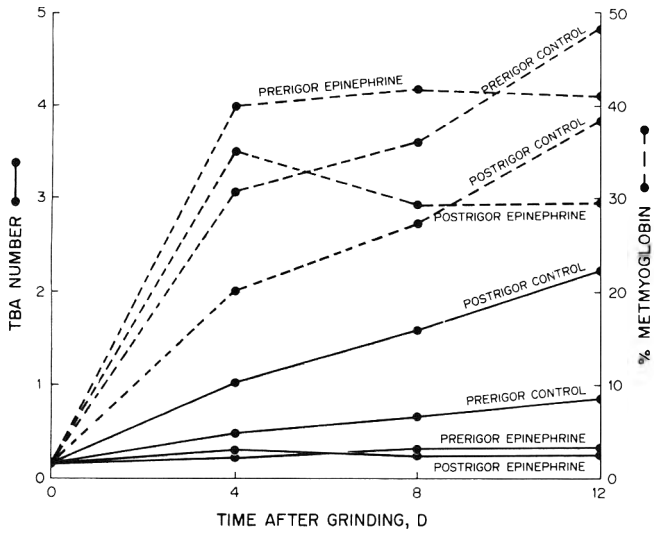

Fig. 4—TBA numbers and metmyoglobin concentrations in prerigor- and postrigor-ground pork after antemortem epinephrine (Experiment 3). SE of least squares means: (TBA) Day 0 = 0.01; 4 = 0.39; 8 = 0.64; 12 = 1.14. (Metmyoglobin) Day 0 = 1.36; 4 = 4.99; 8 = 3.82; 12 = 5.07.

pH rather than the reduction of metmyoglobin to the non-catalytic myoglobin form. Further, the data cast doubt on the proposition that metmyoglobin catalyzes lipid oxidation in fresh, refrigerated meat products.

CONCLUSIONS

THE MECHANISM by which pH controls lipid oxidation in raw meat is unclear but Fig. 3 shows that a critical pH value of ∿6.10 or higher is needed to attain maximum inhibition. Evidence suggests that the inhibition may be the result of pH effects on metal catalysts (Liu and Watts, 1970; Love and Pearson, 1974; Tay et al., 1983). Wills (1965) showed that Fe, Mn, Co, and Cu are catalysts of lipid oxidation. All of these metals are present in varying concentrations in muscle tissue.

Powell-Baker and Saroff (1965) and Appurao and Narasinga Rao (1975) suggested that the imidazole or amino groups of proteins play an integral part in the chelation of metal ions. It could be theorized that the ionization of histidine residues (pK < 6.0) at low pH could alter the net charge and/or tertiary structure of proteins thus reducing their ability to sequester catalytic ions such as Mn, Cu, Co and Fe.

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Differential Scanning Calorimetry of Beef Muscle: Influence of Postmortem Conditioning

C. J. FINDLAY and D. W. STANLEY

ABSTRACT

Differential scanning calorimetry (DSC) was used to follow the changes in the endothermic transitions of beef muscle during conditioning. Sternomandibularis muscle held at 5°C from 2-8 days postmortem resulted in a significant ($P < 0.05$) drop in the total heat of transition (ΔH) from 3.8 to 3.0 J/g. The myosin transition decreased from 57.8° to 55.2°C while the actin transition increased from 81.8° to 83.2°C ($P < 0.05$). Storage time and temperature were varied to generate a response surface of thermal data for psoas major and semimembraneosus muscle. The decrease in ΔH of psoas major was optimal between 10° and 13°C. Total ΔH of semimembraneosus (3.9 J/g) was significantly greater ($P < 0.05$) than that of psoas major (3.4 J/g).

INTRODUCTION

THE PRACTICE OF HANGING BEEF carcasses to improve tenderness was established long before the enzymology of conditioning was scientifically investigated. The enzymes responsible for the degradation of myofibrillar proteins during conditioning have been studied extensively and the literature was reviewed comprehensively by Penny (1980). Calcium activated neutral protease (CANP) has been implicated in the breakdown of the Z-disc and the fragmentation of myofibrils. Cathepsins B and D, which have pH optima below 4, have also been shown to degrade myofibrillar proteins (Yates et al., 1983). The rate and nature of proteolysis varies between muscles depending on their available enzyme concentration and holding temperature. It was recently reported that at 4°C and pH 7 catheptic activity is minimized, while at 37°C and pH 5.4 CANP activity is low (Yates et al., 1983). These researchers also noted the degradation of myosin and related this to a reduction in the rigor crossbridges effecting a mechanical weakening of the myofibril. Smith et al. (1978) concluded that maximum tenderizing was achieved for most beef muscles after 11 days storage at 4°C, but that some muscles reached their minimum shear values in 5 days while others required 28 days. Differential scanning calorimetry (DSC) has been used to relate the denaturation of muscle proteins to the textural changes caused by cooking (Findlay and Stanley, 1984a, Martens et al., 1982). The transition temperature and ΔH of muscle proteins have been shown to be sensitive to changes in pH and ionic strength (Wright et al., 1977, Wright and Wilding, 1984). Because DSC has been shown to be useful in accurately measuring thermal properties of muscle, this study was undertaken to determine the effect of conditioning of beef on DSC thermal curves.

Authors Findlay and Stanley are affiliated with the Dept. of Food Science, Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1.

MATERIALS & METHODS

BEEF NECK MUSCLE (sternomandibularis) was obtained from four 18 month-old Charolais crossbreed heifers immediately postmortem and restrained to prevent shortening. The samples were wrapped in damp paper towel and held at 20°C for 8 hr before holding isothermally at 5°C for 2, 4, 6 and 8 days. Sarcomere length, as measured by laser diffraction, averaged 1.95 μm (sd 0.12 μm).

Beef psoas major (sarcomere length 3.12, sd 0.09 μm) and semimembraneosus (sarcomere length 2.33, sd 0.06 μm) muscles were taken from another group of four 18 month-old Charolais crossbreed heifers 8 hr postmortem, comminuted, blended, divided into 50g samples and sealed in plastic bags. A two variable rotatable design was used to optimize the experiment and yield response surface data on the effect of varying time and temperature of conditioning (Mullen and Ennis, 1979). Temperature levels of 3°, 8°, 15.5°, 23° and 28°C were used for storage times of 8, 22, 56, 90 and 104 hr.

Differential scanning calorimetry was performed using the methods previously described by Findlay and Stanley (1984b). Thermal curves were analyzed for temperatures of maximum transition, T_{max} (T_1, T_2, T_3 and T_4) and corresponding heats of transition (ΔH). The data were analyzed by multiple regression and response surface plots were generated using the Statistical Analysis System (Helwig and Council, 1979).

RESULTS

SEVERAL THERMAL TRANSITIONS changed significantly ($P < 0.05$) during the time course of 5°C isothermal storage of beef sternomandibularis muscle (Table 1). The first transition (T_1), attributed to myosin (Wright et al., 1977; Stabursvik and Martens, 1980), shifted from 57.8° to 55.2°C, while the ΔH decreased from ca. 0.72 to 0.55 J/g. The second transition (T_2) showed a major decrease in ΔH from 1.97 to 1.47 J/g, but remained at ca. 66°C. The actin transition (T_3) increased from 81.8° to 83.2°C and dropped

Table 1—Effect of isothermal conditioning on the T_{max} and ΔH values beef sternomandibularis muscle (n = 15)

Aging (days) +		Transition temperature (T_{max}°C)			Heat of transition (J/g)			
		T_1	T_2	T_3	ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
2		57.82 a*	66.33 a	81.84 a	0.725 a	1.969 a	1.127 a	3.821 a
	(Std dev)	(1.77)	(1.16)	(0.37)	(0.115)	(0.329)	(0.179)	(0.477)
4		56.04 b,c	65.69 b	82.59 b	0.655 a,b	1.875 a,b	1.098 a	3.629 a
		(2.34)	(0.99)	(0.70)	(0.102)	(0.387)	(0.195)	(0.499)
6		56.60 b	66.15 b	82.96 c	0.599 b,c	1.601 b,c	1.027 a,b	3.227 b
		(2.35)	(1.13)	(0.72)	(0.126)	(0.394)	(0.207)	(0.578)
8		55.16 c	66.97 a	83.20 c	0.551 c	1.465 c	0.967 b	2.983 b
		(2.91)	(1.23)	(0.73)	(0.118)	(0.393)	(0.176)	(0.505)

* Means in the same column with the same letter are not significantly different $P < 0.05$, using Duncan's Multiple Range Test.

from 1.13 to 0.97 J/g. The total ΔH over the range 45-92°C dropped from ca. 3.8 to 3.0 J/g. Thermal curves of 2, 4, 6 and 8 days storage, normalized to 10 mg wet muscle, appear in Fig. 1.

During conditioning at 5°C, the ΔH values for all transitions of beef sternomandibularis muscle declined steadily. The decrease in the ΔH of myosin (T_1) was accompanied by a drop in T_{max} while actin (T_3) increased in transition temperature. It is clear that these three major endothermic transitions of beef muscle are not the discrete events attributed to myosin, collagen and actin by Stabursvik and Martens (1980) but a net response of the muscle proteins reflecting their association and environment as discussed below.

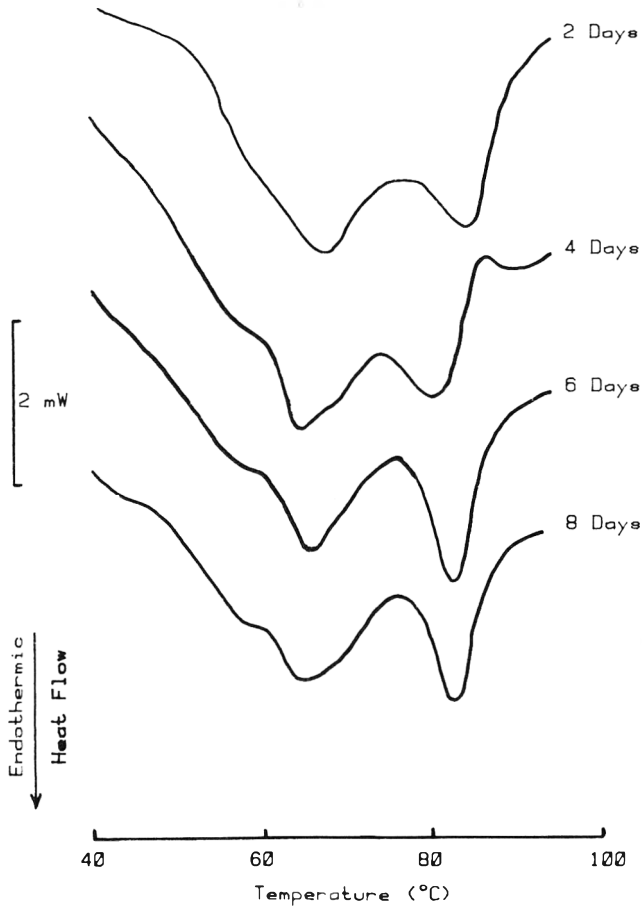

Fig. 1—DSC thermal curves of beef sternomandibularis muscle stored at 5°C for 2, 4, 6 and 8 days postmortem. Each curve is the sum of 10 samples normalized to 10 mg wet muscle.

It was noted by Wright et al. (1977) and Wright and Wilding (1984) that although myosin appeared to have a single transition in fresh muscle, isolated myosin had three transitions that have been related to the three structural subunits, light meromyosin and heavy meromyosins S_1 and S_2 and was sensitive to changes in pH and ionic environment. Proteolysis of the thick filament may be responsible for cleavage of the myosin and the liberation of more labile subunits. Conversely, the dissociation of actin from the actomyosin complex may lead to greater thermal stability and the higher actin transition temperature observed with conditioning.

The interaction of temperature and storage time was investigated using a rotatable design experiment. Psoas major was selected for its tenderness and low connective tissue content while semimembraneosus is known to possess more connective tissue and is a tougher cut of meat (Swatland, 1984). Multiple regression was used to provide an equation to generate a response surface. Table 2 contains the mean, standard deviation, probability and R^2 values for T_{max} and ΔH values for both muscles. The total ΔH for semimembraneosus was 3.9 J/g while that for psoas major was only 3.4 J/g ($P < 0.05$), probably reflecting the lower connective tissue content of psoas major.

The response surface models generated by regression analysis of psoas major thermal curves revealed that the effect of storage temperature and time on ΔH_1 accounted for 68% of the variation at P = 0.0913 (Fig. 2). The model for total ΔH (Fig. 3) accounted for 70% of the variation (P = 0.0714). The T_4 transition gave a significant (P = 0.0145) temperature response (Fig. 4). Analysis of the ther-

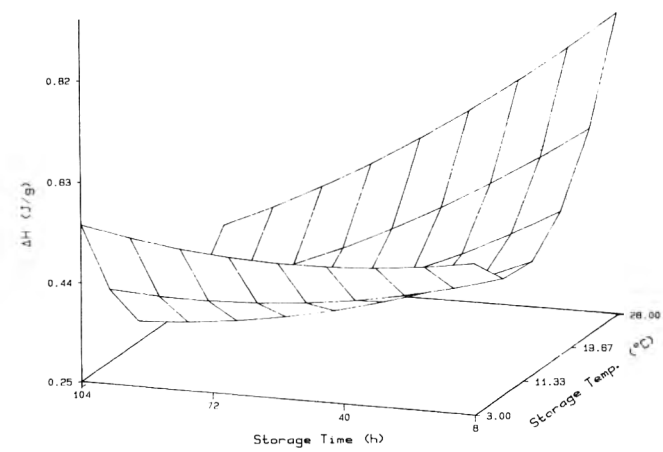

Fig. 2—ΔH_1 (Z) of psoas major as a function of storage time (X) and temperature (Y). ($Z = 0.615 - 0.00107\ X + 0.02528\ Y - 0.000016\ X^2 + 0.00123\ Y^2 - 0.000198\ X*Y$; $R^2 = 0.683$; $P = 0.0913$).

Table 2—Regression analysis of the rotatable design thermal curves of beef muscle (n = 13)

Muscle	Temperature of Transition (T_{max}°C)				Heat of Transition (J/g)				
	T_1	T_2	T_3	T_4	ΔH_1	ΔH_2	ΔH_3	ΔH_4	ΔH_{Total}
Psoas major									
Mean	54.49	66.77	80.79	89.97	0.398	0.516	0.686	0.046	3.413
Std dev	0.91	1.78	0.42	0.43	0.084	0.096	0.082	0.024	0.420
Probability =	0.3867	0.8779	0.3902	0.0145	0.0913	0.2878	0.2295	0.3884	0.0714
R^2	0.4677	0.1921	0.4658	0.8229	0.6829	0.5257	0.5639	0.4668	0.7076
Semimembraneosus									
Mean	55.12	66.62	80.77	89.95	0.481	0.519	0.624	0.038	3.928
Std dev	1.15	1.41	0.36	0.57	0.124	0.150	0.024	0.012	0.581
Probability =	0.1442	0.3900	0.0067	0.2531	0.3541	0.3054	0.0001	0.8321	0.8847
R^2	0.6300	0.4659	0.8597	0.5480	0.4861	0.5148	0.9670	0.2240	0.1870

Regression model: d.f. = 5; $Z = aX + b\ X^2 + cY + dY^2 + eXY + f$; X = storage time (h); Y = storage temperature (°C).

mal curves of semimembraneosus showed a significant response for temperature, P = 0.0067, (Fig. 5) and ΔH, P = 0.0001 (Fig. 6) for the actin transition (T_3).

DSC thermal curves are difficult to analyze in the form of discrete data due to the net heat flow being measured and the probability of interactions between proteins. However, to utilize the power of three dimensional plotting a discrete response is required. The thermal data that proved to be significant demonstrated changes that are consistent with the current understanding of the enzymology of conditioning. The ΔH_1 of beef psoas major (Fig. 2) showed its greatest reduction in ΔH_1 around 12–13°C, the temperature used commercially for carcass conditioning. Almost no change occurred at 3°C, while the initially higher ΔH at higher storage temperature declined rapidly as proteolysis progressed. The total ΔH for psoas major (Fig. 3) had its highest value at 3°C and at the initial time. With an increase in storage temperature there was a rapid decrease in ΔH commensurate with proteolysis. Again, it appears that temperatures of 10–15°C resulted in a more rapid reduction in ΔH to the 3 J/g value that is typical of aged meat.

The currently unidentified (F-actin, desmin ?) T_4 transition of psoas major accounted for less than 2% of the total ΔH. This transition temperature increased gradually (Fig. 4) at low storage temperatures but rose more rapidly at higher initial storage temperature. At higher storage temperatures there was a significant decrease in T_{max} paralleling the catheptic activity noted by Yates et al. (1983).

Semimembraneosus gave highly significant responses for both temperature and heat of the actin transition. The response surfaces (Fig. 5 and 6) displayed the same general shape with maxima at 28°C initially but at 3°C after 104 hr. At high storage temperature there was a steady decline in T_{max} and ΔH of the actin transition resulting from proteolytic activity. At low temperature there was a gradual increase in ΔH and in T_{max} similar to that found in the beef sternomandibularis stored isothermally at 5°C.

SUMMARY & CONCLUSIONS

THE INTERACTIONS of muscle proteins during heating must be understood before the changes in endothermic profiles of meat can be quantitatively related to the degradation observed during conditioning. In general, the total ΔH value for muscle declined during conditioning. The increase in the transition temperature of actin as a result of conditioning in both sternomandibularis and semimembraneosus paralleled the actin shift observed when sarcomere length was increased (Findlay and Stanley, 1984b).

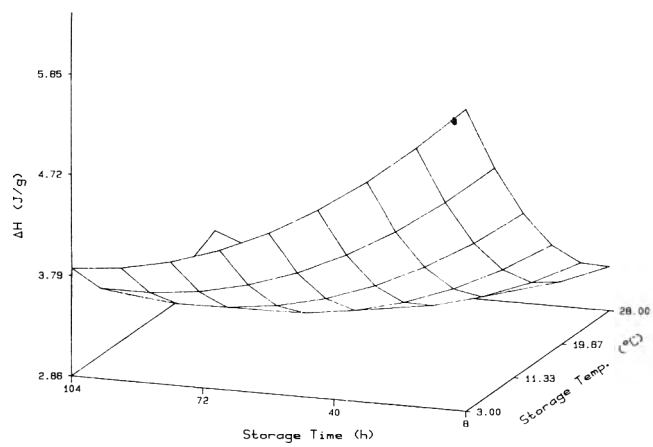

*Fig. 3—Total ΔH (Z) of psoas major as a function of storage time (X) and temperature (Y). (Z = 6.46 − 0.04087 X − 0.1796 Y + 0.000178 X^2 + 0.00253 Y^2 + 0.00075 X*Y; R^2 = 0.708; P = 0.0714).*

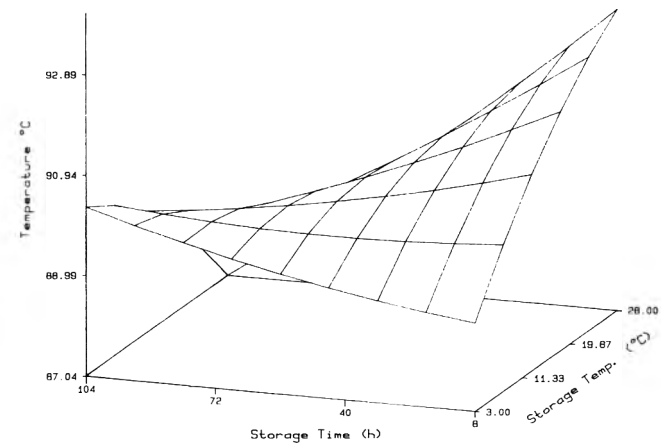

*Fig. 4—T_4 transition temperature (Z) of psoas major as a function of storage time (X) and temperature (Y). (Z = 87.9 + 0.01714 X + 0.2841 Y + 0.000075 X^2 − 0.00302 Y^2 − 0.00308 X*Y; R^2 = 0.823; P = 0.0145).*

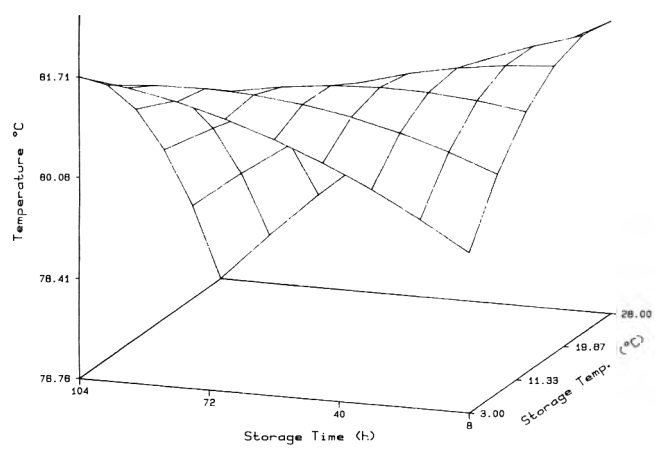

*Fig. 5—T_3 transition temperature (Z) of semimembraneosus as a function storage time (X) and temperature (Y). (Z = 0.0382 + 0.00825 X + 0.0598 Y − 0.000005 X^2 − 0.00113 Y^2 − 0.00054 X*Y; R^2 = 0.967; P = 0.0001).*

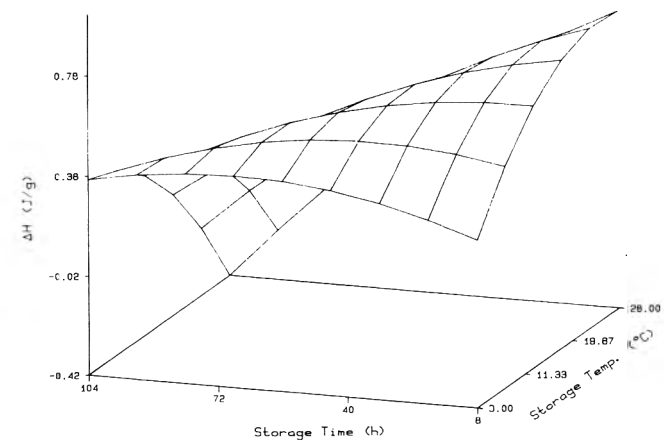

*Fig. 6—ΔH_3 (Z) of semimembraneosus as a function of storage time (X) and temperature (Y). (Z = 78.3 + 0.0529 X + 0.2728 Y − 0.000179 X^2 − 0.00526 Y^2 − 0.00296 X*Y; R^2 = 0.860; P = 0.0067).*

The minor reduction in ΔH of protein transitions during conditioning, less than 1% of the energy required to increase the temperature of meat during cooking, emphasizes the importance of the temperature of transition. A shift towards higher transition temperatures in meat cooked to a specific internal temperature will result in a greater proportion of undenatured myofibrillar proteins and, it would be supposed, concomitantly less myofibrillar toughening. The tenderizing effect of conditioning on beef muscle has been well established; however, the relative importance of CANP or cathepsins B and D in meat tenderness is still not clear. DSC may provide a way to follow the degradation of myofibrillar proteins during conditioning. The contribution and interaction of individual muscle proteins to the endothermic profile of muscle requires further research before its effect on cooked meat tenderness is understood.

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the proportion of nontoxic and very toxic samples received during a season. Over a 5-month period, an autoanalyzer system would have eliminated the bioassays on 70–80% of the approximately 1500 samples routinely analyzed. Another advantage of autoanalyzer screening is the availability of an estimate of toxin level for all samples, thus reducing the number of trials necessary to establish the proper dilution for those samples which are retested by the bioassay.

The results of this study show that the autoanalyzer has great potential as a routine monitoring system for PSP. Additional work is currently being conducted to refine the technique and to determine the exact correlation between the mouse bioassay and the autoanalyzer in this region. Further comparison testing will help to establish an exact relationship between the results of the autoanalyzer and the bioassay and may lead to a predictive mathematical factor, the use of which would allow a closer correspondence between the two techniques. As described earlier, laboratories in other regions of the country will need to do extensive comparison testing to determine the level of correlation between the two techniques in their particular region.

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Assessment of Autoxidation in Freeze-Dried Meats by a Fluorescence Assay

A. R. KAMAREI and M. KAREL

ABSTRACT

A simple and sensitive fluorescence-measuring technique was developed to assess extent of lipid oxidation in freeze-dried meats. Solvent extracts of reconstituted stored samples were assayed by fluorimetry. Spectra of "oxidized" meats show maximum excitation and emission wavelengths of λex = 350 and λem = 440 nm, respectively. At λem of 440 nm, "unoxidized" meats show three peaks in excitation spectrum at λex_1 = 308, λex_2 = 318 (max.), and λex_3 = 350 nm. However, at λex of 350 nm, these samples show a peak at λem = 476 nm. The intensity ratio of λex_3 or λem over λex_2 are useful as sensitive and reliable "internal standards" of lipid oxidation. Presence of 100 ppm TBHQ (monotertiary butylhydroquinone), absence of oxygen, and compression of meat before freeze-drying, which protect against oxidation also result in corresponding reductions of these ratios.

INTRODUCTION

TRADITIONAL METHODS for assessment of the state of oxidation, including peroxide value and TBA tests give good results in model systems and in fresh or frozen meats but do not produce satisfactory results in freeze-dried meats. A technique which seemed promising was assay of fluorescent products of reactions between oxidizing lipids and proteins. Detection of lipid oxidation products by fluorescence technique has been applied mostly in biological tissues (Gray, 1978; Logani and Davies, 1980). In a recent review, Melton (1983) found no reports of studies that used fluorescence to study lipid oxidation in muscle foods. Crawford et al. (1966) found that reaction between malonaldehyde and glycine involves 1,4 addition of the nucleophilic nitrogen atom of glycine to the enolic carbon atom of the α, β unsaturated carbonyl system of the enol form of malonaldehyde, followed by loss of water, to form the enamine, N-prop-2 enal amino acetic acid. They indicated a condensation in a 1 to 1 molar ratio. Chio and Tappel (1969) found that the fluorescent compounds, derived from glycine, leucine, and valine showed excitation wavelength of 370, and emission wavelength of 450 nm. The authors attributed the typical electronic absorption and fluorescence properties to the chromophoric system NC=C-C=N which contains 6 π electrons. They reported that 1 mole of malonladehyde reacts with 2 moles of amino acid esters to yield N,N'-disubstituted 1 amino-3 imino propenes.

Dillard and Tappel (1971) found that fluorescence techniques can measure the Schiff base product at a level of 1 ppb, which on the molar basis is 10 to 100 times more sensitive than the colorimetric TBA assay. This confirms the findings of Sawicki et al. (1963) who reported that spectrofluorometric method is by far the most sensitive and highly selective method for determination of malonaldehyde.

Dillard and Tappel (1973) found that a conjugated Schiff base structure is required for fluorescence. Hence, monomer amino-malonladehyde products are not fluorescent, but low levels of the dimer products are easily detected by sensitive fluorescence technique. Thus, RN=CH-CH=CHOH does not fluoresce, but, RN=CH-CH=CHNHR does. Similar results are reported by Malshet and Tappel (1973) and Trombly and Tappel (1975). Studies using fluorescence technique to investigate the peroxidation products, have been reported on phospholipids (Bidlack and Tappel, 1973), DNA (Reiss and Tappel, 1973), red cells (Goldstein and McDonagh, 1976), hemoglobin (Grossman et al., 1979), brain lipids (Guttridge et al., 1982), and fish muscle during frozen storage (Davis, 1982; David and Reece, 1982).

Investigators have reported that lipid-protein interactions cause browning similar to nonenzymatic browning (Pokorny, 1981) and have used fluorescence as an analytical technique for parallel studies on lipid and carbohydrate-derived carbonyl groups with amino functional groups (Porter et al., 1983).

Considering above reports and lack of any fluorescence study on lipid oxidation in freeze-dried muscle foods, our objective was to use and develop such technique to evaluate the extent of lipid oxidation in stored freeze-dried meats. We further used this technique to evaluate the effectiveness of reduction of headspace oxygen and of presence of an antioxidant (A.O.) in improving the stability of such freeze-dried meats.

MATERIALS & METHODS

Preparation and storage of freeze-dried meat

All steps of meat preparation were conducted in the cold (approx. 4°C), unless otherwise stated. Fresh, choice-grade beef was deboned, chopped, and trimmed to approximately 15 ± 1% fat. Meat was tempered (−2°C, 17 hr), flaked, and divided into two batches for antioxidant (A.O.) treatment.

The control batch received only 0.5% sodium chloride and 0.2% sodium tripolyphosphate (TPP). The other batch was treated in addition with 100 ppm monotertiary butylhydroquinone (TBHQ) (based on final dry weight) as antioxidant. A solution of antioxidant (0.2%) was initially prepared by dissolving TBHQ in a mixture (1 + 9) of absolute ethanol and water. The batches were mixed in stainless steel mixers under vacuum (20 inches) for 12-13 min. The product was then stuffed into polyethylene casings, retempered, compressed, and sliced into 155-160g samples. Samples were frozen in a blast freezer for 4 hr and then freeze-dried for 36 hr to a final moisture of 1%. The final weights of samples were between 53-57g. For half of the samples, to be "stored" in absence of air, the vacuum was released with dry nitrogen. For the other half, to be "stored" under air the vacuum was released with dry air. All control and TBHQ-treated samples were placed in flexible pouches and double sealed in the presence or absence of air (vacuum).

During preparation of freeze-dried beef, three samples (each in duplicate) were removed at various steps of processing and were labeled A (fresh beef), B (mixed with ingredients), and C (processed up to freeze-drying).

Samples were then stored at 37°C for suitable time intervals (up to 30 days), when they were removed from the storage room and the extent of lipid oxidation was determined by the following fluorimetry method.

To study the effect of compression during processing on oxidation during following storage, the additional cold (−40°C) freeze-

Authors Kamarei and Karel are affiliated with the Dept. of Nutrition & Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

dried samples were ground, using a Krups household coffee mill (type 203), for 5 sec at 4°C. Duplicate 1.6-g samples of the resultant homogeneous ground freeze-dried beef were then weighed in 25 ml glass Erlenmeyer flasks and stored at 37°C, under air, for up to 3 days (0, 1, 2 and 3 days).

Preparation of samples for fluorescence assay

For fluorescence assay, freeze-dried samples were designated into four groups as follows:

Group 1 (control, under air);
Group 2 (control, under vacuum);
Group 3 (A.O. treated, under air);
Group 4 (A.O. treated, under vacuum).

Table 1—Step-by-step preparation of freeze-dried (F-D) beef for fluorescence study

Reconstitute F-D beef samples to initial moisture content (1.6g sample + 3.4 mL H_2O)
↓ Vortex and store (15 min @ room temp)
Add 25 mL $CHCl_3$:MeOH (2:1)
↓ Mix (15 min) and transfer to centrifuge tube
Rinse with 10 mL H_2O
↓
Centrifuge (10^4 x *g* for 5 min @ room temp)
↓ Remove aqueous phase (aspiration)
Collect solvent layer
↓ Filter (2X) SS #595
Fluorimetry assay

For each treatment, duplicate samples were analyzed. Preparation of freeze-dried beef samples for assay by fluorescence techniques was performed as follows:

Freeze-dried beef samples were reconstituted to initial moisture content (1.6g sample + 3.4 mL H_2O), vortexed, and stored for 15 min at room temperature. To extract the fluorescent compounds, 25 mL solvent ($CHCl_3$:MeOH, 2:1) were added to the paste, mixed (15 min), transferred to a centrifuge tube, and rinsed with 10 mL water. The mixture was centrifuged at 10,000 x *g* for 5 min at room temperature. The aqueous phase was removed by aspiration and the collected solvent layer was filtered two times (SS #598) and used to obtain the fluorescence spectra. The procedure for preparation of beef samples for fluorescence study is summarized in Table 1. Non-freeze-dried A, B, and C samples, taken during processing line, did not require reconstitution.

Fluorescence spectra were determined with an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Inc.). Standard solution was quinine sulfate (1 ppm in 0.1N H_2SO_4). The slit arrangement was as follows: excitation exit slit 1 mm, emission entrance slit 2 mm, exit slit 2 mm. The meter multiplier setting was dependent upon the fluorescence intensity of each sample and was set at 3 and/or 10. Scanning was performed at 120 nm/min (wavelength) and 5 cm/min (recorder).

RESULTS & DISCUSSION

PURE SOLVENT ($CHCl_3$:MeOH, 2:1) did not show any peak in either excitation or emission range. Scanning of well-oxidized, freeze-dried beef for 10 nm increments in both excitation and emission range indicated that excitation at 350 nm resulted in the "maximum" emission at 440 nm. When emission wavelength was set at 440 nm, the excitation spectrum showed the "maximum" wavelength of 350 nm. Consequently, for obtaining the excitation spectra (200–380 nm) of all samples, the emission wavelength was set at λem • max (440 nm) and for obtaining the emission spectra (380-632 nm), the excitation wavelength was set at λex • max (350 nm). Fig. 1 shows the typical excitation and emission spectra of unoxidized vs oxidized (stored) freeze-dried beef.

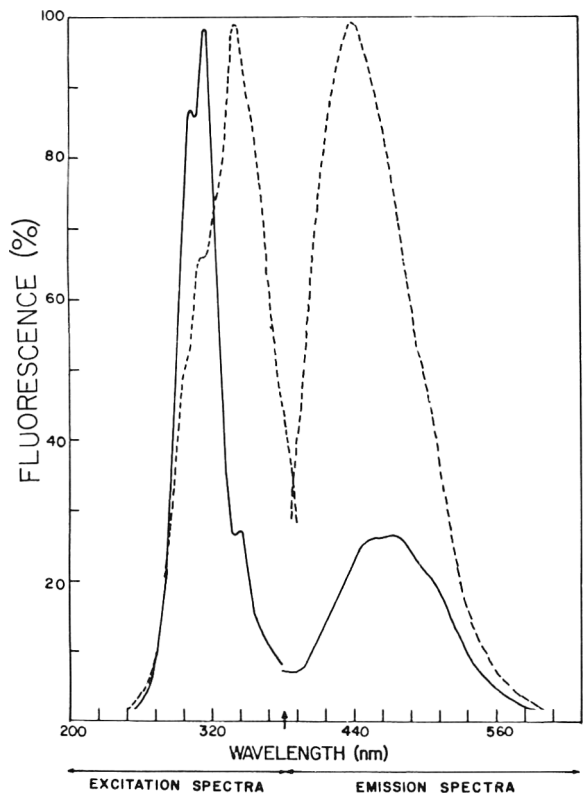

Fig. 1—Typical excitation and emission spectra of unoxidized (——) vs oxidized (---) freeze-dried beef.

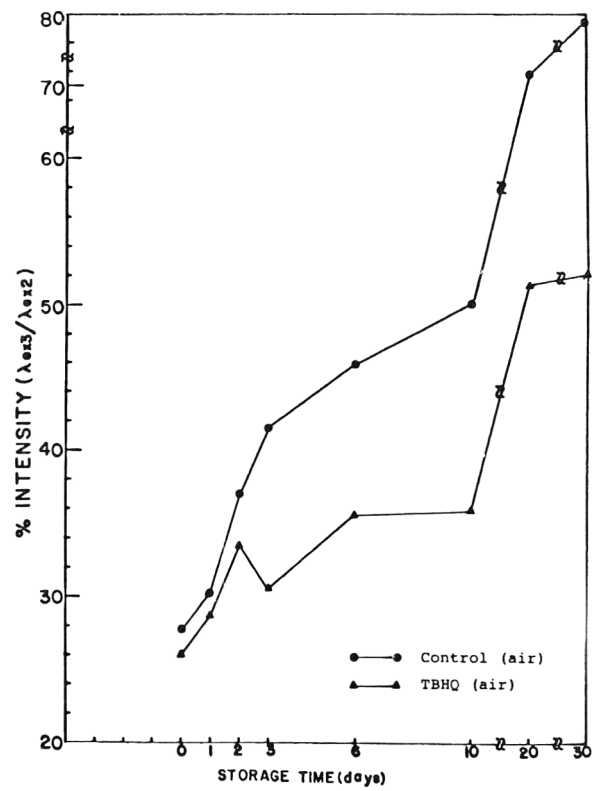

Fig. 2—Increase of $\lambda ex_3/\lambda ex_2$ in compressed freeze-dried beef upon storage (under air) at 37°C.

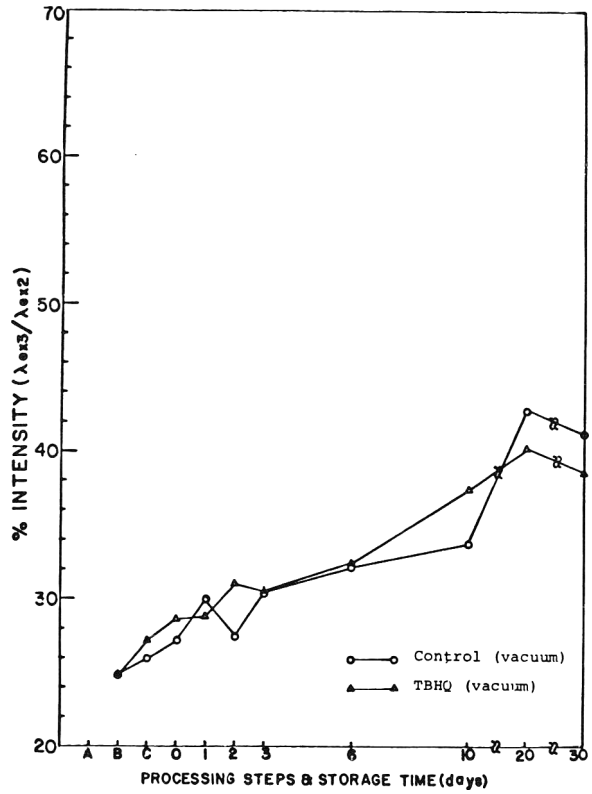

Fig. 3—Increase of $\lambda ex_3/\lambda ex_2$ in compressed freeze-dried beef upon storage (under vacuum) at 37°C.

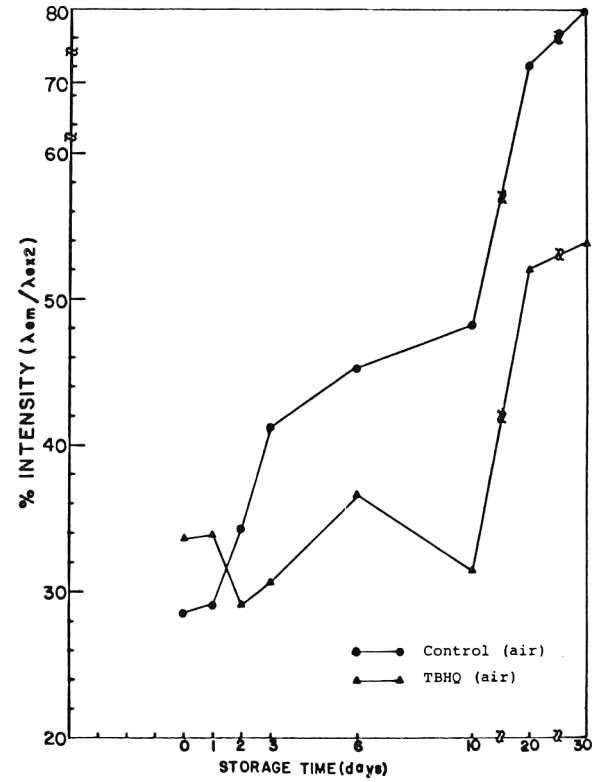

Fig. 4—Increase of $\lambda em/\lambda ex_2$ in compressed freeze-dried beef upon storage (under air) at 37°C.

Fluorescence-derived indices of duplicate samples taken during processing (A, B, and C) as well as freeze-dried samples groups 1, 2, 3, and 4 stored for 0, 1, 2, 3, 6, 10, 20, and 30 days (at 37°C) are presented in Fig. 2 through 5.

It is clear from Fig. 1 that in the excitation spectra, there are essentially three peaks with the following maximum wavelengths: $\lambda ex_1 = 308 \pm 2$ nm; $\lambda ex_2 = 318 \pm 2$ nm; $\lambda ex_3 = 350 \pm 2$ nm. The intensity of these peaks varied, depending on the extent of oxidation. Unoxidized samples (e.g. A, B, C and/or time zero freeze-dried sample) λex_2 and λex_3 had the highest and lowest intensities, respectively. As the extent of oxidation proceeds, λex_2 intensity decreased (progressively appeared as shoulder) while that of λex_3 increased. The shift in characteristic peaks could well be used to assess the degree of changes in oxidation. Consequently, the ratio of $\lambda ex_3/\lambda ex_2$ intensity may serve as an "internal standard" for determination of the extent of lipid oxidation in freeze-dried meats.

In the emission spectra, there was only one broad single peak whose intensity gradually increased upon oxidation while its maximum wavelength shifted from 476 ± 2 nm toward 440 ± 2 nm. Intensity increase of emission peak (λem), or more precisely, its ratio to λex_2, could serve as another index for determination of lipid oxidation by fluorescence technique.

To investigate the effect of antioxidant (100 ppm TBHQ), we calculated and used both internal standards (% intensity $\lambda ex_3/\lambda ex_2$ and $\lambda em/\lambda ex_2$) for each sample and used the mean value of two replicates for graphic representation of the data.

Fig. 2 compares the extent of oxidation, determined from $\lambda ex_3/\lambda ex_2$ ratio (ordinate axis) for samples of groups 1 and 3 (air as headspace) stored at 37°C for up to 30 days. Application of antioxidant (100 ppm TBHQ) had a pronounced effect on the prevention of lipid oxidation as measured by the above ratio. Fig. 3 compares the extent of oxidation, determined from $\lambda ex_3/\lambda ex_2$ ratio, in samples

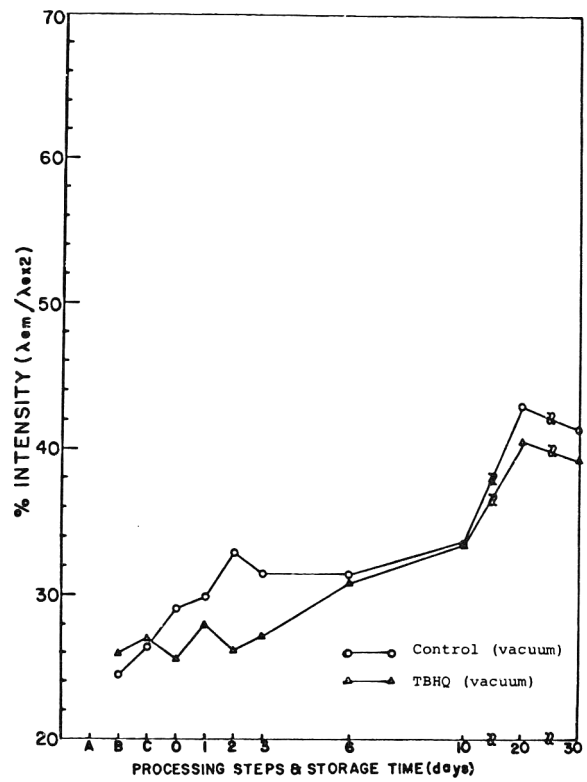

Fig. 5—Increase of $\lambda em/\lambda ex_2$ in compressed freeze-dried beef upon storage (under vacuum) at 37°C.

taken during processing as well as stored samples of groups 2 and 4 (under vacuum, no headspace). The overall rate and extent of lipid oxidation in these samples was much less than when air was present as headspace (Fig. 2). Consequently, this method detected the protection afforded by

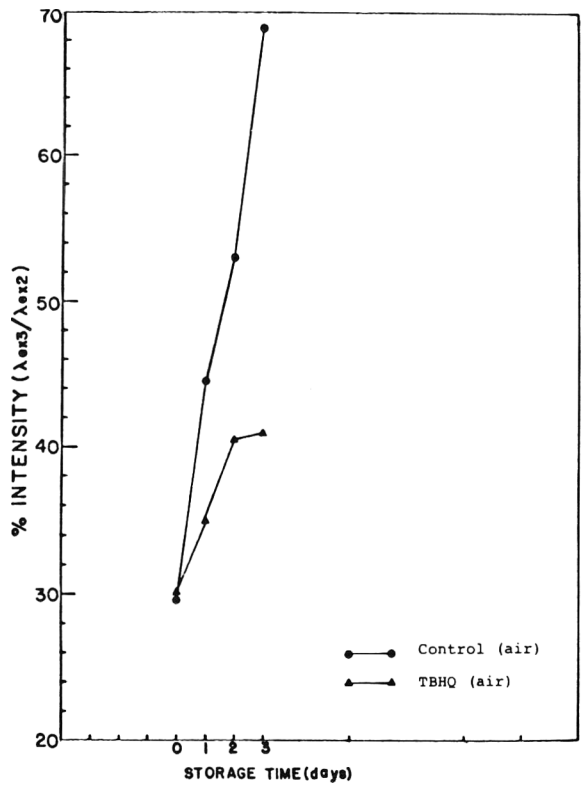

Fig. 6—"Earlier" detection of antioxidant protective effect in ground (not compressed) freeze-dried beef from $\lambda ex_3/\lambda ex_2$ values upon storage at 37°C.

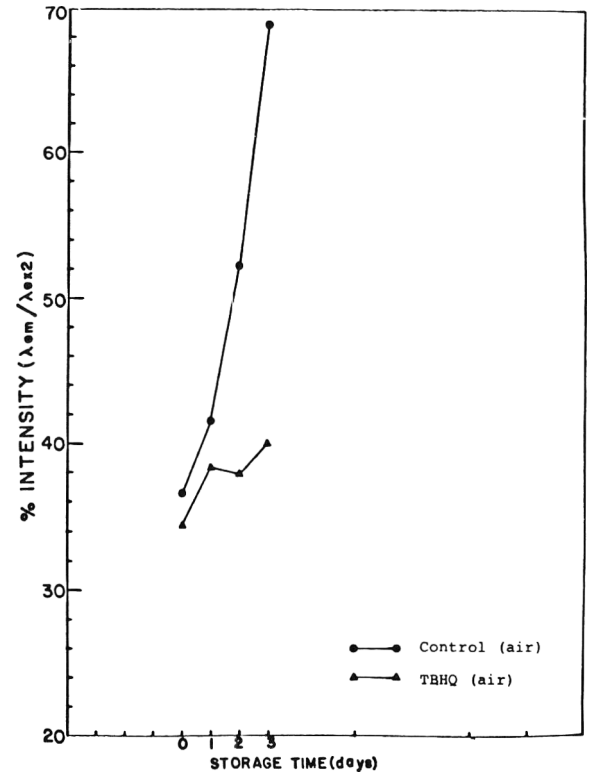

Fig. 7—"Earlier" detection of antioxidant protective effect in ground (not compressed) freeze-dried beef from $\lambda em/\lambda ex_2$ values upon storage at 37°C.

absence of air. When the headspace did not contain any oxygen, there was no effect of antioxidant, again confirming that the ratio was specifically measuring oxidation effects. It is also notable that samples taken during processing (A, B, and C) did not build up any fluorescent oxidation products and in fact there was negligible increase in ordinate axis from samples A up to the time zero freeze-dried samples, reflecting good manufacturing practice in preparation of these samples. Fig. 4 and 5 present the data of Fig. 2 and 3, respectively; the only difference is that the extent of oxidation has been determined from $\lambda em/\lambda ex_2$ ratio (ordinate axis). It is notable that both internal standard methods for detection of lipid oxidation by fluorimetry result in the similar values.

The freeze-dried beef samples in this study, unlike most porous freeze-dried beef samples, were "compressed" just before freeze-drying. Compressed products have far more resistance to oxygen diffusion into the samples which, in turn, result in less oxidation and better stability. To investigate the role of compression in prevention of lipid oxidation during storage, we "ground" the freeze-dried beef and repeated storage and fluorimetry experiments on "ground" samples of groups 1 and 3 (under air). Fig. 6 and 7 show the extent of oxidation, determined from both $\lambda ex_3/\lambda ex_2$ and $\lambda em/\lambda ex_2$ methods, in "ground" samples. Results indicate that when compressed samples were ground, they showed far higher rate and extent of oxidation during storage. For example, comparison of Fig. 6 with Fig. 2 shows that after 3 days of storage, the $\lambda ex_3/\lambda ex_2$ ratio of control (without antioxidant) compressed samples was 42% while the same samples, when ground, showed the value of 69%. Because of higher rate of oxidation in "ground", samples, the effect of applied antioxidant (100 ppm TBHQ) can be assessed at the earlier storage time. Comparison of Fig. 7 with Fig. 4 confirms the protective role of compression in prevention of oxidation. It also indicates that both internal standard methods, when applied to ground homogeneous samples, confirm each other's results.

The above spectra and their interpretation show that fluorescence spectrophotometry can be used as a useful simple and sensitive technique to indicate the extent of lipid oxidation in freeze dried meats.

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Determination of the Amount of *trans*-Octadecenoate and *trans*-9,*trans*-12-Octadecadienoate in Fresh Lean and Fatty Tissues of Pork and Beef

K. C. LIN, M. J. MARCHELLO, and A. G. FISCHER

ABSTRACT

Longissimus muscle and subcutaneous fat from 17 pork and 6 beef carcasses were analyzed for *trans*-octadecenoates and *trans*-9, *trans*-12-octadecadienoate (linolelaidate) content by argentation thin layer chromatography and/or glass capillary gas liquid chromatography. No detectable amount of *trans*-9,*trans*-12-octadecadienoate was found in pork or beef. In pork the amount of *trans*-octadecenoates ranged from 0.0074 - 0.0077 g/100g of fresh Longissimus muscle and 0.1453 - 0.1549 of fresh subcutaneous fat. The content of *trans*-octadecenoates in beef ranged from 0.0782 - 0.0839 g/100g of fresh Longissimus muscle and 2.4280 - 2.7368 g/100g of fresh subcutaneous fat. The content of *trans* fatty acids contributed by pork and beef to the human diet is negligible relative to that derived from other dietary sources.

INTRODUCTION

SINCE THE BEGINNING of this century the partial hydrogenation of liquid vegetable oil into semisolid and solid fats has introduced a substantial amount of *trans* fatty acids into the human diet. *Trans* fatty acids are not only readily absorbed and catabolized by experimental animals, they are incorporated readily into all tissue in the human body (Davignon et al., 1980). There is increasing interest in the biological utilization and effects of *trans* and other isomeric unsaturated fatty acids on human health. Recent reports have shown that *trans* fatty acids interfere with essential fatty acid metabolism (Holman, 1981; Privett et al., 1977) and cause isochemic vascular disease (Kummerow, 1975). Enig et al. (1979) suggested that there is a correlation between dietary fat and cancer due to the increasing ingestion of *trans* fatty acids. These previous studies focused on the *trans* fatty acids of hydrogenated oils. It should be noted that *trans* fatty acids are formed by the rumen microorganisms of ruminant animals (Viviani, 1970) and are found in fat-containing products from ruminants such as cow's milk and butter (Woodrow and DeMan, 1968; Hay and Morrison, 1970; Parodi, 1976) and meats (Lanza and Slover, 1981).

Pork is one of the major meat sources in human diets. Rizek et al. (1974) reported that about 34 kg of pork was consumed per person in 1972 in the United States. The amount of consumption per capital remained constant in 1982. However, there is very little information available as to the amount of *trans* fatty acids present in pork tissue when pigs are fed commercial growing and finishing rations. Ackman et al. (1981) reported that a single retail market pork chop contained *trans*-9,*cis*-11- and *cis*-9,*trans*-11-octadecadienoate as 0.15% of the total fatty acid content. Therefore, the main objective of this research was to determine the amount of *trans* fatty acids in lean and fatty tissue of cattle and hogs fed traditional growing-finishing rations.

Author Marchello is affiliated with the Dept. of Animal Science and Author Fischer is with the Dept. of Biochemistry, North Dakota State Univ., Fargo, ND 58105. Author Lin is affiliated with the Dept. of Animal Science, Texas A&M Univ., College Station, TX 77843.

MATERIALS & METHODS

Sample preparation

Seventeen crossbred swine were randomly selected from pigs fed a regular growing-finishing diet containing 16% protein. Swine were slaughtered at market weight (100 - 109 kg) and the carcasses were chilled in a 3°C cooler 24 hr prior to processing. Outer subcutaneous fat (backfat) over the 11th to 13th ribs and the Longissimus muscle (loin) from the same area were removed from the right side of each carcass at the time of processing.

Six steers, fed a traditional fattening ration, were slaughtered at market weight (approximately 500 kg). The carcasses were chilled in a 3°C cooler for 48 hr prior to processing. The Longissimus muscle was obtained between the 10th and 12th ribs and the subcutaneous fat was removed from the same area from the right side of each carcass at the time of processing.

Lean and fat samples were lyophilized, thoroughly homogenized and stored under nitrogen at −18°C before lipid extraction. The weight of each sample before and after lyophilization were recorded to determine fresh weight calculation. The lipid from both animal samples was extracted and the *trans* fatty acids were separated by preparative argentation thin-layer chromatography and then identified and quantified by glass capillary gas liquid chromatography.

Lipid extraction and lipid content measurement

Lyophilized samples were extracted with a mixture of chloroform and methanol (Bligh and Dyer, 1959). Twenty milliliters distilled water were added to each 5g lyophilized sample to adjust it to 80% water content. The extracted lipids were stored under N_2 and frozen at −18°C for further analysis. The lipid content of lean tissue was measured by the Foss-let apparatus (Foss-let 15310, A/S N. Foss Electric Emask). A separate 5-g lyophilized sample was introduced into the apparatus and the lipid content was determined by the specific gravity of the solvent-lipid solution. The lipid content of fatty tissues (backfat) was determined by the method of Bligh and Dyer (1959).

Transesterification of the extracted lipids

A modification of the procedure described by Metcalfe and Wang (1981) was used. A pork sample (75 - 90 mg) of extracted lipid and 2.0 mg internal standard, *trans*-heptadecenoic acid methyl ester (17:1ω7*t*) (Nu Chek Prep, Inc.) were placed in a 13 x 100 mm test tube with a Teflon-lined cap. Two hundred μL 20% solution of tetramethylammonium hydroxide (TMAH) in methanol (W/V) (Aldrich Chemical Co.), and 3.0 mL diethyl ether (reagent grade) was added and the capped tube was shaken vigorously on a Vortex mixer for 3 min. Deionized water was added and shaken again for at least 30 sec. The diethyl ether layer and water layer were separated by centrifugation at 3200 x rpm (Clay-Adams Co., CT-1000) for 2 min. The clear upper ether layer containing the internal standard and the methyl esters derived from the lipid fatty acids was transferred to another 13 x 100 mm screw test tube, sealed with a Teflon-lined cap and stored at −18°C until analyzed.

Because of the complexity of the beef fatty acid composition (Viviani, 1970), the extracted lipid from the beef samples was transesterified in the presence and absence of internal standard.

Trans fatty acid assay

A modified method of Lanza and Slover (1981) was used for identification and quantification of the *trans* unsaturated fatty acids in the pork and beef tissues. A schematic of the experimental procedure used for analyzing *trans* fatty acids is given in Fig. 1.

Gas chromatography. A 2.0 μL transesterified sample containing the standard and fatty acid methyl esters (FAME) was taken for

direct gas chromatography, the remainder of the sample was subjected to preparatory argentation thin-layer chromatography (Ag-TLC). The gas chromatographic conditions were as follows: injection port temperature, 230°C; flame ionization detector (FID) temperature, 300°C; helium (He) flow rate, 0.6 mL/min (30 psi); split ratio 1/110. The silica fused glass capillary column (Sp2340, 60m x 0.25mm i.d., Supelco, Inc.) was installed in a Hewlett Packard Model 5880A and was temperature-programmed from 170°C to 210°C at 2.0°C/min. Identification of the FAME sample was based on retention time of the known standard FAME. For further confirmation of the *trans* fatty acid present, the sample was spiked by adding the respective authentic standard FAME into the sample which was then re-chromatographed.

Argentation thin-layer chromatography. A 100 μL aliquot of the transesterified lipids was applied to each plate as a 2 mm band at the origin with a 50 μL syringe. Each plate was developed twice in a solvent of petroleum ether-diethyl ether (92:8 V/V) in order to minimize tailing. Five fraction bands were visualized by spraying the argentation TLC plates with 0.1% 2′, 7′-dichlorofluorescein in methanol (Supelco, Inc.) and locating the band under U.V. light (long wave length). The area on the TLC plates corresponding to the *trans* fractions of each samples was scraped off and eluted three times with a total of 8 mL (3 mL, 3 mL, 2 mL) diethyl ether. The eluates after each centrifugation were transferred and combined in a 13 x 100 mm screw-capped test tube. The extracted material was concentrated to dryness by evaporating under a stream of nitrogen and re-dissolved in 50 μL diethyl ether, and then 2.0 μL of the solution were applied to gas chromatography analysis as described previously.

Detectable level limit of the gas chromatography

Authentic FAME standard (18:2ω6*tt*) was used for measuring the detectable limit of the gas chromatographic procedures employed in this study. A 48.25 mg sample of standard was weighed, dissolved in 5.0 mL (9.75 mg/mL) diethyl ether as stock solution and then further diluted in diethyl ether prior to gas chromatography.

Calculation

The amount of *trans* FAME was calculated by comparing the area ratio between the *trans* FAME and the known amount of internal standard methyl ester (17:1ω7*t*). The calculation procedure is described below:

Sample calculation for pork.

$$\frac{a}{S} \times Sm = A$$

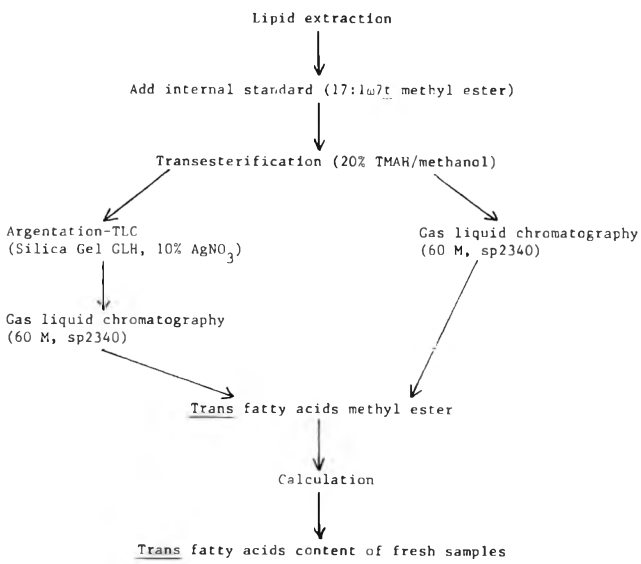

Fig. 1—Schematic for determination of trans-octadecenoic acid and trans,trans-octadecadienoic acid in fresh lean and fatty tissue of pork and beef.

$$A \times \frac{am}{an} = B$$

$$B \times \frac{F\%}{Ft} = C$$

$$C \times \frac{L\%}{100} = \%\ \text{of } \textit{trans}\text{-octadecenoic acid in the fresh sample.}$$

a = Area amount of *trans*-octadecenoic methyl ester;
S = Area amount of internal standard;
Sm = Amount of internal standard added in the sample;
am = 282 g/mole (molecular weight of *trans*-octadecenoic acid);
an = 296 g/mole (molecular weight of *trans*-octadecenoic acid methyl ester);
B = Amount of *trans*-octadecenoic acid in extracted lipid;
F% = Lyophilized sample lipid content (%);
Ft = Amount of extracted lipid transesterified (mg);
C = % *trans*-octadecenoic acid in the lyophilized sample;
L% = Lyophilized sample weight % in fresh sample.

Sample calculation for beef.

$$\frac{Un}{D1} \times Dlm = Y$$

$$\frac{Un}{D2} \times D2m = Z$$

$$\frac{a}{S\text{-}Y} + \frac{a}{S\text{-}Z} \times Sm \div 2 = A.$$

Un = Area amount of the unknown peak without added internal standard;
D1 = Area amount of stearic (18:0) without added internal standard;
D1m = Area amount of stearic with internal standard added sample;
Y = Correction factor of the area amount of the internal standard added sample;
D2 = Area amount of palmitic (16:0) without added internal standard;
D2m = Area amount of palmitic with internal standard added sample;
Z = Correction factor of the area amount of the internal standard added sample.

The value obtained for A is then treated as described for pork sample calculation.

Statistical analysis

The data from each sample, which was run in duplicate, were averaged by duplicate and paired t tests (Snedecor and Cochran, 1980) were used to analyze the difference between the direct gas chromatography and Ag-TLC- gas chromatography combination methods for pork and beef individually. The pooled data (beef and pork samples) were analyzed for the difference between the two experimental methods by the same statistical analysis procedure. Confidence intervals (95%) were calculated for each of the type of tissues, species combination means. The difference between beef and pork *trans* content was analyzed by the independent sample t test (Snedecor and Cochran, 1980).

RESULTS & DISCUSSION

THE AMOUNT of *trans*-octadecenoate determined by direct gas chromatography and preparative Ag-TLC followed by gas chromatography is shown in Table 1. No *trans*-9,*trans*-12-octadecadienoate (linolelaidate) peak was detected at the level of 0.0033 mg/100g of lipid in pork and beef samples in both analytical procedures. The beef *trans*-octadecenoate content is ten and seventeen times higher than pork in Longissimus muscle (lean tissue) and subcutaneous fat (fat tissue), respectively.

Despite the voluminous literature on fatty acids in plants, animals and their products, much of it is qualitative rather than quantitative and is unsuitable for entry in

nutrient tables. Therefore, the *trans*-octadecenoate content in this study is reported on the absolute content of the fresh sample by relating the peak area to the known internal standard (17:1ω7*t*), instead of reporting on the relative percentage distribution of the total fatty acid methyl esters. One can estimate the total consumption of the fatty acid from the respective food product by this method.

No peaks overlapped with the internal standard peak in pork samples. Therefore, the area ratio of internal standard and the *trans*-octadecenoate peak are calculated as the amount of *trans*-octadecenoate in the sample of direct gas chromatographic analysis (Table 1). Because of the complexity of the fatty acid composition in beef tissues the peak of internal standard overlapped with other fatty acids endogenous to the beef tissue, each beef sample (lean and fatty tissue) was directly gas chromatographed before internal standard was added. The area ratios from these chromatograms of the unknown small peak to the stearate (18:0) and palmitate (16:0) peaks were calculated, respectively. These ratios of each beef sample were treated as correction factors for the respective internal standard peak area which was subsequently added to each sample.

A more satisfactory resolution by gas chromatography was obtained by pre-separation of the sample FAME into different fractions (saturates, *cis*-monoenes, *trans*-monoenes, dienes, polyenes) by preparative Ag-TLC. The internal standard (17:1ω7*t*) and *trans*-octadecenoate appeared in the same fraction (second fraction) on the preparative Ag-TLC. This agreed with the work described by Smith et al. (1978). Furthermore, the internal standard overlapping with the endogenous fatty acid in beef was avoided by this preparatory procedure. The difference between direct gas chromatography and preparative Ag-TLC-gas chromatography is shown in Table 2. Higher Longissimus muscle *trans*-octadecenoates values were obtained with the Ag-TLC-gas chromatography method than by direct gas chromatography. The paired t test showed no significant difference ($P < 0.10$) between the two methods of analysis for lean tissue. However, there was a significant difference ($P < 0.02$) between the two methods for fatty tissue. This was especially true for beef in which the Ag-TLC-gas chromatography exceeded the direct gas chromatography by a mean difference of 0.3088. There was very little difference between the two methods for pork fat. The difference was significant because of the low standard error (Tables 1 and 2). Furthermore, the incomplete removal or elution of the sample from TLC plates and the great variability among each individual sample may also contribute to these differences.

Comparing the two different methods of analysis in four food items, Lanza and Slover (1981) reported that the agreement of *trans*-octadecenoates content between direct gas chromatography and Ag-TLC-gas chromatography in these samples ranged from 84% to 96%. Using similar analytical procedures, the agreement of *trans*-octadecenoate content in this study are 89% to 106% in both beef and pork samples (Table 2). More specifically, the agreement of the two methods for analysis of beef sample in this research is 93% in lean tissue and 89% in fatty tissue, while Lanza and Slover (1981) reported 96% in their beef lean sample. Lanza and Slover (1981) indicated that the difference between the two methods may be a result of the incomplete resolution by direct gas chromatography. This study and that of Lanza and Slover (1981) suggest that the preparative Ag-TLC is required for a more complete estimation of the *trans* fatty acid content. Furthermore, the *trans*-octadecenoate content of beef lean tissue reported here, 0.0782% and 0.0839% (g/100g of lean

Table 1—Amount of trans-octadecenoic acids (trans-18:1) in fresh Longissimus muscle and subcutaneous fat of pork and beef.

		Lipid content (g/100g fresh sample)	Direct gas chromatography (g/100g fresh sample)	Argentation TLC and gas chromatography (g/100g fresh sample)
Longissimus muscle	Pork[a]	6.2 ± 2.1%[c]	0.0074 ± 0.0025[c] (0.0006[d], 0.0013[e])	0.0077 ± 0.0024 (0.0006, 0.0013)
	Beef[b]	5.0 ± 1.4%	0.0782 ± 0.0445 (0.0182, 0.0467)	0.0839 ± 0.0443 (0.0181, 0.0465)
Subcutaneous fat	Pork[a]	85 9 ± 3.3%	0.1549 ± 0.0450 (0.0109, 0.0231)	0.1453 ± 0.0437 (0.0106, 0.0225)
	Beef[b]	87.4 ± 3.4%	2.4280 ± 1.1146 (0.4550, 1.1699)	2.7368 ± 1.2144 (0.4958, 1.2746)

[a] n = 17
[b] n = 6
[c] Standard Deviation
[d] Standard Error of Mean
[e] One half width of 95% confidence interval

Table 2—Comparison of the direct gas chromatography (without Argentation TLC) and Argentation TLC-gas chromatography methods by paired t test

		Mean difference[d]	Significance level	Standard error of mean	Agreement of mean (%)[e]
Pork	*Longissimus* muscle[a]	−0.0003	0.19	0.0002	96%
	Subcutaneous fat[a]	+0.0096	0.02	0.004	106%
Beef	*Longissimus* muscle[b]	−0.0057	0.10	0.003	93%
	Subcutaneous fat[b]	−0.3088	0.01	0.064	89%
Overall[c]		−0.0384	0.03	0.017	

[a] n = 17
[b] n = 6
[c] n = 46
[d] Direct minus TLC
[e] Agreement of mean (%) = (Mean of Direct/Mean of TLC) x 100

tissue), are lower than the results reported by Lanza and Slover (1981), 0.24% and 0.23% (g/100g of lean tissue), with and without Ag-TLC preparation, respectively. The difference between the content of *trans*-octadecenoate of the two reports might be due to a different feeding regimen. Ramsey et al. (1972) indicated that cattle raised entirely on hay or pasture have a higher degree of saturated fatty acids in their carcass tissue than grain-fed cattle, which contained higher unsaturated fatty acids. The increase in unsaturation was reflected most consistently by an increase in octadecenoates. Viviani (1970) also indicated that higher unsaturated fatty acid feed ratio facilitates the microbial biohydrogenation and formation of the *trans* fatty acids.

This study found that the authentic FAME mixture containing *trans*-9,*trans*-12,-octadecadienoic methyl ester migrated with the *cis* monoene fraction (third fraction) on preparative Ag-TLC. However, pork or beef samples analyzed by direct gas chromatography or gas chromatography after Ag-TLC preparation indicated no detectable amount of *trans*-9,*trans*-12-octadecadienoate. Ackman et al. (1981) analyzed one retail pork chop and found 0.15% (of total fatty acids) *cis*-9,*trans*-11 and *trans*-9,*cis*-11-octadecadienoates present. The standard of *cis,trans*- and *trans,cis*-octadecadienoate were not available for this research. Therefore, data regarding *cis-trans* dienoic isomers were not obtained.

The content of *trans*-octadecenoate in beef samples is ten- and seventeen-fold greater than in pork lean and fatty tissue, respectively. Although it is known that *trans* fatty acids are usually formed by the rumen microorganisms, Ackman et al. (1981) suggested that the swine intestinal microbial processes form some of the *trans* isomers which contribute to the presence of these acids in pork and attribute to part of the *trans* fatty acids in human foods. However, this study indicated that the amount of the *trans* fatty acid in pork compared to the hydrogenated fat, 10.8% (g/100g of sample) of *trans*- octadecenoate (Lanza and Slover, 1981), are negligible.

This research indicates that a daily consumption of 200g of pork chops (30% fat, 70% lean) and 100g of beef steak (30% fat, 70% lean) would give a human approximately 0.9g of *trans*-octadecenoate, which is relatively negligible in the human diet.

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Functional Properties of Enzymatically Modified Beef Heart Protein

DENISE M. SMITH and CLARK J. BREKKE

ABSTRACT

The effect of enzymatic modification on beef heart protein functionality was examined in model system and frankfurter experiments. Modification of heart myofibrils with ficin was effective in improving protein solubility and emulsification capacity compared to controls. Incorporation of enzyme-modified heart into a meat model system composed of 30% beef heart and 70% beef skeletal meat resulted in improved cooked yields which were equal to controls made with 100% skeletal meat, in both no-salt and 3% salt formulations. Normal (2%) and low (0.5%) salt frankfurters made with 30% enzyme-modified beef heart had significantly greater smokehouse yields and consumer cooked yields than frankfurters made with 30% unmodified heart.

INTRODUCTION

BEEF HEART is a low-cost, yet nutritious protein source which, due to its relatively poor protein functionality, has limited use in processed meat products (Forrest et al., 1975; Porteous, 1979). It is considered to be a low binding meat with poor water retaining (Wiley et al., 1979) and low emulsifying abilities (Forrest et al., 1975). Deficiencies in beef heart functional properties result in the formation of an undesirable processed meat product, leading to processing losses.

Chemical and enzymatic modification have been used extensively to improve the functional properties of low-function proteins and to tailor the functionality of certain proteins to meet specific processing needs (Richardson, 1977; Brekke and Eisele, 1981). Eisele and Brekke (1981) used chemical modification with acid anhydrides to improve the functionality of isolated beef heart myofibrils. Modified myofibrils were superior to controls in model system tests of solubility, emulsification capacity, and emulsion stability. Brekke and Eisele (1981) also reported preliminary experiments on the effect of adding chemically modified myofibrils to formulations composed of 30% heart and 70% beef skeletal meat using the emulsified meat model system of Randall et al. (1976). When myofibrils were modified with either acetic or succinic anhydride and substituted for 8% of the heart component, no significant improvements were observed in cooked yield or fat binding ability when compared to controls. This was due to the inability to incorporate enough modified myofibrils in the model system formulation because of moisture considerations. The use of chemical modification presents other problems as it may decrease the nutritional value of the protein or cause toxicological problems (Eisele et al., 1982).

Enzymatic modification has been found effective in improving the functionality of many plant and animal proteins (Richardson, 1977), and thus may be a viable alternative to chemical modification. Partial protein hydrolysis with proteases is the most common method of enzymatic modification (Brekke and Eisele, 1981). Enzymatic modification does not harm the nutritive value of a protein, is nontoxic, specific, used under mild conditions, and the use of enzymes in foods is accepted by the public (Phillips and Beuchat, 1981). Therefore, the objectives of this research were to determine the effect of enzymatic modification on beef heart protein functionality in model systems and to evaluate the effectiveness of incorporating enzyme-modified beef heart into frankfurters prepared on a pilot plant scale.

Authors Smith and Brekke are affiliated with the Dept. of Food Science & Human Nutrition, Washington State Univ., Pullman, WA 99164-6330.

MATERIALS & METHODS

Preparation of myofibrils

Frozen beef hearts were purchased from the Washington State University (WSU) Meats Laboratory and stored in polyethylene bags at −30°C. Before use the hearts were thawed 24 hr at 4°C, and heart valves, excess fat and connective tissue removed. The remaining muscle was cut into small cubes and ground by a single pass through the 4 mm plate of a chilled food grinder. Myofibrils were isolated in 0.01M NaCl, 0.05M K-phosphate, pH 7.4, as described by Eisele and Brekke (1981). The final pellet was suspended in 0.1M NaCl, 0.05M K-phosphate, pH 7.0 to produce a final protein concentration of 35-45 mg/mL buffer.

Hydrolysis conditions

Ficin (crude, from fig tree latex, 100 casein solubilization units/g solid) was obtained from Sigma Chemical Co. (St. Louis, MO). Ficin (20% w/v) was activated for 5 min in 0.007M mercaptoethanol, 0.001M EDTA, 0.1M K-phosphate, pH 7.0. Hydrolysis was initiated by adding the activated enzyme at a crude ficin:protein ratio of 1:100 (w/w). Hydrolysis was performed at 20°C with slow stirring on a magnetic stirring apparatus. Samples were removed at desired intervals between 0 and 150 min. Ficin was inactivated by adding iodoacetate (IA). Preliminary experiments using beef heart myofibrils as substrate indicated that ficin was completely inactivated when IA was added on an equal weight basis and that this concentration of IA had no effect on myofibril solubility. This concentration of IA was used throughout this study to inhibit ficin. Two myofibril controls were included, one with iodoacetate alone, and one which contained ficin/activation buffer/iodoacetate in combination. Samples were stored in ice until used within 3 days.

Tests of protein functionality

Protein solubility. Hydrolysis was followed by measuring the increase in protein solubility with time of enzyme treatment. Ten milliliters of the protein suspension, pH 7.0, was centrifuged at 10,000 × *g* for 15 min. Total protein and protein in the supernatant were measured using biuret reagent (Gornall et al., 1949). Percentage solubility was calculated by dividing the protein content of the supernatant by the total protein content then multiplying by 100.

Emulsification capacity. Emulsification capacity (EC) of the enzyme-modified myofibrils was determined using a procedure similar to that of Webb et al. (1970), as modified by Eisele and Brekke (1981). Proteins were tested at a concentration of 1.0 mg/mL in 0.1M NaCl, 0.05M K-phosphate, pH 7.0. An ohmmeter (Simpson 260, Simpson Electrical Co., Chicago, IL) was used to monitor an increase in electrical resistance which occurred upon emulsion collapse. Results were expressed as milliliters of oil emulsified per milligram of protein.

Number of trials. All data represent a mean value of triplicate analyses on each of three myofibril preparations.

Meat system experiments

Raw materials. Pork backfat and beef skeletal trimming were purchased from the WSU Meats Laboratory. After thawing at 4°C the pork backfat was ground two times through the 6 mm plate of a grinder and stored in polyethylene bags at −30°C. Beef heart and beef trimmings were ground once through a 6 mm and a 4 mm plate of a food grinder and stored in polyethylene bags at −30°C. The meat ingredients were analyzed for moisture, protein, and fat (AOAC, 1980).

Preparation of enzyme-modified heart. Ground heart equilibrated to 20°C was placed in a Kitchen Aid Mixer (Hobart Corp., Troy, OH) and activated enzyme added at a crude ficin: protein ratio of 1:100 (w/w) while mixing with the paddle attachment at the lowest speed setting. The meat was mixed for 1 min, and thereafter for 30 sec at 10 min intervals. Samples were removed at the desired time intervals, and iodoacetate added to inhibit ficin. The samples were stored at 4°C until used the next day.

Solubility changes were monitored by mixing 2g of modified heart with 8 mL of 0.1M NaCl, 0.05M K-phosphate, pH 7.0, or 0.6M NaCl, 0.05M K-phosphate, pH 7.0 in a Polytron homogenizer (Bronwill Scientific, Rochester, NY) for 15 sec, and assaying as previously described for protein solubility. These two NaCl concentrations were selected to approximate concentrations in low- and high-salt frankfurters.

Emulsified meat model system. An emulsified meat model system described by Randall et al. (1976) was used to evaluate the effectiveness of enzymatic modification. Emulsions were formulated to contain 12.0% protein and 35.0% fat, with or without 3.0% salt. The meat component of the system was composed of a 30:70 blend of unmodified or modified beef heart and beef skeletal meat. Pork backfat and water were added to the meat to achieve the desired formulation.

The meat, water, and salt (if called for in the formulation) were added to a 31.5 mm by 164 mm polypropylene tube, and blended with a Willems Polytron Homogenizer at a speed setting of eight. After blending for 30 sec the pork backfat was added and the ingredients emulsified to homogeneity (ca. 60 sec more). The polypropylene tubes were immersed in an ice/water bath during blending to prevent heating of the meat emulsion.

The prepared emulsion (ca. 36-39 g) was packed into 29 mm by 103 mm preweighed polypropylene centrifuge tubes containing a wire support. The emulsion was cooked for 30 min in a 75°C water bath. After cooking, the meat plug was removed from the tube and weighed. The cook-out liquid was transferred to a 10 mL graduated cylinder. The total cook-out volume and the volume of the fat and aqueous fractions were measured. All data represent a mean value of duplicate analyses on each of three separate emulsion preparations.

Frankfurter preparation. Two treatments were compared: a 30% beef heart/70% beef skeletal meat frankfurter and a 30% ficin-modified beef heart/70% beef skeletal meat frankfurter. The skeletal meat, heart, and fat were thawed overnight at 4°C. Ingredients were weighed out according to each treatment formulation and the fat and meat held another 24 hr at 4°C until completely thawed. The frankfurter batter was formulated to contain 12% protein, 29% fat, 66% water, 1.8% spice mix, and either 0.5% or 2.0% salt. Formulations were designed to give identical proximate compositions among all treatments.

Frankfurters were manufactured in a 12°C work room. Skeletal meat, spice mix, half the water, salt, and heart (unmodified or modified) were placed in a Hobart silent cutter (Model 84142, Troy OH). The ingredients were chopped for 5 min (1725 rpm) until the batter temperature reached 6 - 9°C. The fat and remaining water were added, and the ingredients were chopped for another 5 min until the batter temperature reached 11 - 12°C. Duplicate frankfurter batches were prepared for each treatment.

Immediately after chopping, the batter was stuffed into 25 mm Nojax casings (Union Carbide, Chicago, IL) using a Frey 20 liter Electro-Hydraulic Stuffer (Koch Supplies, Inc., Kansas City, MO). The frankfurters were linked into 13 cm lengths using a Koch manual linking machine (Kansas City, MO) and each batch weighed separately. The frankfurters were then hung on a rack and held until all formulations were prepared. The unprocessed frankfurters were rinsed with a cold water spray and placed in the smokehouse (Enviro-Pak, Portland, OR).

Table 1—Smokehouse schedule for processing frankfurters

Smokhouse temp (°C)	Time (min)	Relative humidity (%)	Smoke
54	30	*	no
63	30	**	yes
77	ca. 100	39	yes

* Dampers Open
** Dampers Closed

The frankfurters were processed according to the smokehouse schedule shown in Table 1. When the frankfurter internal temperature reached 67°C (ca. 100 min) the smokehouse was turned off and the frankfurters cooled in a cold water spray until the internal temperature reached 32°C (ca. 5 min). The frankfurters were held in a 4°C cooler overnight and then reweighed to determine smokehouse yield.

Frankfurter casings were removed by hand, and the frankfurters were stored in polyethylene bags at 4°C until evaluated. The frankfurters were analyzed in triplicate for moisture, protein, fat, and salt following standard AOAC procedures.

Consumer cook-out test. A cook-out test described by Froning et al. (1971) was used to evaluate the cooked stability of the frankfurters. A frankfurter was weighed and placed in 400 mL of boiling water for 6 min. The frankfurter was removed from the cooking water, cooled 5 min at room temperature, blotted dry, and reweighed to calculate cooked yield. The cooking water was transferred to a graduated cylinder and allowed to cool to room temperature. The volume of fat in the cooking water was recorded. Three frankfurters from each treatment were tested.

Statistics. Results were analyzed for significance ($P < 0.05$) using Duncan's new multiple range test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Model system functionality

Protein functionality depends to a great extent on the amount of soluble protein present in the system (Kinsella, 1976). It is for this reason that protein solubility is often measured when evaluating protein functionality. The myofibrillar proteins of muscle are most responsible for the functional responses in a comminuted meat product. Unmodified myofibrillar proteins are insoluble at low ionic strength, requiring 0.6M NaCl or greater for solubilization (Forrest et al., 1975). The relative activity of ficin toward beef heart myofibrils was compared by monitoring the increase in myofibril solubility in 0.1M NaCl, 0.05M K-phosphate, pH 7.0 with time of enzyme treatment when 1% (w/w) enzyme was added (Fig. 1). The solubility of the iodoacetate control did not differ from the unmodified myofibrils. The ficin/activation buffer/iodoacetate control had 1% more soluble protein than the unmodified myo-

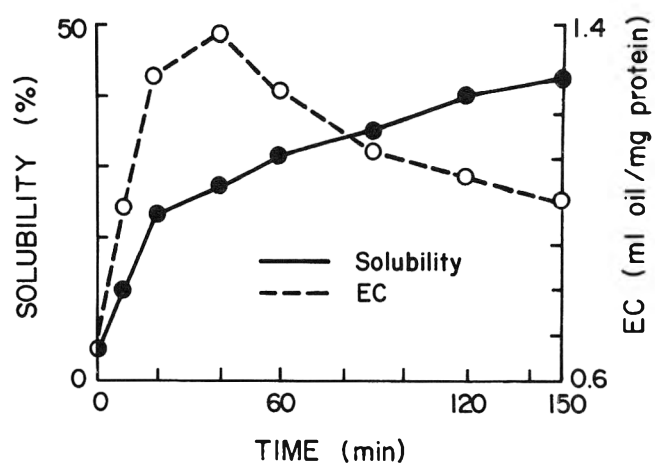

Fig. 1—Effect of duration of enzyme treatment on the solubility and emulsification capacity of beef heart myofibrils in 0.1M NaCl, 0.05M K-phosphate, pH 7.0.

fibrils. Ficin increased the solubility of heart myofibrils to 42% in 150 min. In a previous study, chemical modification improve the solublity of beef heart myofibrils in 0.1M NaCl, 0.05M K-phosphate, pH 7.0 up to 72% depending on the type and quantity of acid anhydride used (Eisele and Brekke, 1981).

Emulsification capacity (EC) is one of the most widely used tests of meat protein functionality, as a comminuted meat product exhibits many characteristics of a true emulsion (Kinsella, 1976; Hamm, 1973; Van den Oord and Wisser, 1973). Enzymatic modification of beef heart myofibrils with ficin increased the EC compared to the control (Fig. 1). Ficin inactivated with iodoacetate and then added to heart myofibrils had the same EC as controls. EC reached a maximum at an extent of proteolysis sufficient to increase myofibril solubility to 28% and then decreased with further increases in solubility. EC increased from 0.67 mL oil emulsified/mg protein to 1.38 mL oil emulsified/mg protein at 28% solubility.

Using the same emulsification procedure, Eisele and Brekke (1981) reported that chemical modification with varios anhydrides doubled the EC compared to controls. Enzymatic modification was successful in increasing the EC of fish myofibrillar proteins (Spinelli et al., 1972), salt-extractable proteins of beef muscle (DuBois et al., 1972), and soy proteins (Puski, 1975). DuBois et al. (1972) reported that the EC increased or decreased from controls depending on the extent of proteolysis and type of protease used.

The effect of protein structure on emulsifying ability has been reviewed by Kinsella (1982). During the preparation of a protein-based emulsion, the formation of an interfacial film occurs in three stages: diffusion of the proteins to the interface, protein unfolding, and rearrangement of the denatured protein molecules to their lowest free energy state to form a film. Protein solubility is important for rapid diffusion to the interface. Intramolecular bonds affect the rate and extent of protein unfolding. Electrostatic, hydrophobic, and steric factors facilitate or inhibit molecular rearrangement and retard coalescence. Enzymatic modification with proteases may alter all of these properties of the original molecule by forming smaller protein fragments with different solubilities, intramolecular bond arrangements, and proportions of charged groups. Consequently, although solubility and EC are usually positively correlated, other properties of the protein may affect emulsification (Nakai, 1983). Detrimental changes in these other properties may be responsible for the decrease in EC above 28% solubility.

Table 2—Mean cooked meat yield and cook-out composition of model system meat emulsions prepared with a 30/70 blend of ficin-modified beef heart and beef skeletal meat

Sample	Protein solubility[a] (%)	Cooked yields[b,c] (%)	Cook-out composition[d] Water (%)	Cook-out composition[d] Fat (%)
No Salt				
100% Skeletal		71.3a	62	38
30/70:control	31	63.5b	53	47
:10 min	39	68.0b	61	39
:30 min	45	68.4b,c	58	42
:60 min	49	72.7a	66	34
:120 min	53	70.3a,c	58	42
3% Salt				
100% Skeletal		75.6d	65	35
30/70:control	44	71.4a	57	43
:10 min	54	74.0a	66	34
:30 min	63	72.5a	67	33
:60 min	70	76.2d	67	33
:120 min	75	76.8d	71	29

[a] Solubility (%) = $\frac{\text{Protein in supernatant after centrifugation}}{\text{Total protein concentration}} \times 100.$

No salt: solubility measured in 0.1M NaCl, 0.05M K-phosphate, pH 7.0 3% salt: solubility measured in 0.6M NaCl, 0.05M K-phosphate, pH 7.0.

[b] Means followed by the same letters did not differ significantly ($P>0.05$).

[c] Cooked yield (%) = $\frac{\text{Weight of meat emulsion after cooking}}{\text{Weight of raw meat emulsion}} \times 100.$

[d] Proportion of fat and water in the cook-out liquid.

Emulsified meat model system

An emulsified meat model system similar to that described by Randall et al. (1976) was also used to evaluate the effectiveness of enzymatic modification. This model system simulates commercial frankfurter composition and preparation more closely than the functional tests, but requires less sample and equipment than a pilot plant study. Meat emulsions were prepared as a 30/70 blend of beef heart and beef skeletal meat. Thirty percent heart was included in the formulation as this amount was sufficient to stress the system and allow for the evaluation of the effectiveness of enzymatic modification. The beef heart fraction was modified with ficin for various times up to 2 hr. The results are shown in Table 2. In both the no-salt and 3% salt formulations, greater cooked yields were obtained for 100% skeletal meat than for the controls containing 30% heart. These results were expected due to the poor functionality of beef heart protein.

In samples made without salt, the cooked yield increased from 63.5% for the 30/70 control (31% soluble protein) to 72.7% for the sample enzyme-modified for 60 min (49% soluble protein). The cooked yield for the 60 min enzyme-modified sample was not significantly different from the no-salt 100% skeletal control. When 3% salt was used in the formulation, the cooked yield increased from 71.4% for the 30/70 control (44% soluble protein) to 76.8% yield after 120 min of modification (75% soluble protein). Again, this yield was not significantly different from the 100% skeletal control containing 3% salt. In the samples made with 3% salt, a decrease in the percentage fat in the cook-out liquid was noted as modification progressed. This indicates an increase in fat binding ability with modification. Maximum cooked yields were obtained when solubility was increased by 18% in the no-salt formulation. This increase in solubility occurred in the range where EC in the functional tests was also increasing with solubility. These results and the model system functional tests indicated that enzymatic modification was able to improve beef heart protein functionality.

The unmodified 100% skeletal plugs and the unmodified 30/70 plugs made without salt had lower cooked yields than the respective samples made with 3% salt. Salt is generally added to processed meat products at a concentration of 2.25 - 2.75% of the formulation in order to solubilize the myofibrillar proteins (Olson and Terrell, 1981). The myofibrillar proteins are responsible for fat binding, water holding capacity, texture formation, and desirable yields. Cooked yields of the 30/70 meat plugs made without salt and modified for 60 and 120 min were not significantly different from the 30/70 control made with 3% salt. This suggests that enzymatic modification, by solubilizing the myofibrillar proteins, may have potential as a partial substitute for salt in the production of processed meat products.

Production of frankfurters

The results of the functional tests and the meat model system indicated that enzymatic modification was effec-

Table 3—Proximate composition of 30% beef heart/70% beef skeletal frankfurters[a,b]

Treatment	Protein (%)	Fat (%)	Moisture (%)	Salt (%)
Unmodified; low salt	16.9a	29.6a	50.2a	0.8a
Unmodified; high-salt	15.9b	31.9a	48.0a	2.3b
Modified; low-salt	16.0b	30.7a	50.0a	0.8a
Modified; high-salt	16.0b	31.8a	48.2a	2.3b

[a] Values are the average of triplicate determinations from duplicate batches.
[b] Means in the same column followed by the same letter did not differ significantly (P>0.05).

tive in improving the functionality of beef heart proteins at both low and normal salt levels. The purpose of this part of the research was to determine if enzymatic modification was effective on a pilot plant scale. Model system results do not necessarily predict performance at the pilot plant level as preparation of frankfurters in a pilot plant requires the use of different equipment, processes, and ingredients.

Frankfurters were prepared using normal (2%) and low (0.5%) salt content. Treatments tested were 30% beef heart/70% beef skeletal controls and 30% ficin-modified beef heart/70% skeletal meat. Enzyme-modified heart was prepared by treating the heart with ficin until a protein solublity of 49% in 0.1M NaCl was obtained. This extent of modification was chosen as it produced maximum cooked yields in the meat model system experiments.

The proximate composition of the finished frankfurters was determined (Table 3). There was no significant differences in proximate composition between the treatments except the low-salt heart/skeletal control frankfurters had significantly more protein than the other treatments. The normal-salt frankfurters contained 2.3% salt, while the low-salt frankfurters contained 0.8% salt.

The product yields of the frankfurter treatments are shown in Table 4. Both the normal and low-salt enzyme-modified frankfurters had significantly greater smokehouse yields and consumer cooked yields than the unmodified frankfurters. In addition, the enzyme-modified frankfurters had significantly less cook-out fat, indicating improved fat binding.

Thus, enzymatic modification with ficin was effective in improving beef heart protein functionality at both low and normal salt contents in an actual processed meat product. The success of the enzyme-modified low-salt frankfurters has special significance as many meat processors are trying to reduce the sodium content in their processed products, due to evidence which links high sodium consumption with high blood pressure in genetically predisposed individuals (Olson, 1982; IFT, 1980). Obviously, due to the toxic effects of iodoacetate, its use as an inhibitor is not practical. However, selection of alternative enzymes and/or inhibitors may make enzymatic modification a feasible procedure. In addition, further research is necessary to determine if enzyme-modified low-salt frankfurters are subject to flavor, texture, or microbial problems.

Table 4—Product yields of 30% beef heart/70% beef skeletal meat frankfurters

Treatment	Smokehouse[a,b] yield (%)	Consumer cooked yield[b,c] (%)	Volume of cook-out fat (mL)
Unmodified; low-salt	80.9a	71.8a	10.0a
Unmodified; high-salt	83.5a	73.2a	9.3a
Modified; low-salt	88.0b	83.9b	6.7b
Modified; high-salt	87.7b	85.8b	6.0b

[a] Values are the average of a single determination from duplicate batches.
[b] Means followed by the same letter did not differ significantly (P>0.05).
[c] Values are the average of triplicate determinations from duplicate batches.

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Differential Scanning Calorimetry of Beef Muscle: Influence of Sarcomere Length

C. J. FINDLAY and D. W. STANLEY

ABSTRACT

Contraction state of beef muscle at onset of rigor influences tenderness of cooked meat. Loss in tenderness during cooking has been related, through use of differential scanning calorimetry (DSC), to thermal denaturation of myofibrillar proteins. Contraction of beef sternomandibularis muscle was controlled at sarcomere lengths of 2.4, 2.1, 1.9, 1.7, and 1.4 μm. Samples were scanned from 25-105°C at 10°C/min; ΔH (change in heat of transition) between 45° and 92°C dropped from ca. 4 J/g muscle at 2.4 μm to ca. 3 J/g at 1.4 μm. This difference ($P < 0.05$) amounts to less than 1% of the total energy required to heat meat from 45° to 92°C. The decrease is attributed to a greater actomyosin contribution to the overall thermal curve resulting from increased overlap of the filaments.

INTRODUCTION

THE STATE OF muscle contraction at the onset of rigor has been associated with meat tenderness since Ramsbottom and Strandine (1949) concluded that shortened beef muscle was tougher than similar muscle that had been stretched before rigor. Subsequent studies have related the loss of tenderness to the increased overlap of thick and thin filaments and the concurrent decrease in sarcomere length (Locker, 1958). Cold shortened beef was found to require four times the shear force of rest length muscle (Marsh and Leet, 1966). The contribution of myofibrillar proteins to overall meat tenderness has received a great deal of attention since commercial conditioning reduces myofibrillar toughness while the background toughness due to connective tissue is largely unaffected (Penny, 1980).

Regardless of the improvement in tenderness caused by conditioning, final assessment of meat quality can only be made after cooking. The effect of cooking on tenderness hass been explained on the basis of denaturation of muscle proteins and dehydration (Hamm, 1966). Recently, differential scanning calorimetry (DSC) has been applied to the muscle system to investigate the fundamental thermodynamic changes associated with the heating of meat. The endothermic transitions observed in muscle have been attributed to the principal protein constituents (Martens and Vold, 1976; Wright et al., 1977; Stabursvik and Martens, 1980). These transitions in normal (pH 5.4) post-rigor beef muscle have been related to sensory panel response (Martens et al., 1982), but in none of these studies has contraction state been considered.

The objective of this study is to examine the effect of the contraction state of beef muscle on DSC thermal curves.

MATERIALS & METHODS

Samples

Beef neck muscle (sternomandibularis) was obtained immediately postmortem from four 18 month-old Charolais crossbreed heifers at the University abattoir. Each muscle was divided laterally into four sections and wrapped in damp paper towel to prevent surface dehydration. A cold shortened sample was prepared by refrigerating at 5°C. The remaining samples were stretched to different tensions, restrained with cord, wrapped in damp paper towel and held at 20°C for 8 hr before refrigerating at 5°C for 2, 4, 6 and 8 days.

Laser diffraction

Individual muscle fibers were teased from the beef samples and diffraction measurements were made using the method of Varcoe and Jones (1983). Ten measurements were taken to provide an average sarcomere length. Five groups of samples were selected at the following sarcomere length; 1.4 μm, sd = 0.09; 1.7 μm, sd = 0.07; 1.9 μm, sd = 0.11; 2.1 μm, sd = 0.09 and 2.4 μm, sd = 0.13.

Differential scanning calorimetry

A portion of muscle (ca. 5 mg) from the same muscle fiber used for laser diffraction measurement was transferred to an aluminum hermetic pan and weighed to within 10 mcg using a Model G Cahn electrobalance. To adjust for small natural variations in pH, ensure an excess of moisture and promote good thermal contact, 5 μL of 0.07M Sorenson's phosphate buffer (pH 5.4) were included in the pan before sealing. The quantity of buffer was selected to give approximately 1:1 proportions. Stabursvik and Martens (1980) showed that pH of the muscle affected the resultant thermal curve; they concluded that buffers could be used to adjust muscle samples of different natural pH values to a common pH. Wright and Wilding (1984) concluded that unless samples are studied under equivalent conditions, the results obtained are not necessarily comparable. Sorenson's phosphate buffer was used for the preparation of beef muscle for electron microscopy. Consequently it was selected for use in DSC at a pH of 5.4 to assure that the muscle was in the lower range of pH of normal post-rigor muscle. The characteristic exotherm between 45° and 55°C found in pre-rigor muscle and disappearing with rigor (Wright et al., 1977) was absent in all samples used in this study. A Dupont Model 1090 Differential Scanning Calorimeter (DSC) with a pressure DSC cell was used to scan the samples from 25-105°C at 10°C/min under ambient pressure with a nitrogen flush of 25 mL/min. The differential heat flow was recorded at 0.2 sec intervals on a magnetic disk for subsequent computerized analysis. Each sample was cooled and rescanned to provide a measure of the reversible transitions and a baseline for analysis. Plots of heat flow versus temperature were obtained for each specimen. Data analysis programs were applied to the thermal curves to provide heat of transitions (ΔH) and temperatures of maximum transition (T_{max}). Rescanned thermal curves were subtracted from initial runs to compensate for the change in baseline. All thermal curves were normalized to 10 mg of whole muscle (80% moisture) to facilitate plotting of thermal curves and permit comparison. Analysis of variance and multiple linear regression were performed using the Statistical Analysis System (Helwig and Council, 1979).

RESULTS

STRETCHING AND RESTRAINING MUSCLE does not produce uniform sarcomere length, but, by taking samples for DSC from the same fiber used for measurement of sarcomere length it was possible to classify samples into sarcomere length groups. Three samples were taken from within each group at 2, 4, 6 and 8 days postmortem for each animal. The thermal curves were analyzed in two different ways. Discrete data for ΔH values and T_{max} were obtained over a range of 45-92°C. Partial area analysis provided the T_{max} and ΔH values for individual transitions

Authors Findlay and Stanley are affiliated with the Dept. of Food Science, Univ. of Guelph, Guelph, Ontario, N1G 2W1, Canada.

that fell within integration limits of 45-60°C (T_1), 60-76°C (T_2) and 76-92°C (T_3). The means and standard deviations of the data for sarcomere length are shown in Table 1. Analysis of variance results are given in Table 2. A linear model was applied to determine the contribution of each source of variation to the regression model (Table 3).

Individual thermal curves for each sarcomere length group were combined and normalized to 10 mg of wet muscle for the baseline corrected scans (Fig. 1). Since muscle thermal curves are not comprised of discrete events, but are a net response resulting from the overlapping transitions of the protein constituents, the second derivative was plotted to help locate subtle changes in heat flow hidden in the shoulders of larger transitions (Fig. 2). To examine the difference between the extremes of sarcomere length the thermal curve of the 2.4 μm sample was subtracted from that of the 1.4 μm sample to yield a thermal curve of the difference (Fig. 3).

The first transition, (T_1), which has been attributed to myosin (Wright et al., 1977; Stabursvik and Martens, 1980), decreased significantly ($P < 0.05$) with increased sarcomere length. The heat of transition (ΔH_1) increased significantly as sarcomere length increased from 2.1 to 2.4 μm. The T_2 results showed no significant difference due to sarcomere length; however, the contribution of sarcoplasmic proteins, actomyosin and connective tissue to this transition makes interpretation difficult. The heat of transition, ΔH_2, increased significantly with sarcomere length. The actin transition, T_3, increased significantly with increased sarcomere length, indicating greater stabilization of this protein. ΔH_3 also increased significantly with sarcomere length. The total heat flow over the range 45-92°C increased significantly from 2.94 J/g at 1.4 μm to 4.04 J/g at 2.4 μm ($P < 0.05$). These enthalpies are in the range of those found by Wright et al. (1977) for muscle and constituent proteins. No significant difference was found between animals for any of the variables tested (Table 2).

The relative contribution of the independent variables sarcomere length, aging and animal to a linear regression model (Table 3) showed that aging had the greatest effect on transition temperatures while sarcomere length influenced the heat of transition, particularly ΔH_3. The contribution of interation to R^2 was relatively small with the exception of T_3.

Second derivative analysis of the thermal curves (Fig. 2) indicated the emergence of a transition below 60°C in the 1.4 μm curve which was not apparent at 2.4 μm. The pattern of the 1.9 μm curve shows a doublet in the vicinity of 54°C which warrants further investigation. A direct subtraction of the 2.4 μm thermal curve from the 1.4 μm thermal curve gave a thermal difference curve that might explain some of the differences due to sarcomere length. The T_{max} values for the thermal curve are 54°, 65°, 80°, and 85°C; the temperatures cited by Wright et al. (1977) for actomyosin are 54°, 65°, and 80°C. Greater overlap of thick and thin filaments will increase the probability of interaction between actin and myosin. Wright et al. (1977) found actomyosin to require 20% less heat than whole muscle and 30% less than myofibrils. On this basis it may be hypothesized that greater overlap associated with shorter

Table 1—Effect of sarcomere length on T_{max} and ΔH values in beef sternomandibularis muscle

Sarcomere length (n = 12)		Transition Temperature °C			Heat of transition J/g			
		T_1	T_2	T_3	ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
1.40		57.03 a*	66.07 a	82.38 a	0.551 a	1.502 a	0.891 a	2.943 a
	(Std. Dev.)	(2.75)	(0.91)	(0.29)	(0.073)	(0.302)	(0.114)	(0.347)
1.70		57.38 a	66.18 a	82.21 a	0.615 a	1.598 a	0.959 a	3.173 a,b
		(2.38)	(1.06)	(0.36)	(0.135)	(0.381)	(0.133)	(0.543)
1.90		56.63 a,b	66.48 a	82.83 b	0.633 a	1.700 a,b	0.990 a	3.323 b,c
		(1.70)	(1.31)	(1.06)	(0.123)	(0.404)	(0.148)	(0.450)
2.10		55.49 b	66.09 a	82.93 b	0.622 a	1.810 a,b	1.156 b	3.588 c
		(3.26)	(1.35)	(1.10)	(0.135)	(0.379)	(0.153)	(0.469)
2.40		55.59 b	66.62 a	82.88 b	0.742 b	2.028 b	1.277 c	4.047 d
		(1.99)	(1.37)	(0.72)	(0.116)	(0.473)	(0.140)	(0.571)

* Means in the same column with the same letter are not significantly different $P < 0.05$ using Duncan's Multiple Range Test.

Table 2—Analysis of variance of beef sternomandibularis DSC data

Source	d.f.	T_1	T_2	T_3	ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
					Probability			
Sarcomere length	4	0.1765	0.6882	0.0032	0.0008	0.0050	0.0001	0.0001
Aging	3	0.0199	0.0303	0.0001	0.0001	0.0005	0.0056	0.0001
Animal	3	0.1541	0.3288	0.1560	0.4602	0.6197	0.8487	0.9215
Error	23							
Total	59							

Table 3—Contributions to R^2 for a linear model composed of sarcomere length, age and animal

Source*	T_1	T_2	T_3	R^2 (%) ΔH_1	ΔH_2	ΔH_3	ΔH_{Total}
Sarcomere length	6.70	1.64	8.39	19.42	18.53	49.59	38.92
Aging	11.10	5.13	37.86	25.10	22.99	10.13	29.80
Animal	0.98	0.96	2.36	0.04	8.73	1.33	6.14
Total**	20.82***	7.02	56.17***	48.17***	42.68***	60.18***	68.73***

* d.f. for linear model = 10; ** Includes interactions; *** $P < 0.01$.

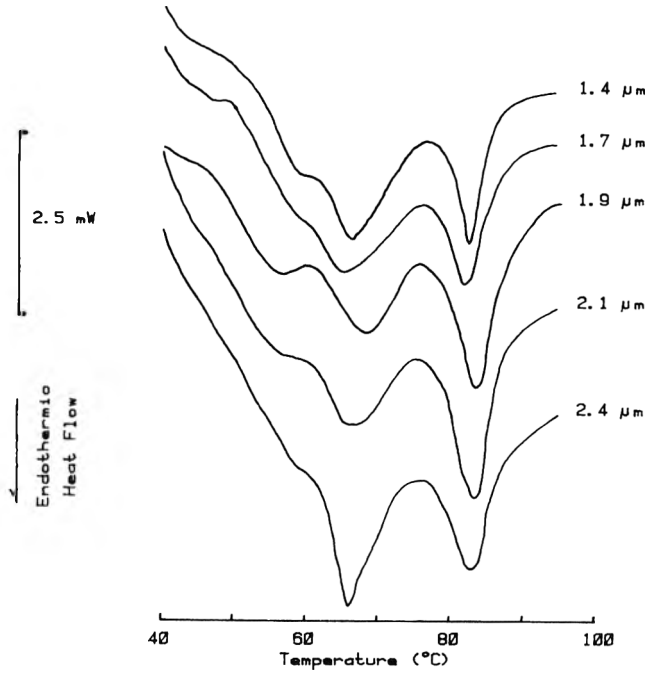

Fig. 1—DSC thermal curves of beef sternomandibularis muscle as a function of sarcomere length. Each curve represents the addition of 12 samples normalized to 10 mg of wet muscle.

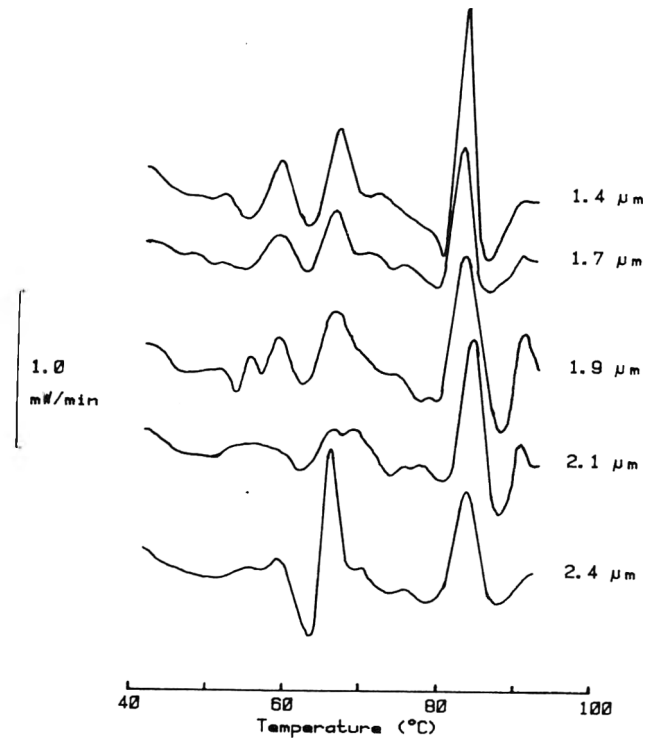

Fig. 2—Second derivative curves of beef sternomandibularis muscle DSC thermal curves as a function of sarcomere length.

sarcomere length leads to a greater interaction between actin and myosin. Thereby resulting in a greater actomyosin contribution to the overall muscle thermal curve reducing the total heat of transition. The heat contribution of the actin transition is affected the most since F-actin has a higher T_{max} (Wright et al., 1977) and thus a concomitant higher stability.

The contribution of aging to the shift in transition temperature and decrease in ΔH is demonstrated by the data in Table 3. The magnitude of the effect on R^2 suggests that the tenderization due to proteolysis also causes a reduction in the ΔH values in meat.

SUMMARY & CONCLUSIONS

THE MAJOR THERMAL EFFECT of the contraction state of beef sternomandibularis muscle, measured as a decrease in sarcomere length from 2.4 to 1.4 µm, is a reduction in ΔH from 4 J/g to 3 J/g. This change appears to be due to the increase in overlap of thin and thick filaments. The major contribution to this decrease in ΔH is found in the actin transition (from 1.3 J/g to 0.9 J/g, $P < 0.05$) indicating a reduction in the stability of actin when it interacts with myosin as actomyosin, instead of aggregating with itself.

The effect of aging on the thermal properties of muscle is in keeping with the current understanding of the enzymology of meat conditioning (Penny, 1980). Proteolysis breaks down the structure of the sarcomere making it more heat labile. However, a more extensive examination of the effect of conditioning on the thermal properties of beef is warranted.

Meat is usually cooked at a fixed external temperature for a predetermined time or by monitoring the internal temperature. It is apparent that contraction state will have an influence on the temperature at which the proteins undergo denaturation and the resultant loss in tenderness. The difference in ΔH value between contracted and rest length muscle is approximately 1 J/g. The heat capacity of beef is related to its moisture content and is in the vicinity of 3.3 J/g °C (Mohsenin, 1980). Over the temperature range used

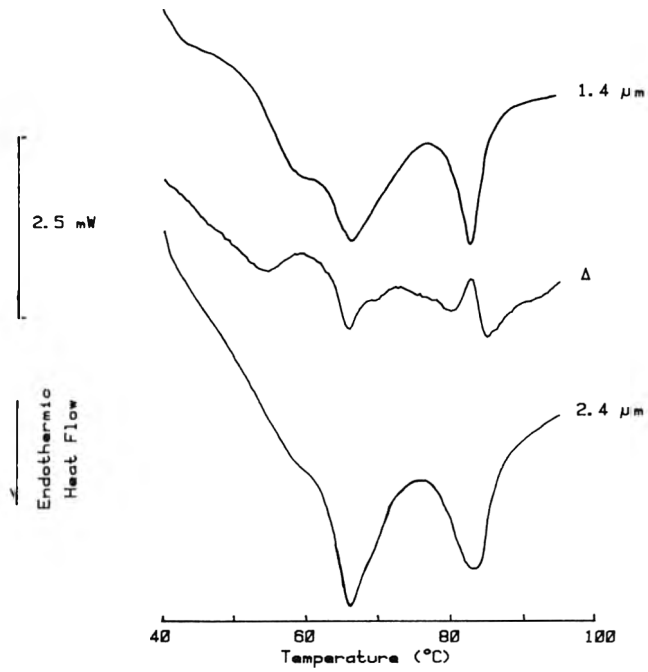

Fig. 3 — The difference in net heat flow between beef sternomandibularis muscle of sarcomere length 2.4 and 1.4 µm.

for integration of ΔH, 45-92°C, the total heat required to increase the temperature would be 155 J/g. It is clear from this that the variation in heat required for denaturation of protein will contribute less than 1% to the total heat required in cooking. However, the stabilizing shift of the actin transition towards a higher denaturation temperature that was observed with increased sarcomere lengths may have a noticeable effect on the tenderness of cooked meat. This relationship shall be the subject of further investigation.

—Continued on page 1534

Effects of Dielectric and Steam Heating Treatments on the Pre-Storage and Storage Color Characteristics of Pecan Kernels

S. D. SENTER, W. R. FORBUS JR., S. O. NELSON, and R. J. HORVAT

ABSTRACT

Pecan kernels were subjected to steam conditioning and dielectric heating treatments and evaluated initially and during 16 wk of accelerated storage to determine temperature effects on color characteristics. Steam conditioning treatments, which raised kernel temperature to 93°C, caused significantly greater darkening of the kernels initially and during storage than did dielectric heating to 88, 136, and 156°C. Comparison of mean Hunter color values of stored dielectrically heat-treated kernels and kernels that were frozen and maintained as control samples showed a stabilizing influence on kernel darkening by these heat treatments. Lightness (L values) of the kernels was increased by dielectric heating, Hunter a values did not differ significantly from the control, and the hue (θ) of the kernels was increased only by the 156°C dielectric heating treatment.

INTRODUCTION

KERNEL COLOR is an important characteristic in determining pecan kernel quality. Preference is for a light color for which the market will pay a premium price. A dark or "amber" color is often associated with substandard quality, being related to exposure of the pecans to adverse harvesting, processing, and storage conditions. This generalization, however, is not valid, because the hue and intensity of kernel color varies with growing conditions (Woodroof and Heaton, 1967), with time of harvest (Heaton, 1974) and curing methods (Heaton et al., 1975), with cultivar (Kays and Wilson, 1978; Forbus et al., 1983) and by conditioning treatments preceding shelling (Forbus and Senter, 1976; Forbus et al., 1979, 1983).

The effect of temperature on pecan kernel color has not been investigated adequately. It is generally acknowledged that exposure of kernels to temperatures greater than 37.8°C, or to prolonged heating periods, damages kernel color (Heaton et al., 1979). Damage in this context refers to initial darkening of kernels by application of heat, but does not relate treatments to rates or degree of color change during storage. Forbus and Senter (1976) and Forbus et al. (1983) found that exposure of in-shell pecans to steam conditioning treatments caused an initial darkening of kernels; however, the degree of change was not enough to cause downgrading in quality or selling price. With steam conditioning, the kernels were more uniform in color, and the kernels seemed to lighten in storage.

In a recent study, Senter et al. (1984) found that exposure of pecan kernels to heating treatments up to 156°C improved flavor quality during accelerated storage, presumably through inactivation of oxidative systems in the kernels. Optimum levels of heating appeared to be within the range of 90–100°C and were achieved by exposure of shelled and in-shell nuts to dielectric heating and steam conditioning treatments, respectively. The present study was conducted to compare the effects of similar levels of dry and wet heating treatments on the color characteristics of pecan kernels, both initially and during accelerated storage. Results will provide a basis for the development of processing methods which will improve kernel color stability. Wet heat was applied to in-shell pecans in combination with steam conditioning whereas the dry heat treatment involved applying dielectric heat to the shelled kernels. These treatments are possible methods which could be used to apply heat to kernels under commercial operating conditions.

The authors are affiliated with the USDA-ARS, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30613.

MATERIALS & METHODS

Samples

Ninety kg of cleaned and sized in-shell pecans [*Carya illinoensis* (Wang.) K. Koch, cv. Schley] were obtained from a commercial sheller in Georgia in December, 1981, and were representative of combined harvests of this cv. from this area. The pecans were subdivided into two 45 kg lots for duplication of analyses and placed in −30°C storage until February, 1982. At that time, a 45 kg lot was removed from storage and equilibrated to 4°C in a controlled temperature room. Twenty-seven kg were then shelled without further conditioning with commercial cracking and shelling machinery. Perfect halves were selected and returned to 4°C storage until used in dielectric heating treatments and as control samples. The remaining in-shell pecans were used in steam heat treatments. Treatments were repeated 2 wk later with the remaining 45 kg in −30°C storage.

Dielectric heating treatments

Dielectric heating treatments were obtained by placing ca 135g of pecan halves in Pyrex petri dishes (15 cm wide X 2 cm deep) that were positioned between parallel-plate electrodes of an electronic dielectric heater operating at a frequency of 43 MHz (Nelson and Whitney, 1960). The pecans were exposed as loose halves rather than in-shell to obtain a more uniform treatment for the experiment. Average field intensity in the pecans was 0.8 Kv/cm, which was calculated as previously described by Pour-el et al. (1981) from the measured radiofrequency electrode voltage, electrode spacing, and the dimensions and dielectric properties of the pecans and Pyrex glass. For each of the replicate samples, 16 petri dishes full of halves were treated for exposure periods of 1, 2, and 2.5 min. The internal temperature of the pecan kernels was measured immediately after exposure by inserting a hypodermic needle containing a copper-constantan thermocouple (Medical-Electronics Dev. Co., Type TC-1A) into kernels through matching holes in the side of the petri dish and lid.

Steam conditioning treatments

In-shell pecans were exposed to 100°C steam in a pre-heated, vented, upright retort for 4 min, then spread and allowed to cool to ambient temperature before cracking and shelling. Kernel temperature was measured with a thermocouple that was inserted into the kernel through a hole drilled through the shell. The measured temperature is expressed as the mean of duplicate measurements. After being shelled, perfect halves were selected for storage and analysis.

Sample storage

Kernel halves were combined by treatment and then subdivided into aliquots of ca 350g for sampling at 4 wk intervals during 16 wk of storage. Samples of similar size from the 4 treatments and untreated controls were placed in perforated polyethylene bags and stored in the dark at 21°C and 65% RH. These conditions have been shown to be ideal for accelerating quality deterioration of stored kernels (Forbus and Senter, 1976).

Color analysis

Hunter color values were determined with a Hunterlab D25-2 colorimeter using a 5.08 cm viewing port on the optical head. Forty halves per treatment were randomly selected from the subsamples designated for analysis at the particular date. Twenty halves were positioned in a 5.08 cm diameter plexiglass sample holder in 4 layers of 5 halves each with the cleavage resulting from separation of the halves facing up. Four L (lightness), a (red), and b(yellow) values were determined on each of the two replicate subsamples by rotating the sample holder 90° between readings.

Analysis of variance was performed on the data sets to determine whether there were significant differences by treatment in the color values with time. Differences in treatment means at the 5% probability level were evaluated by LSD tests (Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE EFFECTS of steam and dielectric heating on the surface color of the pecan kernels initially (T_0) and after 16 wk storage (T_{16}) are readily apparent from the Hunter color data shown in Fig. 1. Dielectric heating treatments (designated D-1, D-2, and D-3 and corresponding to kernel temperatures of 88, 136, and 156°C, respectively) caused L values to increase initially which indicates a lightening of the kernels in relation to the untreated control. Steam treatments raised kernel temperature to 93°C and caused an initial decrease in kernel lightness that continued throughout the 16 wk of storage. Mean L values over weeks (Table 1) differed significantly by treatment at the 5% level. These L values indicate that the dielectrically heat treated kernels were as light or lighter than the control sample. Steam-treated kernels had significantly lower L-values than all other treatments indicating that steam had a darkening effect on kernel color.

Increases in Hunter a values during storage indicate increases in redness of pecan kernels (Fig. 1). The lower a values at T_0 for the dielectrically heated kernels (D-1, D-2, and D-3) indicate an initial decrease in redness in relation to the control; however, steam heating caused an increase in redness at T_0. The amount of change in redness over time was greater for the control and dielectrically heated kernels than for steam treated kernels. The a values differed signifi-

Table 1—Means of Hunter color values of steam and dielectrically heated pecan kernels over 16 wk storage at 21°C, 65% RH

	Hunter color values				
Treatment[a]	L	a	b	Hue	S. I.
Control	32.1^{c}	10.0^{c}	13.4^{b}	53.2^{b}	16.8^{b}
D-1	32.4^{bc}	9.8^{c}	12.9^{c}	52.8^{b}	16.3^{c}
D-2	33.1^{b}	9.8^{c}	12.8^{cd}	52.5^{b}	16.1^{c}
D-3	32.6^{bc}	10.1^{c}	12.3^{d}	50.5^{c}	16.0^{c}
Steam	30.3^{d}	10.6^{b}	12.6^{cd}	49.8^{c}	16.6^{b}

[a] D-1, D-2, and D-3 treatments refer to dielectric heating treatments at 43 MHz for 1, 2, and 2.5 min with attained temp of 88, 136, and 156°C, respectively. Steam treatments were for 4 min with attained temp of 93°C.

[b-d] Mean separations by LSD analysis; numbers within columns followed by a common letter are not significantly different at the 5% level.

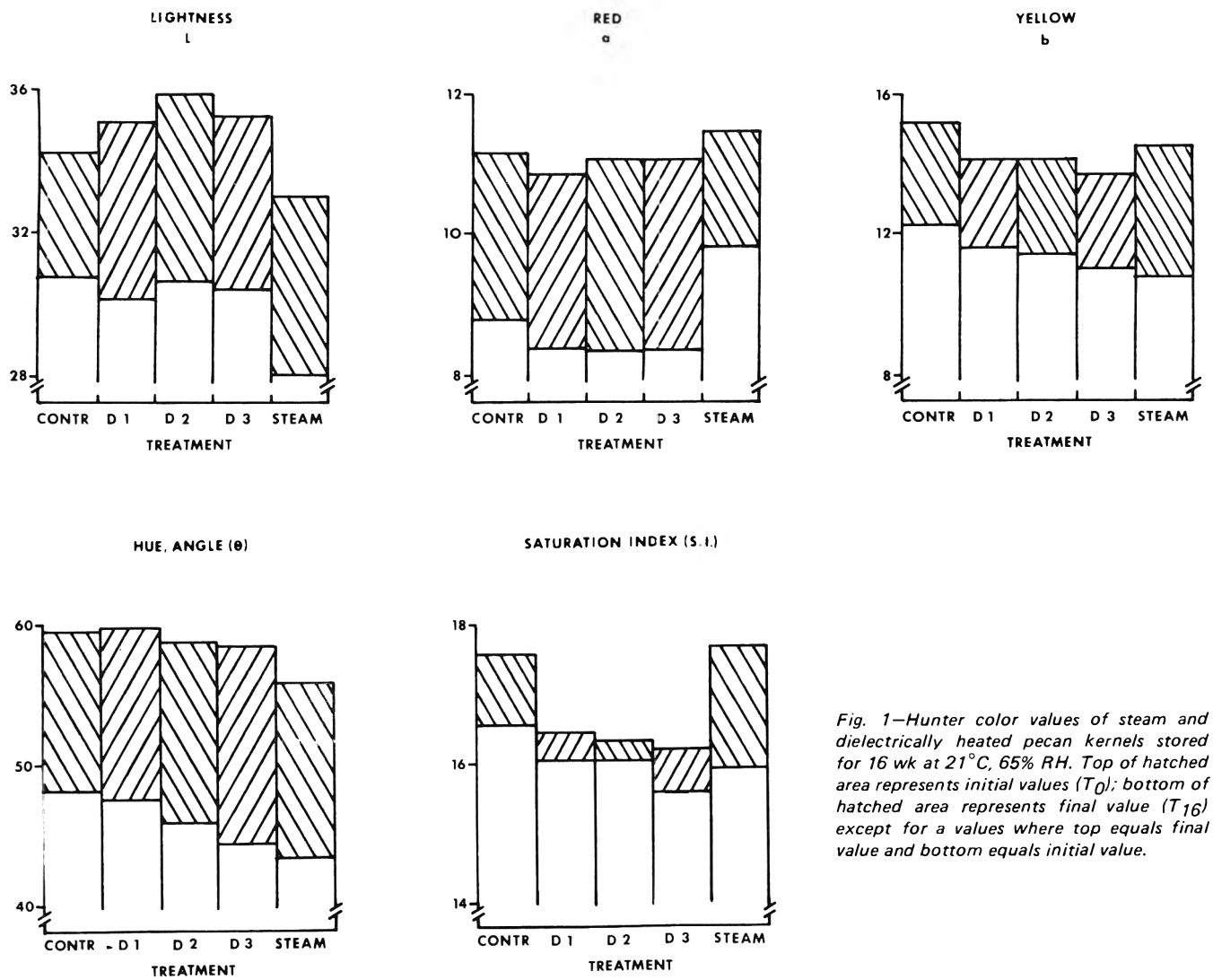

Fig. 1—Hunter color values of steam and dielectrically heated pecan kernels stored for 16 wk at 21°C, 65% RH. Top of hatched area represents initial values (T_0); bottom of hatched area represents final value (T_{16}) except for a values where top equals final value and bottom equals initial value.

cantly at the 5% level by time and treatments with mean values for the steam treated kernels being significantly greater than values for the other treatments (Table 1).

The b values (Fig. 1) indicate that all heat treatments caused an initial decrease in yellowness of the kernels in relation to the control. Mean b values for all treatments over time were significantly less than the control, but b values for D-1, D-2, and steam treated kernels were not significantly different.

The hue angle ($\theta = \tan^{-1} b/a$) and saturation index [SI = $(a^2 + b^2)^{1/2}$] are more effective for predicting visual color appearance than either the L, a, or b values alone (Little, 1975). Values of θ for pecan kernels fall within the first quadrant of the Hunter a, b diagram; therefore, kernels with higher values of θ appear more golden while kernels with lower values of θ appear more reddish brown (Forbus et al., 1983).

Initial values of θ for dielectrically heated kernels were considerably higher than for the steam treated kernels (Fig. 1), again indicating the darkening and reddening effects of the steam heat treatment. Hue of the kernels changed significantly with time and treatment, with mean values for the D-3 and steam treated kernels being significantly lower (or more reddish brown) than kernels subjected to the other treatments. Values for θ at T_{16} decreased progressively with increasing kernel temperature of the dielectric heating treatments, resulting in final values of 48, 46.5 and 45°, respectively (Fig. 1). Values for all treatments and the control at T_{16} fell within the "medium brown" color classification as related to values established previously (Forbus et al., 1983).

The differences in kernel color saturation are indicated in Fig. 1 by values of SI at T_0 and T_{16} for the various treatments. SI values for the steam treated kernels and the control kernels were significantly higher initially and indicated a more saturated reddish brown and golden color, respectively, for these treatments than was apparent in the dielectrically heated kernels. However, the control and steam treated kernels underwent a greater loss in color saturation with time than did the dielectrically heated kernels at T_{16}. Mean values of SI for all treatments over time (Table 1) indicate nonsignificant differences in color saturation for kernels receiving the dielectric heating treatments.

CONCLUSION

OUR RESULTS show that the heating of pecan kernels in a high moisture atmosphere causes greater changes in kernel color initially and during storage than equivalent treatment in a low moisture atmosphere. Differences are apparently due to enhancement of the oxidative transformation of color precursors in the testa of the kernels (Senter et al., 1978) by heat in the presence of high moisture since leaching of tannins from the shells of the steam treated pecans was not apparent in this study.

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Relative Tryptic Digestion Rates of Food Proteins

N. D. HUNG, M. VAS, E. CSEKE, and G. SZABOLCSI

ABSTRACT

The kinetics of tryptic digestion of different food proteins were studied by measuring trichloroacetic acid soluble peptide release and peptide bond splitting either by proton titration at constant pH or by recording the pH drop in nonbuffered suspensions. The theoretical basis of the pH drop assay was described. Application of this method for comparative studies requires complementary determinations of buffering capacities of the samples and the time course of the reference protein digestion. It was shown that milk powder preparations differed in digestion rates by a factor of two and various lots of commercial soy meals differed as much as by a factor of three. Relative digestion rates of some food proteins from different sources could not be characterized by a single figure.

INTRODUCTION

THE NUTRITIVE VALUE of food/feed proteins has great economic importance both in the food industry and animal husbandry. Data which would provide additional information on the nutritive quality besides the well known in vivo and in vitro methods would be highly desirable.

The in vitro proteolytic degradation of food proteins is frequently used as an index for the evaluation of their nutritive value (Sheffner, 1956; Akeson and Stahman, 1964; Buchanan, 1969; Saunders et al., 1973). Maga et al. (1973) attempted to relate the initial rate of digestion to the acceptibility of food and a relatively rapid in vitro multienzyme method was described (Hsu et al., 1977) based on the measurement of the time course of pH decrease following peptide bond splitting in a nonbuffered digestion mixture. The data obtained at 10 min digestion could be correlated with in vivo apparent digestibilities of animal and plant proteins using different proteolytic enzymes (Marshall et al., 1979; Rich et al., 1980; Bodwell et al., 1980). The effect of buffering capacities of the samples on the time course of pH decrease was noted by Pedersen and Eggum (1981).

For the quantitative evaluation of comparative in vitro digestion rate studies, the kinetics of the digestion process of food proteins should be known. This was formerly investigated only in the case of isolated native proteins. The enzymic hydrolysis of peptide bonds could be described by the Michaelis-Menten equation and was found to follow pseudo-first order kinetics (Richards, 1955; Ottesen, 1956; Biszku et al., 1973; Solti et al., 1975).

The aim of the present study was the determination of relative digestion rates of food proteins on the basis of kinetic principles and the elaboration of the theoretical basis of the pH drop method.

MATERIALS & METHODS

Foods tested

Commercial defatted soy meals were kindly supplied by the Agricultural Cooperative, Füzesgyarmat, and the different lots are named according to the Cooperative's code number. The protein content of the meals (N x 6.25) varied between 47 and 50% of the dry meal. Commercially available lentil seeds were subjected to microwave treatment in our Institute for 5 min in the presence of added water (sample L-Mw-5). Irradiation was performed in a microwave oven (Toshiba, Japan) at 1140 watts according to Benedek et al. (1983). Protein content was 30% of the dry meal. Casein (Hammersten) was purchased from the Reanal Fine Chemical Co. (Budapest). Skimmed milk powder (Dairy Product Research Institute, Mosonmagyaróvár) and DUSI milk powder (Milk Powder Factory, Cserna) contained 34.7 and 75% protein and 1.1 and 2% fat, respectively.

The authors are affiliated with the Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest H-1502, P.O. Box 7, Hungary. Author Hung's permanent address is Dept. of Biochemistry, Agricultural Univ., Hanoi, Vietnam.

Chemicals

BAEE (benzoyl-L-arginine ethyl ester) (Merck). Solutions of 0.934 mM were prepared before use in 0.5M glycine-NaOH buffer pH 8 containing 20 mM $CaCl_2$.

BAPA (DL-benzoyl-arginine-p-nitroanilide) (Merck). 0.92 mM solutions were freshly prepared as suggested by Kakade et al. (1969).

Trypsin (beef pancreas, Boehringer) contained 33 Tu_{BAEE}/mg dry weight. According to our measurements (cf. trypsin activity assay) this corresponds to 1833 TU_{BAPA}/mg dry weight. Stock solutions of about 30 mg/mL were stored at 5°C in 10^{-3}N HCl.

Bovine serum albumin (N-content 87%) and hydrogen peroxide were purchased from Merck and all other reagents were Reanal Fine Chemical Co. (Budapest) preparations of reagent grade.

N-content of foods was determined by Dr. G. Koppány, Institute for Animal Breeding and Feed Control, by the micro-Kjeldahl method. This kind collaboration is highly appreciated.

Digestion

Meals and seeds were ground to pass a 30 mesh sieve. Digestion of food suspensions was carried out at 37°C. Milk powders were solubilized within a few minutes after addition of trypsin at any concentration used (cf. legends to figures).

Determination of peptide bond cleavage

The principle of the method was described by Jacobsen et al. (1957). Suspensions in 50.5 mL 0.1M KCl were adjusted to pH 8.0 in an automatic titrator under continuous N_2 flow while stirring, essentially as described by Biszku and Szabolcsi (1964). Following 15 min stirring the reaction was started by addition of trypsin solutions of pH 8.0 and the base consumption (usually 0.1N NaOH was used) was automatically recorded. Stirring continued throughout the entire measurement. The degree of digestion was calculated by assuming that the investigated food proteins contained on average 12.5 mole per cent of Arg + Lys. Taking the average residue weight of an amino acid as 114 and the degree of dissociation (α) of the $R\text{-}NH_3^+$ ions formed during digestion as 0.715 at pH 8.0 and 37°C (Richards, 1955; Biszku and Szabolcsi, 1964), the maximum base consumption when 1 mg/mL protein is completely digested by trypsin is:

$$\frac{HO^-}{\text{mg protein}} = \frac{1 \times 0.125 \times 0.715}{0.114} = \frac{0.784\ \mu\text{moles } HO^-}{\text{mg protein}} \quad (1)$$

i.e. 7.84 μL 0.1N NaOH/mg protein.

Determination of pH decrease during the course of digestion

The pH decrease method described by Hsu et al. (1977) was slightly modified. Following adjustment of the suspension in 0.1M KCl to pH 8.0 and an additional 15 min stirring the reaction was initiated by addition of trypsin solutions of pH 8.0 and the time course of pH decrease was automatically recorded. Stirring continued throughout the whole procedure. No N_2 atmosphere was

required since the pH decrease in the absence of trypsin was negligible even if prolonged reactions were studied (cf. Fig. 6B).

Determination of trichloroacetic acid (TCA) soluble peptides

The whole procedure was carried out with continuous stirring. Following adjustment to pH 8.0 of suspensions made up in 0.5M glycine-NaOH buffer pH 8.0 and after 15 min preincubation, digestion was started by addition of trypsin solutions of pH 8.0. At time intervals, 3 mL aliquots were withdrawn and pipetted into test tubes containing 1.5 mL 30% TCA solutions. After 10 min incubation at 0°C the precipitate was centrifuged at 10,000 x *g* for 15 min. The concentration of peptides soluble in 10% TCA was determined by biuret reaction (Layne, 1957). The original method was modified by a tenfold increase in reagent concentration to improve the sensitivity of the method and to permit application to peptide bond determination in food suspensions. In certain cases reducing sugars were released from the foods and the absorption of the Fehling reaction interfered with the absorbance of the biuret reaction. Therefore hydrogen peroxide was added to the acidic supernatants to oxidize the sugars. The following standard reaction mixture was used: to 3 mL TCA supernatant 0.2 mL H_2O_2 (10%) was added, and after at least 2 min, 0.3 mL 7.5N NaOH and 0.53 mL biuret reagent (15g $CuSO_4 \cdot 5H_2O$, 60g K,Na-tartrate $\cdot 4H_2O$ in 1L 7.5N NaOH) were added. The mixture was allowed to stand for 20 min at room temperature. If opalescent, the solutions were centrifuged at 10,000 x *g* for 15 min. The zero time aliquot was used as a blank and the difference in absorption was read at 540 nm. Protein concentration was chosen so that the absorbance differences were between 0.050 and 0.400. Above this value in the assay system used ΔE_{540} was no longer proportional to protein concentration. Initial velocities could be reliably determined only if protein concentration exceed 4 mg/mL. A calibration curve made up with bovine serum albumin was used for calculating the concentration of peptide bonds. The degree of digestion was calculated on the same assumption of trypsin susceptible peptide bond content of food proteins as mentioned above. Thus

$$\Delta E_{540}^{max} = \frac{\text{Protein concentration (mg/mL)} \times 0.260 \times 0.875}{2} \quad (2)$$

where 0.260 is the absorbancy of 1 mg/mL bovine serum albumin, the factor 0.875 accounts for the loss in peptide bonds split during digestion, and 2 is the dilution factor of the digestion mixture during manipulations.

$$\text{Degree of digestion (\%)} = \frac{\Delta E_{540}^{measured} \times 100}{\Delta E_{540}^{max}} \quad (3)$$

Exhaustive tryptic digestion

This was performed with 600 TU/mL trypsin at pH 8.0 and 37°C for 24 hr if peptide release was determined. When peptide bond splitting was titrated at pH 8.0 and 37°C, first the time course was determined up to about 40% digestion with a given trypsin concentration. Then additional trypsin was added and base consumption was recorded until it became maximal.

Buffering capacity of food suspensions

Buffering capacity is defined as the amount of proton which decreases the pH of a suspension from 8.0 to the value at which relative digestion rates are calculated; in the present work pH 7.3. Since any other pH range between 9 to 7 can be used in the pH drop assay, buffering capacity should be determined according to the requirements of the experimental set up. Since the value is proportional to any component of the suspension we chose as a reference the protein content. Buffering capacity is given as μL 0.1N HCl/mg protein. The determinations were carried out with at least five samples of different suspension concentration. The amount of 0.1N HCl used for adjusting the pH of KCl, although negligible, was taken into account.

Trypsin activity assay

This was performed with BAEE as substrate according to Schwert and Takenaka (1955) and with BAPA substrate according to Kakade et al. (1969). Trypsin activities are given in TU_{BAPA}/mL digestion mixture as defined by Kakade et al. (1969) and The American Association of Cereal Chemists (1976). According to our measurements 1 TU_{BAPA} = 0.018 TU_{BAEE}. The stock solutions of trypsin were diluted and adjusted to pH 8.0 immediately before use.

Trypsin inhibitor (TI) activity assay

Total TI activity of food suspensions was assayed at pH 10 according to Kakade et al. (1974). The activity of the suspensions is given as TIU/mg dry meal or TIU/mg protein. TI activity solubilized at pH 8.0 was also determined and was found to be practically constant between 15 and 60 min incubation. It is given as TIU/mg protein or TIU/ml digestion mixture.

Standard error

Standard error was calculated according to Draper and Smith (1976) by the aid of a HP-9825A desktop calculator.

Titration and pH decrease experiments were carried out in a PMM 62 pH meter equipped with a TTC 60 titrator, REC 61 recorder and an automatic ABU 12 burette (Radiometer, Denmark). For spectrophotometric assays a Varian Techtron 635 and a Unicam SP 500 spectrophotometer were used.

RESULTS & DISCUSSION

Kinetics of tryptic digestion of food proteins

In order to derive the proper equations for describing the relative digestion rates of food proteins the kinetics of digestion were studied. To obtain appropriate quantitative data it is preferable to use a single pancreatic endopeptidase for digestion. This can be rationalized with the fact that in one-stomach animals the greatest part of digestion occurs in the intestinal tract and the action of exopeptidases is limited by the action of endopeptidases. Trypsin was chosen as a protease since it is highly specific and thereby the number of cleavable peptide bonds can be calculated. Furthermore, since its broad pH optimum is between 7 and 9 (cf. Laskowski, 1955), comparison of the results obtained with different methods is feasible.

Digestion experiments were carried out with food samples from which, at pH 8.0, no appreciable TI activity was released. The time course of tryptic digestion of two plant proteins (a commercial defatted soy meal and a microwave treated lentil), as well as that of casein, is shown in Fig. 1. Digestion was followed both by measuring the release of TCA-soluble peptides and the peptide bond cleavage at constant pH. In the case of the two plant proteins the rate, expressed in degree of digestion, was proportional to trypsin concentration and was independent of substrate (protein) concentration in the range of 1–10 mg/mL (Fig. 1A and 1B). This means that the rate of digestion was proportional also to protein concentration indicating that digestion followed pseudo-first order kinetics. Similar kinetics were observed with isolated native proteins (Richards, 1955; Biszku and Szabolcsi, 1964; Solti et al., 1975).

Casein however, behaved differently. The rate of digestion was proportional to trypsin concentration, but it was not proportional to protein concentration in the range of 1.5–10 mg/mL. It will be shown below that this phenomenon restricts the experimental setting to be used in comparative studies. If the degree of digestion was plotted versus time, digestion seemed to slow down with increasing casein concentration (Fig. 1C). The same phenomenon was observed if commercial milk powder proteins were subjected to the action of trypsin (not shown).

The effect observed with casein is possibly related to the saturation of trypsin with this protein as indicated by the Lineweaver-Burk plot (Fig. 2). The straight lines obtained suggested that the requirement of $[S] \ll K_M$ was not fulfilled even at protein concentrations as low as 1.5 mg/mL and thereby the progress curves of Fig. 1C deviated from the pseudo-first order reaction. The same holds for milk powder proteins since more than 80% of the milk proteins consists of casein.

In addition to the reciprocal values of velocity calculated from the first minute of digestion, those of base consumption at 5 min are also presented in Fig. 2. This was done to

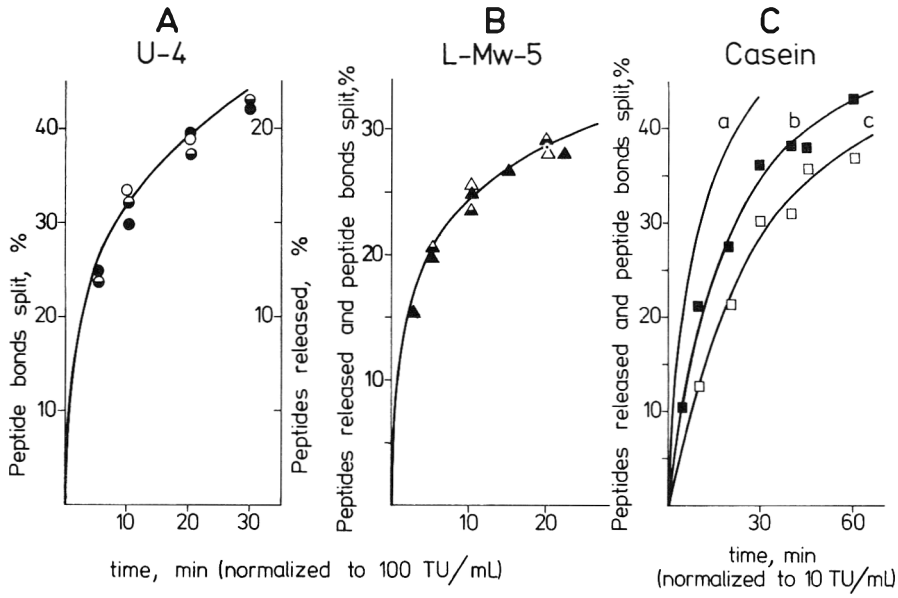

Fig. 1—Time course of peptide bond splitting and peptide release during tryptic digestion of different food proteins. Trypsin concentrations in experiments A and B varied from 100–200 TU/mL; in C., between 10–20 TU/mL. Solid lines represent averages of data derived from peptide bond splitting measurements carried out with protein concentrations of 1.5, 4.5 and 10 mg/mL. Symbols stand for TCA soluble peptide determinations: full symbols 4.5, semifull symbols 7 and open symbols 10 mg/mL protein, respectively. (A) commercial soy meal U-4 (TI activity at pH 8.0 was 1.1 TIU/mg protein). (B) microwave treated lentil seed, L-Mw-5 (TI activity at pH 8.0 was practically not detectable). (C) casein: curves a, b and c refer to protein concentrations 1.5, 4.5 and 10 mg/mL, respectively.

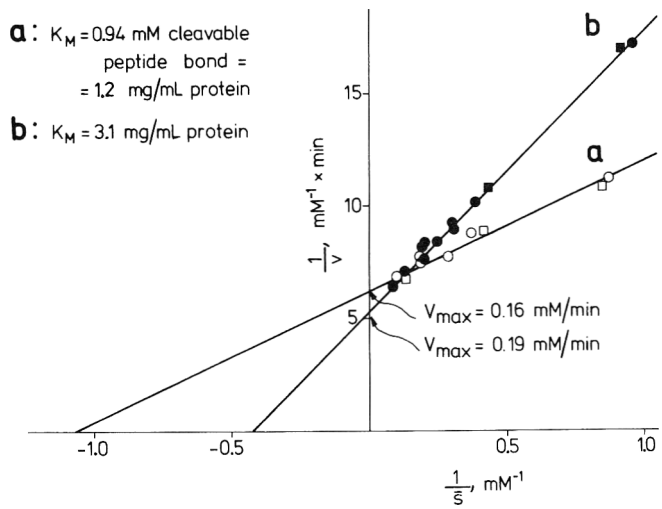

Fig. 2—Lineweaver-Burk plot of casein and skimmed milk powder protein digestion. In peptide bond splitting determinations the amounts of base consumed at 1 min and 5 min after addition of 15 TU/mL trypsin were read on the automatically recorded titration curves. the double reciprocal plots were calculated as suggested by Lee and Wilson (1971): $[\bar{S}] = ([S_o] + [S_t])/2$, where $[S_o]$ equals the initial concentration of cleavable peptide bonds as expressed in mM NaOH [cf. Eq. (1)]; $[S_t]$ equals the remaining noncleaved peptide bond concentration at time t as expressed in mM NaOH; $\bar{v} = ([So] - [St]/t$, mM NaOH x min^{-1}. Full and open symbols represent the values calculated from the base consumption data at 1 min and 5 min, respectively: □ and ■, casein; ○ and ●, skimmed milk powder. The highest degree of digestion reached at 5 min did not exceed cleavage of 27% of all the cleavable peptide bonds.

detect whether the differences in the multiple forms of casein (cf. McKenzie, 1970) were reflected in the rate of digestion. Lee and Wilson (1971) have shown that in contrast to the prevailing belief that "initial velocities" must be measured in order to use the double reciprocal equation, the K_M and V_{max} values can be quite accurately determined from higher velocity data as long as substrate conversion does not exceed 50% of the total. The data of Fig. 2 plotted according to Lee and Wilson (1971) showed that under the experimental conditions two apparent K_M values could be determined which by no means were characteristic of all the casein fractions. Thus the time course of casein digestion represented parallel lysis of different casein fractions, although the V_{max} values calculated from base consumption at 1 min and 5 min respectively, seemed to be similar.

Foods, in general, consist of several protein components of presumably different susceptibilities towards trypsin. Assuming e.g. three protein components in a food, the overall digestion rate can be derived from the Michaelis-Menten equation and that of parallel pseudo-first order reactions according to Eq. (4).

$$v = \left(x \frac{k^1}{K_M^1} + y \frac{k^2}{K_M^2} + z \frac{k^3}{K_M^3}\right) [\text{Protein}]\,[T] \qquad (4)$$

where [Protein] is the total protein concentration, for proteins of unknown molecular weight in mg/mL; [T] equals trypsin concentration in TU/mL; x, y and z stand for the relative proportion of the three protein components, $x + y + z = 1$; k^1, k^2 and k^3 are the first order rate constants of ES decomposition in the Michaelis-Menten equation. The values of k/K_M are characteristic of the tryptic susceptibility of each protein component.

Differences in the fragmentation pattern of food proteins

The fragmentation pattern of digestion was studied by comparing the time course of peptide bond splitting and TCA soluble peptide release as expressed in degree of digestion (cf. Fig. 1). During the digestion of all the lots of soy proteins investigated up to about 40% peptide bond splitting, practically 20% TCA soluble peptides were released (Fig. 1A).

In contrast, peptide solubilization and peptide bond splitting followed the same time course in the case of lentil and milk protein digestion (Fig. 1B and 1C). An

apparently similar stepwise degradation of the polypeptide chain following cleavage of a few intrachain peptide bonds was shown during the course of aldolase-T formation (Biszku et al., 1973). At the present state of the studies we cannot unequivocally decide whether the phenomenon observed with milk and lentil proteins reflects the real mechanism of degradation, or whether solubility and charge effects interfere with the determination of peptide release.

Determination of relative digestion rate (RDR) of food proteins

RDR is defined as the ratio of times corresponding to the same degree of digestion of two protein samples, one of them chosen as a reference. Since the rate of digestion is always proportional to enzyme concentration, the time required to attain the same degree of digestion is inversely proportional to the pseudo-first order rate constant. Thus RDR is defined as

$$RDR = \frac{k_{sample}}{k_{ref}} = \frac{t_{ref}}{t_{sample}} \quad (5)$$

where k is the overall rate constant of digestion at a given trypsin concentration (cf. Eq. 4); t_{ref} and t_{sample} are times required to reach the same degree of digestion. The value of RDR can be determined at any protein concentration if digestion is of first order with respect to protein concentration. If this is not the case, e.g. when the rates of digestion of casein and milk powder preparations are compared, the time courses will depend on the protein concentration used in the individual experiments, thus RDR should be calculated from experiments performed with the same protein concentrations.

RDR of food proteins of different origin

A comparison of the time course of tryptic digestion of two plant proteins (commercial soy meal U-4 and microwave treated lentil L-Mw-5) is shown in Fig. 3. In the evaluation of RDR two problems were encountered:

(1) If digestion of the compared samples proceeded according to different degradation patterns (e.g. soy and lentil proteins), the relative digestibility apparently depended on the method used. As seen in Fig. 3, opposite results were obtained if peptide bond cleavage or TCA soluble peptide release was compared. Calculation of RDR from pH decrease experiments will be presented below.

(2) The divergence of time courses shown in Fig. 3 indicated that, irrespective of the method used, the RDR values changed with the progress of digestion.

This effect was most probably due to the fact that the relative amounts of protein components exhibiting different susceptibilities towards trypsin vary from food to food. This means that relative tryptic digestion rates of food proteins of different origin cannot be unequivocally determined. On the basis of the present kinetic studies work is in progress to elucidate whether this phenomenon also occurs if a mixture of proteases is used for digestion.

As a comparison, the progress curve of tryptic digestion of casein is also documented in Fig. 3. Digestibilities of different foods are often compared to that of casein. However, casein is a good reference only if exhaustive digestibilities are compared. On the basis of the above arguments and of the fact that its digestion rate is not proportional to protein concentration, casein cannot be used as a reference for RDR determinations of different food proteins.

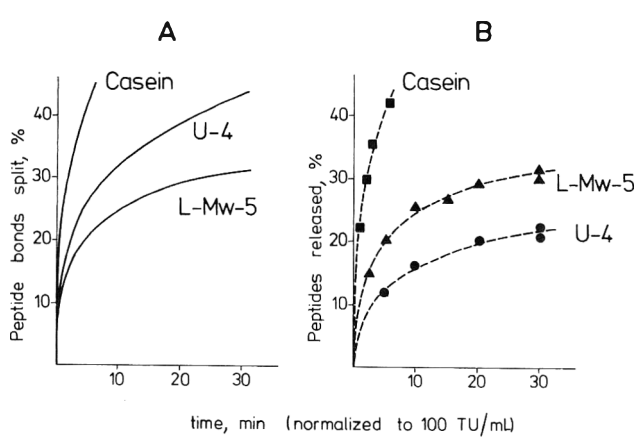

Fig. 3—Comparison of digestion of food proteins of different origin. Typical time courses of peptide bond cleavage (A) and peptide release (B). Protein concentration: 4.5 mg/mL.

RDR of food proteins of the same origin

Determination of RDR did not encounter the above mentioned problems if the relative tryptic digestion rates were calculated for proteins of the same origin. In these cases the RDR value was constant at any degree of digestion (Table 1) i.e. the tryptic susceptibilities of the protein components and their relative amounts in the soy meals tested were similar. These results are in agreement with those obtained for 22 different lots of commercial defatted soy meal samples.

The RDR values of different lots of commercial soy meals differed as much as by a factor of three, even if practically no TI activity was solubilized at pH 8.0. If high TI activity was released from a sample, as in the case of Br-1, the RDR value determined directly from the progress curves with a given trypsin concentration was not correct. The correct value was obtained if time was calculated for TU_{active}/mL digestion mixture. $TU_{active} = TU_{added} - TIU$.

To ascertain that TI activity was not solubilized during tryptic digestion, which would reduce trypsin activity during the course of the reaction, the following experiments were devised. Food samples of the same protein concentration were digested with different concentrations of trypsin. Then the reciprocal values of the times reached at different degrees of digestion were plotted versus trypsin concentration (Fig. 4). Since digestion follows pseudo-first order kinetics according to Eq. (4), the plot of 1/t versus trypsin concentration yields a straight line.

If samples U-4 and others which, in the absence of trypsin, did not release appreciable TI activity at pH 8.0 were tested, the intercept of the straight lines with the abscissa

Table 1—Relative digestion rates of different lots of commercial soy meal[a]

Sample	TI activity pH = 10	TI activity pH = 8	RDR at different degrees of digestion[b] 14%	23%	30%
	TIU/mg protein				
U-4	10.8	1.1	1	1	1
Fgy-24	12.6	2.1	0.72	0.70	0.71
82-12	–	1.2	0.50	0.55	0.50
83-13	11.2	8.2	0.35	0.30	0.28
CH-1	7.1	4.9	0.30	0.30	0.30
Br-1	16.8	15.3	0.02[c]	0.02[c]	–

[a] Digestion rates of soy meals were compared with that of sample U-4. Protein concentrations were about 10 mg/mL. RDR values were calculated from experiments performed with 400 TU/mL digestion mixture. Upon exhaustive tryptic digestion about 80–85% of the total trypsin susceptible peptide bonds of the samples were split. TI activities are average values of triplicate determinations. Standard error of relative digestion rate determinations was ± 5%.

[b] Data calculated from both peptide bond cleavage and pH crop measurements. In the latter case 14, 23 and 30% degree of digestion correspond to a drop in pH from 8.0 to 7.5, 7.3 and 7.2, respectively.

[c] RDR calculated for 1 TU_{active}/mL = 0.04.

was at or near the origin (Fig. 4A). This means that, in the case presented, up to 23% peptide bond cleavage practically no TI activity was released from the sample. Similar results were obtained up to 40% digestion (not shown).

Fig. 4B shows the results obtained with sample Br-1 from which 190 TIU/mL suspension was solubilized in the absence of trypsin. The abscissa intercepts of the straight lines obtained for different degrees of digestion were practically the same and equaled the TI activity released in the absence of trypsin. It follows that no additional TI activity was solubilized during the course of digestion. Were this not the case, the intercepts on the abscissa should be shifted towards higher and higher trypsin concentrations as the degree of digestion proceeded.

RDR of casein and two commercial milk powder preparations could also be reliably determined from the data of Fig. 5A. In this case casein was taken as a reference. The rate of digestion of skimmed milk powder proteins was found to be practically the same as that of casein. The RDR of DUSI milk powder proteins as related to casein was equal to two.

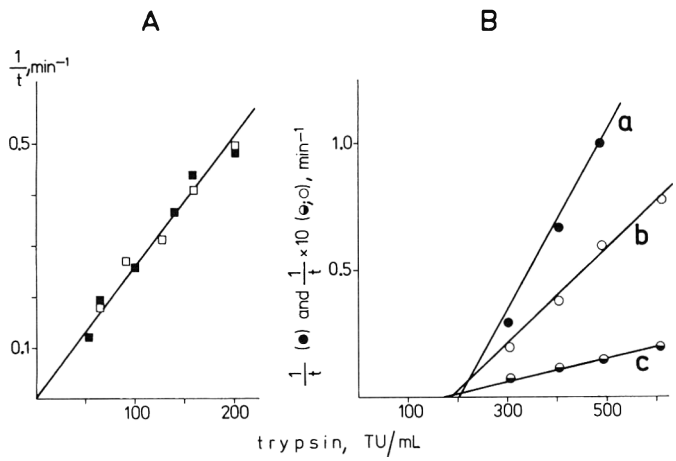

Fig. 4—Graphic determination of possible TI release into digestion mixtures. (A) Commercial soy meal U-4. The ordinate represents the reciprocal values of times reached at 23% peptide bond splitting (open symbols) and the same values derived from pH drop experiments (Full symbols). 23% peptide bond splitting corresponds to a pH drop from pH 8.0 to 7.3 Protein concentration was 5 mg/mL. (B) Commercial soy meal Br-1. Protein concentration was 12 mg/mL. Data derived from peptide release experiments. Digestion degrees: ●, 4; ○, 14, and ⊖, 18%, respectively.

Theoretical basis of the pH drop assay

The pH drop in a nonbuffered digestion mixture is due to the dissociation of the protonated α-amino groups formed during digestion (Eq. 6).

$$R - NH_3^+ \overset{K_D}{\rightleftharpoons} R\text{-}NH_2 + H^+ \qquad (6)$$

Since the protons can be titrated at constant pH, the derivation of the equations (cf. Appendix) is based on the relationship existing between these two methods. The experimental support was performed as follows. Peptide bond cleavage (Fig. 1) was determined at pH 8.0. In another series of experiments, carried out with the same samples under the same conditions, the time course of pH decrease of the digestion mixtures from pH 8.0 to 7.3 was also recorded (Fig. 6).

The prerequisite for the use of the "pH decrease" method is that the observed drop should be within the pH optimum of the protease applied. The considerations will be described for the pH range of 8.0 to 7.3. In addition, the influence of two other factors on the time course of pH decrease has to be considered.

(1) As a result of decreasing pH the proton dissociation from $R\text{-}NH_3^+$ ions is gradually suppressed. Since the pK of the reaction at 37°C is 7.6 (Richards, 1055; Biszku and Szabolcsi, 1964) the degree of dissociation (α) is 0.715 and 0.334, at pH 8.0 and pH 7.3, respectively.

(2) The other factor will be the known buffering capacity of proteins due to several groups of the polypeptide chain with pK values around this pH range. The salt content and the nonprotein components of the samples also contribute to the buffering capacity.

Although the amount of the peptide bonds split is the same in both types of experiments, it can be experimentally determined only by titration at constant pH. Because of the buffering capacity and the rebinding of protons by $R\text{-}NH_2$ groups, only a negligible fraction of the protons titrated at pH 8.0 will decrease the pH of the digestion mixture.

At a given trypsin concentration during time t the relationship of Eq. (7) held for the phenomenon observed in the two kinds of experiments (cf. Eq. A/6 of Appendix).

$$[H^+]^t_{pH\ 8.0} = [a]_{pH\,8.0 \rightarrow 7.3} + [H^+_{NH_2}]^t \qquad (7)$$

where index t refers to the time required to reach pH 7.3 in the pH drop experiment; $[H^+]^t_{pH\ 8.0}$ is the amount of proton dissociated from the $R\text{-}NH_3^+$ ions formed during digestion at pH 8.0 in a 1 mg/mL protein solution/suspension and is measured by the amount of base consumet at pH 8.0 during the same time (μL 0.1N NaOH/mg protein);

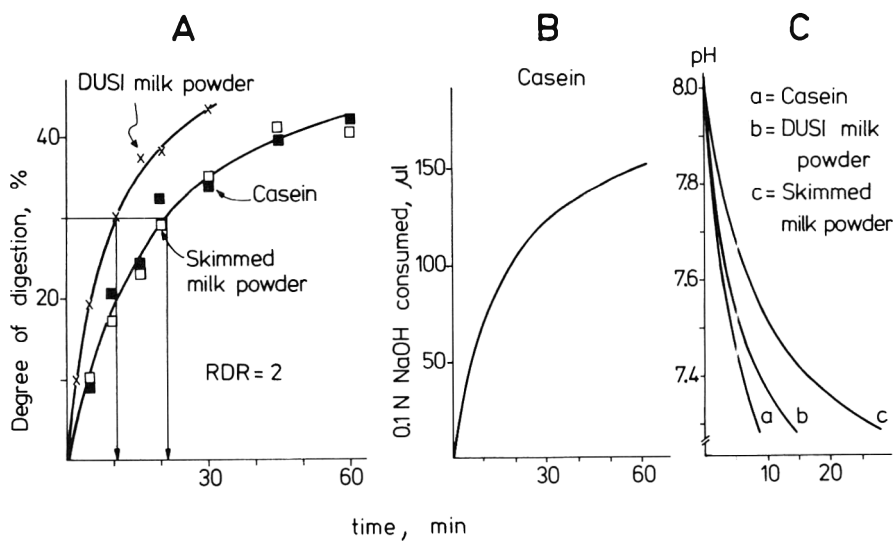

Fig. 5—Relative digestion rates of casein and two commercial milk powder preparations. Protein and trypsin concentration was 4.5 mg/mL and 10 TU/mL, respectively. (A) time courses of peptide bond cleavage (solid lines) and of peptide release (symbols). (B) progress curve of titration of casein used as a reference. (C) time courses of pH decrease.

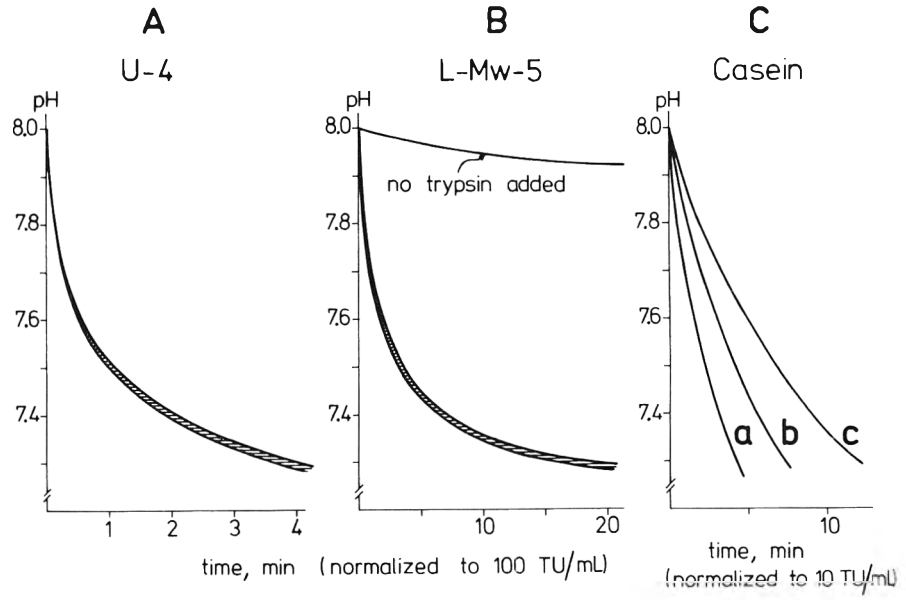

Fig. 6—Time course of pH decrease during digestion of different food proteins. Trypsin and protein concentration are the same as in lengends to Fig. 1 for peptide bond splitting determinations. Hatched areas represent deviation from the average. (A) commercial defatted soy meal U-4. (B) microwave treated lentil L-Mw-5 and C, casein.

$[H^+_{NH_2}]^t$ is the amount of proton rebound to the R-NH_2 groups due to the suppression of dissociation during the time period of pH decrease; a is the buffering capacity of a suspension containing 1 mg/mL protein in the pH range between 8.0 and 7.3 (μL 0.1N HCl).

It can be demonstrated (cf. Eq. A/10 of Appendix) that there is a simple relationship between the amount of base consumed at pH 8.0 during time t and the buffering capacity of the sample. If at time t the pH decreased from 8.0 to 7.3, Eq. (8) is valid.

$$[HO^-]^t_{pH\ 8.0} = [H^+]^t_{pH\ 8.0} = 2.146\ [a]_{pH\ 8.0 \rightarrow 7.3} \quad (8)$$

It follows from the above equations that:

(1) Even if two protein samples are digested at the same rate, the higher the value of a, the higher will be the degree of digestion needed to decrease the pH of the digestion mixture to pH 7.3. In other words, the higher the value of a the longer is the time required to reach the same pH.

(2) If digestion is of first order with respect to protein concentration, the time course of pH decrease will be independent of protein concentration. This is easily conceivable in the following way. If suspension concentration is doubled, the value of a will be doubled by definition. According to Eq. (8) if a is increased by a factor of two, the base consumption measured at pH 8.0 should be doubled, irrespective of the kinetic order of the reaction. If however, digestion is of first order with respect to protein concentration, in case of digestion of a double amount of protein a double amount of base will be consumed during the same time period. Therefore the time course of pH decrease will not change with increasing protein concentration. As shown in Fig. 6A and 6B, digestion of soy and lentil proteins obeys this rule.

If digestion is not of first order with respect to protein concentration, by doubling the protein concentration the value of a being increased by a factor of two, the double amount of base will be consumed during a longer period. Thus the time required to reach pH 7.3 will be prolonged and the pH decrease progress curve will slow down. This is the case for casein (Fig. 6C) and milk powder protein digestion (not documented).

(3) If we know the buffering capacity of a sample in a certain pH range and the time required to produce a pH decrease in this range, we can predict the value of $[HO^-]^t_{pH\ 8.0}$ from which the degree of digestion at this time can be calculated (cf. Eq. 8). Table 2 summarizes the buffering capacities and the measured and calculated base consumption when digestion of different food proteins was tested.

Table 2—Experimental demonstration of the relationship between buffering capacity and base consumption at constant pH

Sample	Buffering capacity $a_{pH\ 8.0 \rightarrow 7.3}$ μL 0.1 N HCl / mg Protein	Base consumption at pH 8.0 at time t[a] μL 0.1N NaOH/mg protein: Measured	Calculated from Eq. (8)
Coml defatted soy meals	0.84 ± 0.04[b]	1.80 ± 0.08[d]	1.80
Lentil-Mw-5	1.03 ± 0.06[c]	2.20 ± 0.07[e]	2.21
Casein	0.54 ± 0.03	1.12 ± 0.07[f]	1.16
Skimmed milk powder	1.18 ± 0.07	2.55 ± 0.13[f]	2.53
DUSI milk powder	1.19 ± 0.08	2.55 ± 0.16[g]	2.55

[a] The time required to reach pH 7.3 in the pH drop experiment.
[b] Average for six lots of soy meals characterized in Table 1.
[c] Average for two processings.
[d,e,f,g] Data derived from 25, 20, 10, and 6 experiments, respectively, performed with different protein and trypsin concentrations.

The above considerations show the quantitative relationship between the time course of pH decrease and titration experiments carried out at constant pH. The conclusions described in this section must be kept in mind if RDR of food proteins is calculated from pH decrease determinations.

Calculation of RDR from pH decrease experiments

As pointed out above, RDR of two samples should be determined from the ratio of times corresponding to the same degree of digestion. If RDR is calculated from pH decrease experiments, it is most convenient to compare the times required to reach a certain pH value, e.g. pH 7.3. During this time period, however, the extent of digestion of the samples being compared is not necessarily the same. Since there is a definite relationship between the buffering capacity of a sample and the degree of digestion attained at the time when pH reaches a given value (cf. Eq. 8), the ratio of times will be affected by the relative value of buffering capacities (A) is defined by Eq. (9) for the pH range 8.0 to 7.3.

$$A = \frac{a^{pH\ 8.0 \rightarrow 7.3}_{sample}}{a^{pH\ 8.0 \rightarrow 7.3}_{ref}} \quad (9)$$

Accordingly, RDR can be directly calculated from Eq. (5) only if A = 1. This was found if the rates of digestion of

Table 3—Calculation of relative digestion rates from pH drop experiments if A ≠ 1[a]

Sample	pH Drop experiment $t_{pH\ 7.3}$[b] (min)	Buffering capacity, $a_{pH\ 8.0 \rightarrow 7.3}$ μL 0.1N HCl / mg Protein	Calculated base consumption at time t for the titration exp.[c] (μL 0.1N NaOH)	RDR calculated from Eq. (10)
Casein	7.5[d]	0.50	–	1
Skimmed milk powder	27.0	1.18	114[e]	0.93
DUSI milk powder	13.5	1.19	114	1.85

[a] Digestion rates of skimmed milk powder and DUSI milk powder proteins were compared with that of casein. Samples containing 45 mg protein were digested in 10 mL 0.1N KCl with 10 TU/ml trypsin. $A = a_{milk\ powder}/a_{casein} = 2.36$.
[b] Data taken from Fig. 5C.
[c] Time t denotes the time required to reach pH 7.3 in the pH drop experiment.
[d] Note that this value cannot be used for RDR calculations since A ≠ 1.
[e] 1.18 x 2.146 x 45 = 114 μL 0.1N NaOH, cf. Eq. (8). The time of 114 μL base consumption was read on the progress curve of casein digestion in Fig. 5B, i.e. t'_{ref} in Eq. (10) equals 25 min.

different lots of commercial soy meal were compared (cf. Table 1).

If A ≠ 1 the following considerations have to be taken into account. As shown in Eq. (8), the higher the buffering capacity of a sample the higher will be the amount of base consumed at pH 8.0 i.e. the degree of digestion at the time when pH reaches the value of 7.3. Therefore the ratio of times at which pH 7.3 is attained cannot be directly used for calculations. The derivation of RDR and the mode of calculation will be presented with the experiments performed with casein and skimmed milk powder proteins.

If Fig. 5A it is seen that casein and skimmed milk powder proteins exhibit the same digestibility whether peptide bond splitting or peptide release is measured. In the pH decrease experiments digestion of casein is apparently much more rapid than that of skimmed milk powder proteins (Fig. 5C curves a and c). If RDR were simply calculated from Eq. (5), taking casein as a reference, the value would be 0.28. The apparent contradiction between the results obtained from the data of Fig. 5A and 5C is due to the difference in the buffering capacities of the two samples, the value of A being 2.36 (cf. Table 3).

It will be shown that in such cases RDR can be calculated only if the progress curve of titration at pH 8.0 of the reference is also determined.

It follows from Eq. (8) that if digestions of two samples of the same protein concentration are compared, the ratio of the amounts of base consumed at times when the two samples reach a given pH value, equals A (cf. also Eq. A/11 of Appendix).

The RDR value of samples as casein and skimmed milk powder can be calculated from Eq. (10):

$$RDR = \frac{t'_{ref}}{t^{pH\ 7.3}_{sample}} \qquad (10)$$

where t'_{ref} is the time when the reference attains the same degree of digestion as the sample tested. t'_{ref} can be easily determined if the value of the buffering capacity, $a^{pH\ 8.0 \rightarrow 7.3}_{sample}$ and the time course of peptide bond splitting of the reference are known. If samples of the same protein concentration are studied (irrespective of the kinetics of digestion) the base consumption of the sample at time t can be calculated from Eq. (8). Time t'_{ref} corresponding to the calculated base consumption can be directly read on the progress curve of reference digestion. Calculations for casein as a reference (titration curve presented in Fig. 5B) and the two commercial milk powders are given in Table 3 resolving the apparent contradiction between the data of Fig. 5A and 5C.

Taking into account that the buffering capacities of skimmed milk powder and DUSI milk powder are equal, the digestion rate of the latter preparation is twice that of casein or skimmed milk powder.

Differences in the buffering capacity of samples were also found when soy and lentil proteins were digested and when the effectiveness of treatments on the digestibility of food proteins was tested (cf. Hung et al., 1984).

The data and the considerations presented demonstrate the importance of buffering capacity on the determination of RDR if digestion is followed by pH decrease measurements. The fact that besides the pH decrease determination an additional experiment is also needed by no means decreases the elegance of the method.

APPENDIX

Relationship between pH decrease and peptide bond cleavage at constant pH during tryptic digestion of proteins

The calculations are valid for the pH range pH 7–9 where the activity of trypsin is constant. For the sake of simplicity the calculations will be presented for peptide bond cleavage at constant pH (pH 8.0) and pH decrease from pH 8.0 to 7.3 in experiments performed under otherwise the same conditions. The calculations refer to digestion of 1 mg/mL protein with 1 TU/mL trypsin at 37°C.

The degree of dissociation of $R\text{-}NH_3^+$ groups (pK = 7.6) formed after peptide bond splitting can be calculated from Eq. (A/1) and (A/2).

In the following, time t denotes the time when pH 7.3 is reached in the pH decrease experiments. The index of pH 8.0 and pH 7.3 refers to experiments performed at constant pH (8.0) and pH decrease experiments in the range pH 8.0–7.3, respectively.

(a) At time t if pH decrease is followed:

$$K_D = 10^{-7.6} = \frac{[R\text{-}NH_2]_{pH\ 7.3} \times 10^{-7.3}}{[R\text{-}NH_3^+]_{pH\ 7.3}} \qquad (A/1)$$

(b) At time t, if digestion is carried out at pH 8.0:

$$K_D = 10^{-7.6} = \frac{[R\text{-}NH_2]_{pH\ 8.0} \times 10^{-8.0}}{[R\text{-}NH_3^+]_{pH\ 8.0}} \qquad (A/2)$$

The concentration of peptide bonds split at time t is given in Eq. (A/3):

$$[P]^t_{pH\ 8.0} = [P]^t_{pH\ 7.3} = [R\text{-}NH_3^+]^t_{pH\ 7.3} + [R\text{-}NH_2]^t_{pH\ 7.3} = [R\text{-}NH_3^+]^t_{pH\ 8.0} + [R\text{-}NH_2]^t_{pH\ 8.0} \qquad (A/3)$$

Eq. (A/4) and (A/5) take into account the suppression of proton release from $R\text{-}NH_3^+$ groups during the change of pH from 8.0 to 7.3:

$$[R\text{-}NH_3^+]^t_{pH\ 8.0} = [R\text{-}NH_3^+]^t_{pH\ 7.3} - [H^+_{NH_2}]^t \qquad (A/4)$$

and

$$[R\text{-}NH_2]^t_{pH\ 7.3} = [R\text{-}NH_2]^t_{pH\ 8.0} - [H^+_{NH_2}]^t \quad (A/5)$$

where $[H^+_{NH_2}]^t$ is the difference in proton concentration bound to the alfa-amino groups formed at time t, at pH 8.0 and pH 7.3.

The relationship between the $[H^+]$ dissociated at pH 8.0 and that rebound to $R\text{-}NH_2$ groups at time t as well as the buffering capacity of the sample is

$$[H^+]^t_{pH\ 8.0} = [H^+_{NH_2}]^t + [a]_{pH\ 8.0 \rightarrow 7.3} \quad (A/6)$$

where $[H^+]^t_{pH\ 8.0}$ stands for the protons dissociated at pH 8.0 and is equivalent to the $[HO^-]^t_{pH\ 8.0}$ consumed in titration experiments carried out at pH 8.0; $[a]_{pH\ 8.0 \rightarrow 7.3}$ is the buffering capacity of the sample, i.e. the $[H^+]$ needed to decrease its pH from 8.0 to 7.3.

The relationship between $[H^+]^t_{pH\ 8.0}$ and the concentration of the peptide bonds split is given in Eq. (A/7).

$$[H^+]^t_{pH\ 8.0} = [HO^-]^t_{pH\ 8.0} = \alpha[P]^t_{pH\ 8.0} \quad (A/7)$$

where $\alpha = 0.715$ is the degree of dissociation of $R\text{-}NH_3^+$ groups at pH 8.0 as calculated from the pK value.

From Eq. (A/1) to (A/5) it can be derived:

$$[P]^t_{pH\ 8.0} = [P]^t_{pH\ 7.3} = 2.62\,[H^+_{NH_2}]^t \quad (A/8)$$

Taking also into account Eq. (A/6) and (A/7), the following relations are obtained:

$$[P]^t = 3.00\,[a]_{pH\ 8.0 \rightarrow 7.3} \quad (A/9)$$

where $[P]^t$ stands for the concentration of the peptide bonds split in both kinds of experiments, and

$$[HO^-]^t_{pH\ 8.0} = 2.146\,[a]_{pH\ 8.0 \rightarrow 7.3} \quad (A/10)$$

Eq. (A/10) was also solved for the pH ranges of 8.0 → 7.5 and 8.0 → 7.2:

$$[HO^-]^t_{pH\ 8.0} = 1.62\,[a]_{pH\ 8.0 \rightarrow 7.5} \quad (A/10a)$$

$$[HO^-]^t_{pH\ 8.0} = 2.53\,[a]_{pH\ 8.0 \rightarrow 7.2} \quad (A/10b)$$

It follows from Eq. (10) that if digestions of two samples of the same protein concentration are compared, the ratio of base consumption at times when the two samples reach pH 7.3 equals the relative buffering capacities of the samples (A).

$$\frac{[HO^-]^{t(sample)}_{pH\ 8.0}}{[HO^-]^{t(ref)}_{pH\ 8.0}} = \frac{[a]^{sample}_{pH\ 8.0 \rightarrow 7.3}}{[a]^{ref}_{pH\ 8.0 \rightarrow 7.3}} = A \quad (A/11)$$

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Processed Protein Foods Characterized by In Vitro Digestion Rates

N. D. HUNG, E. CSEKE, M. VAS, and G. SZABOLCSI

ABSTRACT

In vitro tryptic digestion assays and determination of relative digestion rates of food proteins described previously were used to evaluate changes in the rate of digestion following processing technologies. If pH drop measurements are used, a simple mode for the determination of relative digestion rates is suggested based on the comparison with a single reference of the same protein source. The effect of processing technologies could be tested in vitro. Significant increase in the velocity of digestion was found on microwave treatment of lentil and raw soy bean as well as on acid treatment of commercial defatted soy meals. Increase in the in vitro digestion rates was reflected in decreased feed consumption per weight gain in pig feeding experiments.

INTRODUCTION

IN OUR PREVIOUS WORK, in vitro methods for the comparative determination of food protein digestion rates were described (Hung et al., 1984). The methods were based on the time course measurements of trichloroacetic acid (TCA) soluble peptide release, peptide bond cleavage at constant pH or pH decrease during tryptic digestion. The latter method, i.e. recording the pH decrease of the digestion mixture as a consequence of peptide bond splitting, introduced by Hsu et al. (1977) and extended by others (Marshall et al., 1979; Rich et al., 1980; Bodwell et al., 1980; Pedersen and Eggum, 1981) was quantitated on a kinetic basis. It has been shown that relative digestion rates could be unequivocally determined if digestion of different lots of food proteins of the same origin were compared.

In the present work the methods and mode of calculations described previously were applied to test the efficiencies of new food processing technologies. These were devised: (1) to decrease the high trypsin inhibitor activity (TI) of raw soy bean and commercial lentil seeds, and (2) to enhance the digestion rate of commercial defatted soy meals. Feeding experiments were devised to control the in vitro digestion rate determinations.

MATERIALS & METHODS

THE CHEMICALS used are listed in the previous paper (Hung et al., 1984).

Microwave treatment of commercial lentil seeds and raw soy bean was carried out in the Institute of Enzymology according to Benedek et al. (1983), (cf. also Hung et al., 1984). Microwave treated lentil seeds are denoted as Lentil-Mw-2 and Lentil-Mw-5 (2 and 5 min irradiation, respectively). Raw soy bean irradiated for 6 min is denoted as EnPro-40 soy. Acid treatment of commercial defatted soy meals (SuproSoy) are partly done in the above Institute on Laboratory scale and partly at the Agricultural Cooperative, Füzesgyarmat, in pilot plant experiments according to Dévényi et al. (1982). Mineral acids were used at room temperature and according to the patent a treatment was regarded as efficient (i.e. it improved the weight gain and feed ratio in feeding experiments) if the pH of a 10% w/w water suspension was about 2. SuproSoys are denoted with the letter S following the code number of the nontreated sample given by the Cooperative.

The time course of tryptic digestion was measured at pH 8.0 and 37°C if peptide release or peptide bond cleavage was studied in 0.5M glycine-NaOH buffer and 0.1M KCl, respectively. The pH drop experiments also were carried out at 37°C in 0.1M KCl, starting from pH 8.0. Protein and trypsin concentrations in the individual experiments varied from 5–12 mg/mL and 50–250 TU/mL, respectively.

Exhaustive tryptic digestion was performed with 600 TU/mL trypsin at pH 8.0 at 37°C for 24 hr if peptide release was measured. When peptide bond splitting was titrated at pH 8.0 and 37°C, first the time course was determined up to about 40% digestion then additional trypsin was added and base consumption was recorded until it became maximal.

The detailed description of the in vitro digestion methods applied as well as calculation of the degree of digestion can also be found in the previous paper (Hung et al., 1984).

Feeding experiments with SuproSoys were carried out by the Agricultural Cooperative, Füzesgyarmat using TETRA hybrid pigs. The diet, aside from cornmeal, fishmeal and additives contained 10% commercial soy meal, Fgy-24, or the same amount of its SuproSoy variant, Fgy-24/S2.

Relative digestion rates (RDR) determined on the basis of calculations described previously (Hung et al., 1984) are summarized in the following. RDR of food proteins is the ratio of times required to attain the same degree of digestion as the reference sample proteins according to Eq. 1:

$$RDR = \frac{t_{ref}}{t_{sample}} \quad (1)$$

If the pH decrease method is used, apart from the times required to reach a certain pH value (e.g. pH 7.3) in the digestion mixtures the buffering capacities of samples in the pH range 8.0 → 7.3 were also taken into account for the calculations. The ratio of buffering capacities is defined by $A = a_{sample}/a_{ref}$. If the buffering capacities of the sample and the reference are the same, RDR is directly given by Eq. (2):

$$RDR = \frac{t_{ref}^{pH\ 7.3}}{t_{sample}^{pH\ 7.3}} \quad (2)$$

where $t_{ref}^{pH\ 7.3}$ and $t_{sample}^{pH\ 7.3}$ are the times when pH 7.3 is measured if starting from pH 8.0.

If the buffering capacities of the sample and the reference are different, the following equation should be used:

$$RDR = \frac{t'_{ref}}{t_{sample}^{pH\ 7.3}} \quad (3)$$

where t'_{ref} is the time when the reference attains the same degree of digestion as the sample tested. t'_{ref} can be calculated if the buffering capacity of the sample (i.e. $a_{sample}^{pH\ 8.0 \rightarrow 7.3}$) and the time course of peptide bond splitting of the reference sample are known, as described previously (Hung et al., 1984).

RESULTS & DISCUSSION

Effect of microwave treatment on the digestion rate of lentil proteins

The commercially available lentil contains significant trypsin inhibitor (TI) activity, part of which is solubilized at pH 8.0 (Table 1). (We did not investigate whether the inhibitor activity was due to the presence of the protein known as TI). The microwave treatment elaborated by Benedek et al. (1983) besides decreasing the TI activity (cf. Table 1), enhanced the digestibility of the proteins as

The authors are affiliated with the Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest H-1502, P.O. Box 7, Hungary. Author Hung's permanent address is Dept. of Biochemistry, Agricultural Univ., Hanoi, Vietnam.

Table 1—TI activity of commercial and processed protein foods[a]

Sample	Protein content/ dry meal (N x 6.25), %	TI activity, TIU/mg dry meal pH 10	pH 8
Lentil meals			
Commercial lentil meal	30.0	9.5 ± 0.8	6.3 ± 0.2
Lentil-Mw-2		3.0 ± 0.2	0.1 ± 0.1
Lentil-Mw-5		0.2 ± 0.1	0.1 ± 0.1
Soy meals			
U-4	50.5	5.4 ± 0.3	0.6 ± 0.2
U-4/S		3.3 ± 0.2	0.1 ± 0.1
Fgy-24	47.6	6.0 ± 0.3	1.2 ± 0.5
Fgy-24/S1[b]		4.2 ± 0.2	1.0 ± 0.5
Fgy-24/S2[b]		4.2 ± 0.2	1.0 ± 0.5
82-12	51.0	–	0.6 ± 0.6
82-12/S		–	0.5 ± 0.5
CH-1	47.1	3.3 ± 0.2	2.3 ± 0.2
CH-1/S		2.7 ± 0.2	0.2 ± 0.1
EnPro-40[c]	40.1	3.8 ± 0.2	0.1 ± 0.1

[a] Data are average values of at least three determinations. Mw denotes microwave treatment. The letter S followed by the code number of untreated soy meals stands for the SuproSoy variants.
[b] The pH of the 10% w/w water suspensions of the SuproScys Fgy-24/S1 and Fgy-24/S2 was 4.5 and 1.9, respectively.
[c] Microwave treated raw soy bean.

demonstrated by measuring either the TCA soluble peptide release or the peptide bond cleavage (Fig. 1A). Similar effect was detected if pH decrease in the digestion mixture was followed (Fig. 1B). In all three kinds of samples investigated both peptide release and peptide bond splitting followed the same time course (Fig. 1A) as observed with Lentil-Mw-5 (Hung et al., 1984). Thus processing did not cause detectable change in the degradation pattern of digestion. Similarly, digestion followed pseudo-first order kinetics which became evident if digestion rates of nontreated lentil proteins were calculated for the active trypsin concentrations ($TU_{active} = TU_{added} - TIU$).

It should be mentioned also that after prolonged incubation with trypsin the progress curves levelled off even in the case of Lentil-Mw-5. This indicated that the time course observed exhibited complex kinetics reflecting difference in the tryptic susceptibility of the protein components.

If RDR was calculated from pH drop experiments similar to those presented in Fig. 1B (Table 2) the changes in buffering capacities of the samples (cf. Table 3) were taken into account. For calculations Eq. (3) was applied, nontreated lentil being taken as a reference.

Because of the TI activity solubilized from the meal of nontreated lentil, RDR values calculated directly from the data of Fig. 1 would lead to misleading conclusions. In

Table 2—Effect of microwave treatment on the rate of digestion of lentil proteins[a]

(A) RDR calculated from peptide bond splitting and peptide release determination

Degree of digestion, %	Coml lentil time, min	RDR	Lentile-Mw-2 time, min	RDR	Lentil-Mw-5 time, min	RDR
11.5	13.5 ± 0.7	1	9.8 ± 0.8	1.4	0.9 ± 0.03	14
17.3	50.0 ± 2.6	1	28.0 ± 2.3	1.8	2.8 ± 0.09	18
28.2	400.0 ± 20.8	1	not determined		17.5 ± 0.58	23

(B) RDR calculated from pH drop assays

Sample	$t^{pH\ 7.5}$[b] (min)	RDR[c]	$t^{pH\ 7.3}$[b] (min)	RDR[d]
Commercial lentil	13.5 ± 0.95	1	70 ± 5.2	1
Lentil-Mw-5	2.7 ± 0.11	18.7	17 ± 0.7	23.6

[a] Times are calculated for 100 TU_{active}/mL digestion mixture. The data derived for commercial lentil and Lentil-Mw-2 are average of triplicate experiments, each; those for Lentil-Mw-5 were calculated from 8 experiments; ± stands for standard error. Commercial lentil is taken as a reference
[b] $t^{pH\ 7.5}$ and $t^{pH\ 7.3}$ denote the times needed to reach these pH values starting from pH 8.0.
[c] cf. Eq. (3): t'_{ref}, corresponding to 17.3% peptide bond splitting (cf. Table 3), equaled 50.4 min.
[d] cf. Eq. (3): t'_{ref}, corresponding to 28.2% peptide bond splitting (cf. Table 3), equaled 402 min.

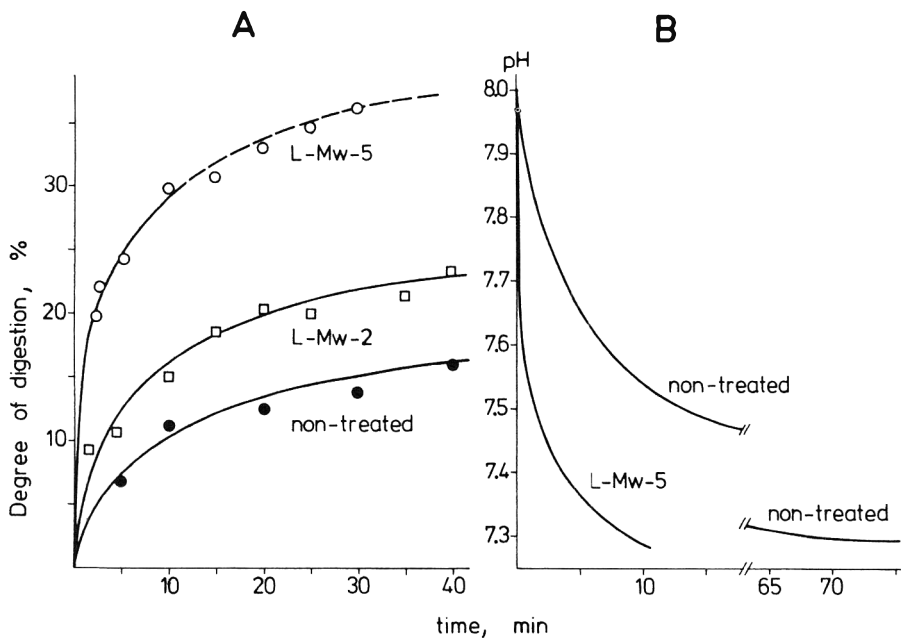

Fig. 1—Time course of digestion of lentil and processed lentil proteins. Protein and trypsin concentration of digestion mixtures was 5.45 mg/mL and 200 TU/mL, respectively. TI activity for the untreated lentil meal suspension at pH 8.0 in the absence of trypsin was 100 TIU/mL. The processed meals did not release detectable TI activity at pH 8.0 (cf. Table 1). (A) Digestion was followed by titration of peptide bond splitting (solid lines) and TCA-soluble peptide release (symbols) determinations. (B) Digestion was followed by recording the pH drop of the mixtures.

Table 2 the RDR values were calculated for active trypsin concentrations. These calculations revealed that the treatment MW-2 only slightly increased the rate of digestion of lentil proteins although irradiation reduced the TI activity. The treatment Mw-5 resulted in a more than 10-fold enhancement in the rate of digestion. The data of Table 2 draw attention to a further effect of the treatments. The RDR values characteristic of processed lentil increase with the progress of digestion. This suggests that the treatment affects differently the digestibility of the lentil protein components: the tryptic susceptibility of the less susceptible protein component(s) being increased to a greater extent than that of already rapidly digestible ones.

It should be noted that the above mentioned positive effect of processing can be directly calculated from the time course of peptide bond cleavage or peptide release. If, however, the pH drop assay is used RDR values corresponding to several pH values should be calculated with the aid of Eq. (3) in order to detect such a phenomenon.

Effect of processing on the in vitro rate of digestion of soy proteins

The effect of two processing technologies was studied. The in vitro digestion following an efficient and non-efficient acid treatment of commercial soy meal Fgy-24 is presented in Fig. 2A and 2B. (The evaluation of RDR from pH drop assays will be shown below). The time course of digestion of the microwave treated sample EnPro-40 is shown in Fig. 3 and, as a comparison, protein digestion of a commercial soy meal of mediocre quality (CH-1) as well as that of its SuproSoy variant (CH-1/S) are also shown. The RDR values characterizing acid treatment of several lots of commercial soy meal as calculated from peptide bond splitting determinations are summarized in Table 4, columns 1 and 2. The RDR values of EnPro-40 relative to sample U-4 and CH-1 are also included. The data show that acid treatment increased the rate of digestion of commercial soy meal proteins by a factor of about two. As to the sample

Table 3—Relationship between buffering capacity and degree of digestion[a]

Sample	pH range	Buffering capacity $\frac{\mu L\ 0.1N\ HCl}{mg\ protein}$	$A = \frac{a_{sample}}{a_{ref}}$	Degree of digestion (%) at time t[b]	
				Peptide bond splitting	Peptide release
Coml lentil meals	8.0 → 7.5	0.55 ± 0.03	Ref	11.2	11.2
	8.0 → 7.3	0.68 ± 0.04		18.5	18.5
Lentil-Mw-5	8.0 → 7.5	0.84 ± 0.05	1.53	17.3	17.3
	8.0 → 7.3	1.03 ± 0.06	1.52	28.2	28.2
Coml soy meals	8.0 → 7.5	0.67 ± 0.03	Ref	13.8	6.9
	8.0 → 7.3	0.84 ± 0.04		23.0	11.5
	8.0 → 7.2	0.93 ± 0.04		30.0	15.0
SuproSoy meals	8.0 → 7.5	0.85 ± 0.05	1.27	17.5	8.8
	8.0 → 7.3	1.06 ± 0.06	1.26	29.0	14.5
	8.0 → 7.2	1.17 ± 0.07	1.26	37.9	19.0
EnPro-40	8.0 → 7.5	0.40 ± 0.02	0.60	8.5	4.3
	8.0 → 7.3	0.57 ± 0.03	0.68	15.8	7.8

[a] The data refer to the meals listed in Table 1. Average of at least 12 determination; ±, standard error for five lots of commercial soy meals and their SuproSoy variants as well as commercial lentil and microwave treated Lentil-Mw-5, respectively.
[b] t is the time required to reach pH 7.5, 7.3, and 7.2, respectively, in the pH-drop assays.

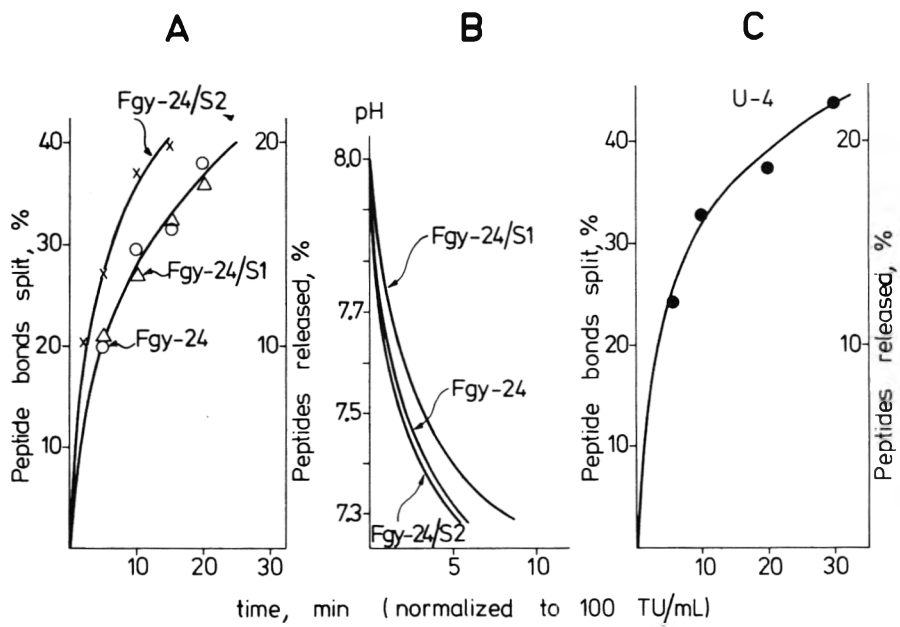

Fig. 2—Effect of acid treatment on the digestion rates of commercial soy meal proteins. Protein concentrations varied between 5–12 mg/mL; trypsin concentrations were 50–200 TU/mL. Average values are presented. Solid lines: titration of peptide bond splitting and pH-drop assay; symbols: peptide release determination. (A) Time course of digestion of sample Fgy-24 and of two of its SuproSoy variants (cf. footnote b to Table 1). (B) pH decrease experiments carried out with samples as in Fig. 2A. (C) Time course of digestion of commercial soy meal U-4, which was used as a reference in the RDR calculations.

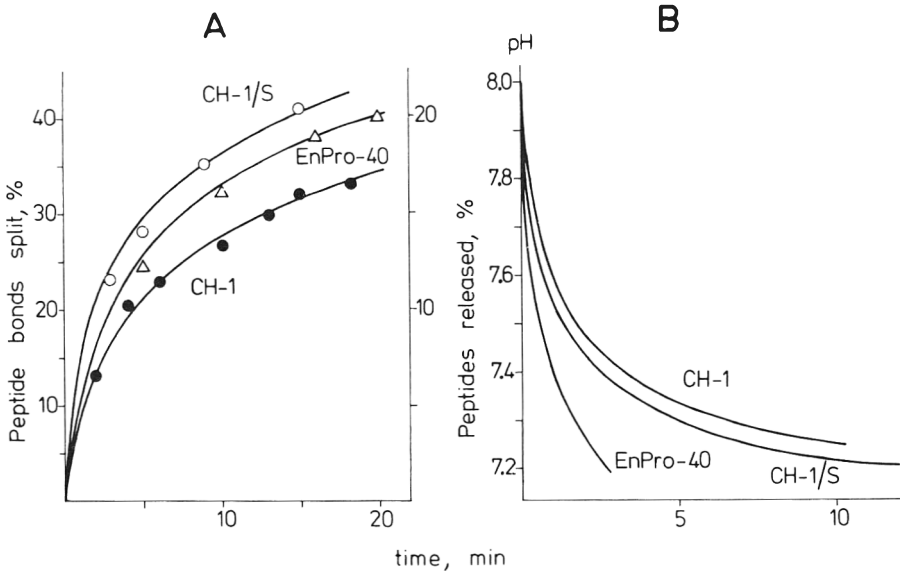

Fig. 3—Effect of different processings on the digestibility of soy proteins. Protein and trypsin concentration was 5 mg/mL and 200 TU/mL, respectively. Solid lines and symbols as in the legends to Fig. 2. CH-1, commercial soy meal; CH-1/S, SuproSoy meal; EnPro-40, microwave treated raw soy bean. TI activity in the absence of trypsin was 25, 2 and 3 TIU/mL for CH-1, CH-1/S and EnPro-40, respectively. (A) Time course of peptide bond cleavage and peptide release. (B) Time course of pH decrease.

Table 4—Characterization of processing technologies of commercial soy meals and raw soy bean[a]

	RDR[b] calculated from peptide bond splitting		RDR calculated from pH drop			
	U-4	Untreated	$t^{pH\ 7.3}$	U-4[c]	U-4[d]	Untreated[e]
Sample	Sample	Processed	min x 1 TU/mL	Untreated	Processed	Processed
U-4	1	1	390	1	1	1
U-4/S	2.85	2.85	300	–	2.50	2.50
Fgy-24	0.67	1	532	0.73	–	1
Fgy-24/S1	0.67	1	835	–	0.88	1.21
Fgy-24/S2	1.55	2.31	490	–	1.53	2.10
82-12	0.50	1	780	0.50	–	1
82-12/S	1	2	780	–	0.96	1.92
CH-1	0.33	1	1250	0.31	–	1
CH-1/S	0.83	2.52	980	–	0.77	2.47
CH-1	0.33	1	1250	0.31	–	1
EnPro-40	0.50	1.52	300	–	0.50[f]	1.61

[a] Data are average values of at least four determinations.
[b] For calculations according to Eq. (1), times corresponding to 23% and/or 29% digestion were used.
[c] cf. Eq. (2): $t_{ref}^{pH\ 7.3}$ corresponds to 23% peptide bond splitting (cf. Table 3).
[d] cf. Eq. (3): t'_{ref}, corresponding to 29% peptide bond splitting (cf. Table 3), equaled 750 min x 1 TU/mL.
[e] cf. Eq. (4).
[f] cf. Eq. (3): t'_{ref}, corresponding to 15.5% peptide bond splitting (cf. Table 3), equaled 150 min x 1 TU/mL.

Table 5—Comparison of a SuproSoy (Fgy-24/S2) with its parent untreated soy meal (Fgy-24) in pig feeding experiments[a]

	Series 1		Series 2	
	Untreated soy meal	SuproSoy meal	Untreated soy meal	SuproSoy meal
Number of animals	549	1 124	79	79
Starting weight, kg	7 430	15 112	1 014	1 038
Average starting weight/animal, kg	13.5	13.5	12.8	13.1
Fattening period, days	76	70	146	146
Weight gain, kg	18 340	40 254	5 169	5 802
Average weight/animal, kg	47	49.3	78.3	86.6
Feed consumption, kg per kg weight gain	3.24	2.99	3.60	3.10

[a] The in vitro RDR value was 2.2.

EnPro-40 its in vitro determined digestion rate was comparable to those of commercial defatted meals.

It should be noted that in the experiments presented and in all other cases investigated, for the release of one TCA soluble peptide the cleavage of an average of two peptide bonds was observed. Thus processing did not change the mechanism of digestion of the proteins. Similarly, tryptic digestion followed pseudo-first order kinetics in all cases tested. In contrast to the phenomenon revealed with processed lentil seed the RDR values did not change appreciably with the progress of digestion. This suggested that the treatments exerted practically the same effect on the rate of digestion of the protein components of the soy.

The acid treatments, exhibiting an RDR value of about two gave good results in pig feeding. Two series of experiments carried out with samples Fgy-24 and Fgy-24/S2 are presented in Table 5. Although both samples showed practically the same value upon exhaustive digestion (about 90%), Fgy-24/S2, which showed a higher rate of digestion in vitro, proved to be a much better feed component.

Calculation of RDR from pH-drop assays with the aid of a single reference titration curve

As shown previously (Hung et al., 1984), the determination of the buffering capacities of the samples and that of the time course of peptide bond splitting of the reference sample is required for calculations of RDR from pH decrease measurements. The buffering capacities of commercial,

—Continued on page 1551

Methionine Sulfoxide Determination After Alkaline Hydrolysis of Amino Acid Mixtures, Model Protein Systems, Soy Products and Infant Formulas

J. M. TODD, N. L. MARABLE, and N. L. KEHRBERG

ABSTRACT

Two previously reported methods (2M NaOH, 18 hr, 100°C; 3M NaOH, 16 hr, 110°C) for alkaline hydrolysis of proteins containing methionine sulfoxide (MetSO) were compared in free amino acid and model protein systems. Recoveries of MetSO from amino acid mixtures after 2M NaOH hydrolysis and ion-exchange chromatography were higher than after 3M NaOH hydrolysis. Recoveries of methionine (Met), MetSO and methionine sulfone ($MetSO_2$) from model proteins after 2M NaOH hydrolysis suggested destruction of Met, no production of $MetSO_2$ and, in the presence of glucose, possible production of small amounts of MetSO. Except for one soy isolate, measured MetSO was ≤ 7% of total methionine (oxidized plus unoxidized) in soy products. In milk- and soy-based infant formulas, measured MetSO ranged from 7 – 32% of total methionine.

INTRODUCTION

A VARIETY of chemical and physical changes which could affect protein nutritional quality may occur during the processing of soy or milk protein. Some changes, such as amino acid destruction, have frequently been studied with well-established methodology. Other changes, such as sulfur amino acid oxidation, which also may be nutritionally important, can be expected to result from heat and/or alkali-treatment of proteins (Walker et al., 1975; Cheftel, 1977). A limited number of studies on the oxidation of sulfur amino acids during the processing of protein food products have been published. Casein, milk, sunflower protein, fish protein, rapeseed flour, egg white, soy isolate, and legume proteins have been studied, usually in model systems (Cuq et al., 1978; Sjoberg and Bostrom, 1977; Slump and Schreuder, 1973; Anderson et al., 1975; Chang et al., 1982; Marshall et al., 1982). Methionine sulfoxide has been detected in meat (Happich, 1975), soy concentrate (Happich et al., 1975), untreated fish protein (Sjoberg and Bostrom, 1977), untreated fish protein and casein (Slump and Schreuder, 1973), native porcine pepsin (Kido and Kassel, 1975), and casein, egg, gelatin, fish, milk, beans, gluten, and soy and rapeseed meals (Njaa, 1980).

Most current methodologies for measuring oxidized forms of methionine such as methionine sulfoxide are questionable. Although difficulties with methodology have frequently been mentioned (Ray and Koshland, 1962; Pieniazek et al., 1975a, b, Gjoen and Njaa, 1977; Njaa, 1980), few method proving experiments have been published. The most widely used methods for determining methionine sulfoxide involve ion-exchange chromatography after basic hydrolysis with approximately 50% NaOH or 2M $Ba(OH)_2$, or an alkylation technique followed by acid hydrolysis and ion-exchange chromatography (Neumann, 1967). Lunder (1972), Kehrberg (1976), Lipton and Bodwell (1977), and Neuman (1967) reported losses in methionine sulfoxide during basic hydrolysis, especially with barium hydroxide. Cuq et al. (1973) reported lower measured levels of methionine sulfoxide in oxidized casein using the alkylation technique than with the 'direct' alkaline hydrolysis method, but no experimental details were given which substantiate the statement that these lower values were the result of incomplete carboxymethylation. Lunder (1972) proposed a method for determining methionine sulfoxide based on the rearrangement of sulfoxide with acetic anhydride, but no evidence was presented to show that the reaction proceeds quantitatively and specifically with methionine sulfoxide in intact proteins. The calibration curve for the acetic anhydride reaction was prepared using free methionine sulfoxide, and thus the data on the methionine sulfoxide content of some proteins may be incorrect. Recently, Njaa (1980) reported the use of a colorimetric method for the determination of methionine sulfoxide. Hydrolysis with barium hydroxide was reported to give better recovery than hydrolysis with 5M NaOH.

Because of the losses of methionine sulfoxide reported in connection with hydrolysis with 50% NaOH or 2M $Ba(OH)_2$, less severe conditions using NaOH have sometimes been utilized. Cuq et al. (1977) and Sjoberg and Bostrom (1977) reported hydrolysis conditions using 3M NaOH at 110°C for 16 hr or 2M NaOH at 100°C for 18 hr, respectively. Neither group reported specific data on recoveries of methionine sulfoxide from the hydrolysis medium. The objectives of the present study were to compare recovery data using these two previously reported hydrolysis methods for measurement of methionine sulfoxide, and to determine the amounts of oxidized forms of methionine in selected soy products and infant formulas.

MATERIALS & METHODS

Materials

One soy flour (Soyafluff 200W), one soy concentrate (Promosoy 100), and two soy isolates (Promine D and Promine F) were obtained from Central Soya Company (Chicago, IL). Five infant formulas were purchased locally and included three milk-based formulas [powdered Similac fortified with iron and concentrated liquid Similac, Ross Laboratories (Columbus, OH) and powdered SMA, Wyeth Laboratories, Inc. (Philadelphia, PA)] and two soy isolate-based formulas in concentrated liquid form [Prosobee, Mead Johnson and Company (Evansville, IN) and Isomil, Ross Laboratories (Columbus, OH)]. Animal Nutrition Research Council reference casein was obtained from Humko-Sheffield Chemical Company (Memphis, TN).

Lysozyme, DL-methionine, DL-methionine sulfoxide, DL-methionine sulfone, cysteic acid, and cystine were purchased from Sigma Company (St. Louis, MO). Ninhydrin, thiodiglycol, and a standard amino acid mixture (Type H) were obtained from Pierce Chemical Company (Rockford, IL). Ethylene glycol monomethyl ether was purchased from Fisher Scientific Company (Fairlawn, NJ). All other chemicals were reagent or chromatographic grade.

Basic hydrolysis

Free amino acids. Methionine, methionine sulfoxide and methionine sulfone were hydrolyzed: (a) individually, (b) in combination with each other and other amino acids (footnote 3, Table 1), (c) individually with glucose present at a level 5 times greater than that

Author Todd is affiliated with the Dept. of Human Nutrition & Foods, Virginia Polytechnic Institute & State Univ., Blacksburg, VA 24061. Author Marable, to whom correspondence should be directed, is with the Dept. of Nutrition & Food Sciences, Univ. of Tennessee, Knoxville, TN 37996-1900. Author Kehrberg is with the Dept. of Home Economics, Western Carolina Univ., Cullowhee, NC 28723.

of the amino acids, and (d) in combination with other amino acids (footnote 3, Table 1) and in the presence of glucose at a level 5 times greater than that of the amino acids. Individual amino acids or amino acid combinations were subjected to two sets of hydrolytic conditions: (1) 2M NaOH for 18 hr at 100°C (Sjoberg and Bostrom, 1977), and (2) 3M NaOH for 16 hr at 110°C (Cuq et al., 1977). One mL of the solution of 20 amino acids (Table 1) or of a 3 - 6 μmole/mL solution of each individual amino acid was placed in polypropylene screw-cap centrifuge tubes (Dynalab, Inc., Rochester, NY). One milliliter of either 4M NaOH or 6M NaOH was added with mixing; the vials were flushed with nitrogen immediately prior to capping. Following hydrolysis, the contents were allowed to cool, the pH adjusted to 1.9 with 6M HCl, and the volume brought to either 10.0 or 25.0 mL with 0.2M sodium citrate buffer, pH 1.9.

Model proteins. One milliliter of a 10 mg/mL solution of lysozyme or 100 mg of casein were hydrolyzed in 2M NaOH (final concentration) as described for free amino acids. Lysozyme and casein were also hydrolyzed using 2M NaOH in the presence of glucose at a level 5 times greater by weight than the respective proteins.

Oxidized lysozyme and casein samples were prepared using H_2O_2 (Cuq et al., 1973; Chang et al., 1982). A 5% casein solution, pH 8, was treated with 0.5% H_2O_2 at 53°C for 45 min. A 2% solution of lysozyme was oxidized without pH adjustment with 0.6% H_2O_2 at 55°C for 1.5 hr. Oxidized proteins were hydrolyzed with and without glucose using 2M NaOH, and oxidized casein was hydrolyzed without glucose using 2M NaOH, and oxidized casein was hydrolyzed without glucose using 3M NaOH as previously described.

Food products. All food products were hydrolyzed with 2M NaOH for 18 hr at 100°C. Dry samples containing 100 mg of protein were weighed directly into polypropylene centrifuge tubes to which 3.0 mL of 4M NaOH and 3.0 mL of deionized water were added. In the case of liquid samples, 3.0 mL of the sample and 3.0 mL of 4M NaOH were pipetted directly into the polypropylene tubes. The contents of all tubes were then mixed and flushed with nitrogen and hydrolyzed as previously described.

Acid hydrolysis

All materials which were base-hydrolyzed were also subjected to acid hydrolysis. In the case of the amino acids and lysozyme, 1 mL of each solution was placed in a 10 mL heat-sealable glass ampoule and ampoule and 4 mL of 6M HCl were added. Approximately 100 - 500 mg of other samples were weighed into 20 mL glass ampoules, and 10 mL 6M HCl were added. The vials were flushed with nitrogen, sealed under a slight vacuum, and heated at 110°C for 24 hr. After filtration through glass wool, aliquots were dried in a vacuum desiccator and resuspended in 0.1M citrate buffer, pH 1.9, 0.2M Na^+.

Performic acid oxidation

Total methionine (methionine plus oxidized methionine) was determined in lysozyme, casein, oxidized lysozyme, oxidized casein, and the food products using the performic acid method of Moore (1963).

Chromatographic conditions

Samples were chromatographed on either a Technicon NC-2P or TSM Amino Acid Analyzer. For the NC-2P, a 0.5 cm x 23 cm column with Technicon Type C-3 resin at 55°C was used; the flow rate was 0.5 mL/min. Citrate buffers (0.2M Na^+) were used for elution as follows: Buffer 1, pH 2.40, 35 min; Buffer 2, pH 3.25, 16 min; Buffer 3, pH 3.61, 30 min. The TSM had a 0.5 cm x 40.0 cm column with Technicon Type C-3 resin at 52°C and a flow rate of 0.41 mL/min. Citrate buffers (0.3M Li^+) were used for elution as follows: Buffer 1, pH 2.68, 23 min; Buffer 2, pH 2.6., 59 min; Buffer 3, pH 3.30, 28 min; Buffer 4, pH 4.15, 55 min. All other solutions and conditions for the operation of both analyzers were described in the Technicon Corporation TSM or NC-2P Operations Manuals (Technicon Instruments Corp., Tarrytown, NY, 1973).

Determination of total nitrogen

Casein and all food products were analyzed for total nitrogen using the macro-Kjeldahl-Gunning-Arnold method (AOAC, 1970).

Food product calculations

Total methionine, methionine sulfoxide and methionine sulfone in the food products were measured after 3 oxidation-hydrolysis or hydrolysis procedures (performic acid oxidation followed by acid hydrolysis, basic hydrolysis with 2 M NaOH, and acid hydrolysis respectively). Unoxidized methionine was calculated by difference (total methionine minus methionine sulfoxide minus methionine sulfone).

Statistical methods

Variances of a set of data were checked for homogeneity using the F_{max}-test (Sokal and Rohlf, 1967). When the variance in a set of data containing only two groups was homogeneous, Student's t-test was done to compare means of the groups. When the variances in a set of data containing three or more groups were homogeneous, one-way analysis of variance was used to analyze the data and the range simultaneous test procedure (Sokal and Rohlf, 1967) was used to detect differences between specific means.

When the variances in a set of data were not homogeneous, a modified t-test for comparing means of groups with unequal variances, described by Sokal and Rohlf (1967), was used to compare means.

Means were considered different from each other if the probability of such a difference was 0.05 or less.

RESULTS & DISCUSSION

Free amino acids

Recoveries of methionine, methionine sulfoxide and methionine sulfone following hydrolysis under various conditions are presented in Table 1. When the amino acids were base-hydrolyzed individually, no form of methionine other than the one being hydrolyzed was recovered (e.g., methionine sulfoxide was not recovered after hydrolysis of methionine). Hydrolysis conditions had little effect on the recovery of methionine sulfone (Table 1).

Table 1—Hydrolysis behavior of selected amino acids

	Percent Recovery[a,b]					
	3M NaOH, 16 hr 110°C		2M NaOH, 18 hr 100°C			
			Hydrolyzed individually		Hydrolyzed in a mixture[c]	
Amino acid	Hydrolyzed individually W/O glucose[d]	Hydrolyzed in a mixture[c] W/O glucose[d]	W/O glucose[d]	W/glucose[d]	W/O glucose[d]	W/glucose[d]
Methionine	93.7 ± 4.0^{w}	83.4 ± 4.5^{x}	94.8 ± 3.3^{w}	99.2 ± 4.5^{w}	89.1 ± 3.2^{x}	90.9 ± 4.3^{x}
Methionine sulfoxide	67.6 ± 2.0^{w}	90.5 ± 3.9^{x}	89.8 ± 3.6^{y}	85.6 ± 2.9^{y}	100.3 ± 2.2^{z}	101.8 ± 3.0^{z}
Methionine sulfone	100.5 ± 4.5^{wx}	97.8 ± 4.3^{x}	101.1 ± 3.8^{w}	103.4 ± 5.2^{w}	104.7 ± 2.1^{w}	102.8 ± 6.4^{w}

[a] All samples were chromatographed on an NC-2P Amino Acid Analyzer.
[b] Values shown are means and standard deviations of three to seven determinations. Where comparisons were made, numbers in horizonta rows having the same superscript letter are not significantly different at 0.05 level. Comparisons were made between columns 1 and 2, 1 and 3, 2 and 5, 3 and 4, 5 and 6, 3 and 5, 4 and 6.
[c] Mixture of 20 amino acids including glycine, asparagine, alanine, valine, serine, glutamic acid, tyrosine, proline, lysine, threonine, histidine, tryptophan, phenylalanine, leucine, arginine, cystine, cysteic acid, methionine, methionine sulfoxide, and methionine sulfone; origina solution concentrations = 0.4-2.8 μmoles/mL.
[d] Without (W/O) glucose, or with (W/) glucose present in the hydrolysis mixture at a level five times greater by weight than the amount of amino acids in the sample.

Methionine sulfoxide recovery was significantly lower ($p < 0.05$) following hydrolysis of individual amino acids with 3M NaOH compared to hydrolysis with 2M NaOH (columns 1 vs 3, Table 1). Sulfoxide recovery was also lower following individual hydrolysis compared to hydrolysis in a mixture of amino acids with either 2M or 3M NaOH both with and without glucose (columns 1 vs 2, 3 vs 5, 4 vs 6, Table 1). Recoveries of methionine sulfoxide after 2M NaOH hydrolysis of an amino acid mixture with or without glucose were excellent (columns 5 and 6, Table 1).

Methionine recovery did not differ significantly between the 2M and 3M NaOH individual hydrolysis conditions, but was significantly decreased when methionine was hydrolyzed in a mixture of amino acids (columns 1 vs 2, 3 vs 5, 4 vs 6, Table 1). Methionine recovery was not significantly affected by the presence of glucose (columns 3 vs 4, 5 vs 6, Table 1).

Model proteins

Recovery of methionine from lysozyme following 2M NaOH hydrolysis was 88% of the total methionine determined after performic acid oxidation. Recovery of methionine from casein following the 2M NaOH hydrolysis was 60% of total methionine (Table 2). This low methionine recovery after alkaline hydrolysis may reflect destruction of methionine during alkaline hydrolysis, as suggested by the hydrolysis of free amino acid mixtures (Table 1), or incomplete release of methionine from the proteins, or both. Cuq et al. (1977) found that methionine was destroyed during alkaline hydrolysis of a tripeptide containing methionine. A small amount of methionine sulfoxide was recovered after hydrolysis of casein with 2M NaOH with or without glucose (1.5 or 0.8 mm/100g respectively, Table 2); however, no sulfoxide was recovered after hydrolysis of lysozyme without glucose (Table 2). The presence of glucose in the hydrolysis mixtures did not affect the recovery of methionine from lysozyme but increased methionine sulfoxide recovery from both casein and lysozyme.

When oxidized lysozyme was hydrolyzed with 2M NaOH, 52% of the theoretical amount of methionine was recovered as methionine and 39% as methionine sulfoxide (column 3, Table 2). Acid hydrolysis apparently reduced all of the methionine sulfoxide present in the oxidized lysozyme to methionine (column 2, Table 2). The acid hydrolysis results for oxidized methionine in intact protein differ from those observed when free methionine sulfoxide was acid-hydrolyzed alone, since only 16% of the free methionine sulfoxide was reduced to methionine in that case (Marable, unpublished data). These differences reaffirm the frequently observed fact that a given amino acid in the free form need not react in the same way or to the same extent as the same amino acid in peptide-bound form.

After 2M NaOH hydrolysis of oxidized casein, only trace amounts of methionine were recovered, and 75% of the total methionine as determined by performic acid oxidation was recovered as methionine sulfoxide (Table 2). Recoveries of methionine sulfoxide from oxidized casein were not significantly different after hydrolysis in 2M and 3M NaOH (13.5 ± 0.7 and 13.7 ± 0.4 mm/100g respectively). Acid hydrolysis of oxidized casein resulted in the recovery of only a trace of methionine sulfoxide. These results again confirm reduction of protein-bound methionine sulfoxide to methionine during acid hydrolysis.

One possible explanation for incomplete recovery of the total methionine in oxidized casen and oxidized lysozyme could be incomplete oxidation with hydrogen peroxide during the preparation of the oxidized proteins, followed by methionine destruction during alkaline hydrolysis. Incomplete oxidation of methionine in casein after H_2O_2 treatment has been observed by Chang et al. (1982) who measured 60 - 70% oxidation at 40 - 90°C using 0.5% H_2O_2 at pH 6.7. Alkaline destruction of methionine has been reported by Cuq et al. (1977) and was indicated in studies with unoxidized casein as discussed above.

Oxidized lysozyme and oxidized casein were also hydrolyzed with 2M NaOH in the presence of glucose (Table 2). The amount of methionine sulfoxide recovered from oxidized casein under these conditions was not significantly different from that recovered after hydrolysis without glucose. The recovery of methionine sulfoxide from oxidized lysozyme hydrolyzed in the presence of glucose was determine only once; thus statistical comparisons of the effect of glucose on recovery from oxidized lysozyme were not made.

Methionine sulfone was not detected in the four protein materials, except for a trace in casein after acid hydrolysis,

Table 2—Methionine, methionine sulfoxide, and methionine sulfone measured in model protein systems[a]

Protein material[b]	Treatment and/or Hydrolysis Method			
	Performic Acid/6N HCl	6N HCl	2M NaOH	
			No Glucose	Glucose[c]
	Millimoles/100g ± standard deviation			
Casein				
Methionine	0	17.7 ± 2.1	11.5 ± 0.8[x]	9.1 ± 0.01[x]
Methionine sulfoxide	0	Trace	0.8 ± 0.1[x]	1.5 ± 0.01[y]
Methionine sulfone	19.1 ± 2.1	Trace	0	0
Oxidized casein				
Methionine	0	14.8 ± 0.9	Trace	Trace
Methionine sulfoxide	0	Trace	13.5 ± 0.7[x]	12.8 ± 1.0[x]
Methionine sulfone	18.1 ± 0.8	0	0	0
Lysozyme				
Methionine[d]	0	12.6 ± 1.1	11.6 ± 1.0[x]	11.1 ± 2.5[x]
Methionine sulfoxide	0	0	0[a]	1.0 ± 0.3[y]
Methionine sulfone	13.2 ± 0.8	0	0	0
Oxidized lysozyme				
Methionine	0	14.6 ± 0.6	7.3 ± 0.2[x]	8.2 ± 1.2[x]
Methionine sulfoxide	0	0	5.4 ± 0.5	4.5
Methionine sulfone	15.3 ± 0.6	0	0	0

[a] The values shown are means and standard deviations of two to eight determinations, except for methionine sulfoxide in oxidized lysozyme with glucose, one determination. Values in horizontal rows having the same letter superscript are not significantly different at the 0.05 level. Comparisons of numbers without superscripts were not made.
[b] See text for source or oxidation method.
[c] Amount of glucose added was five times that of the protein by weight.
[d] Theoretical methionine: 13.97 mmoles/100g (Sober, 1970).

Table 3–Methionine, methionine sulfoxide, and methionine sulfone content of selected soy products and infant formulas[a]

Product	Total methionine[b]	Methionine sulfoxide[c]	Methionine sulfone[d]	Methionine[e]
		Millimoles/16g Nitrogen ± SD		
Soyafluff	10.3 ± 0.6	0.5 ± 0.1	<0.2	9.3
Promosoy	11.0 ± 0.4	0.5 ± 0.1	0	10.5
Promine D	8.8 ± 1.0	5.2 ± 0.7	0.5 ± 0.1	3.1
Promine F	8.7 ± 1.2	0.6 ± 0.1	0.3	7.8
Powdered Similac	17.1 ± 1.7	5.4 ± 0.1	1.5 ± 0.4	10.2
Liquid Similac	14.8 ± 0.6	2.1 ± 0.4	1.6 ± 0.3	11.1
Liquid Prosobee (freeze dried)	12.7 ± 1.6	1.6 ± 0.3	1.5 ± 0.6	9.6
Liquid Isomil	15.2 ± 0.6	1.1 ± 0.4	1.2 ± 0.1	12.9
SMA (powdered)	14.8 ± 1.4	3.1 ± 1.0	<0.2	11.5

[a] Mean and standard deviation of 2-6 independent analyses, except for methionine sulfone in Promine F and Soyafluff, one determination.
[b] Performic acid oxidation, followed by acid hydrolysis.
[c] 2M NaOH hydrolysis.
[d] Acid hydrolysis.
[e] Methionine = Total methionine – (methionine sulfoxide + methionine sulfone).

indicating that the alkaline hydrolysis conditions used in this study did not generate methionine sulfone nor was any methionine sulfone formed during the procedure used to oxidize casein and lysozyme.

Food products

Hydrolysis in 2M NaOH was chosen for methionine sulfoxide determination in food products on the basis of the higher recoveries of sulfoxide when this procedure was used with free amino acids (Table 1) and equal recoveries of sulfoxide from oxidized casein when 2M and 3M NaOH hydrolyses were compared as discussed above.

On the basis of hydrolysis studies with free amino acids, methionine sulfone was stable during hydrolysis with 2M NaOH (Table 1). However, under the chromatography conditions used in this study, methionine sulfone co-eluted with an acid-labile compound after basic hydrolysis. Therefore, methionine sulfone values reported in Table 3 were determined after acid hydrolysis. Since methionine sulfoxide was reduced to methionine during acid hydrolysis, a methionine was apparently destroyed during basic hydrolysis as discussed above, unoxidized methionine was calculated by difference as shown in Table 3 and Materials & Methods.

The soy flour (Soyafluff), soy concentrate (Promosoy), and one soy isolate (Promine F), had, respectively, methionine sulfoxide contents of 5%, 4%, and 7% of total methionine (Table 3). There was little or no methionine sulfone in these products. Another soy isolate (Promine D) had 59% of its total methionine in the form of methionine sulfoxide and approximately 2% as methionine sulfone. Higher levels of sulfoxide in Promosoy have previously been reported (Happich et al., 1975). However, no special basic hydrolysis conditions were reported for the determination of sulfoxide (Happich, 1975), which suggests that some other substance eluting in the sulfoxide position may have caused this apparently higher result.

Methionine sulfoxide levels in infant formulas ranged from 7 – 32% of the total methionine content. The formulas contained about 15% carbohydrate (W/V) in the undiluted liquid formulas and about 56% by weight in the dry formulas. Since the presence of glucose sometimes had an influence on the measured amount of sulfoxide in model proteins (Table 2), these numbers (7 – 32%) may not represent the absolute amount of sulfoxide in the formulas. However, independent evidence for the presence of methionine sulfoxide in SMA was obtained. Feeding of powdered infant formula (SMA) to adult human subjects resulted in the presence of methionine sulfoxide in the plasma within 1 – 3 hr; no sulfoxide was observed in the plasma of control subjects who consumed similar amounts of protein from nonfat dry milk or unoxidized soy (Marable et al., 1980, 1981). The determination of significant levels of oxidized methionine in some infant formulas is of interest since these formulas may provide 90% of an infant's protein needs for the first 6 months of life (Fomon and Zeigler, 1979). Although recent preliminary reports indicate probable availability of methionine sulfoxide to adult humans (Marable et al., 1980, 1981), availability to infants has not been investigated.

SUMMARY

BETTER RECOVERIES of methionine sulfoxide from amino acid mixtures were obtained after hydrolysis with 2M NaOH at 100°C for 18 hr; with or without glucose added (102 ± 3% and 100 ± 2%, respectively) than after hydrolysis with 3M NaOH without glucose (90.5 ± 3.9%). Equal recoveries of methionine sulfoxide from oxidized casein were obtained after 2M and 3M NaOH hydrolysis (13.5 ± 0.7 and 13.7 ± 0.4 mm/100g, respectively). Methionine recoveries from amino acid mixtures (90%) and model proteins (60 – 88%) after 2M NaOH hydrolysis were incomplete. Hydrolysis of model proteins (casein and lysozyme) with 2M NaOH in the presence of glucose may generate small amounts of methionine sulfoxide. Since acid hydrolysis, with or without performic acid oxidation (Moore, 1963), prevents the detection of methionine sulfoxide, and basic hydrolysis destroys methionine, unoxidized methionine in amino acid mixtures or proteins should be calculated by difference as shown in Table 3. Except for one soy isolate, measured methionine sulfoxide was ≤7% of total methionine (oxidized plus unoxidized) in soy products. In milk-based and soy-based infant formulas, measured methionine sulfoxide ranged from 7 – 32% of total methionine. Two powdered infant formulas and one soy isolate contained 21%, 32%, and 59% of total methionine as methionine sulfoxide.

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SuproSoy and EnPro soy meals were significantly different (Table 3). In the RDR evaluation of these samples a further problem arose viz. the efficiency of each processing had to be compared with the starting material. It is not necessary to determine the reference titration curve for each untreated sample. As a simplification, a single untreated sample may be chosen as a reference for the comparison of the digestion rates of all other untreated and treated samples. Thus first $RDR_{ref/untreated}$ and $RDR_{ref/treated}$ should be determined according to Eq. (2) and (3). From these data the RDR reflecting the effect of the treatment can be derived from Eq. (4):

$$RDR_{untreated/treated} = \frac{RDR_{ref/treated}}{RDR_{ref/untreated}} \qquad (4)$$

The titration curve of peptide bond splitting of the commercial soy meal U-4 was taken as a reference (Fig. 2C). It should be emphasized that RDR calculations using a single reference are possible only if the treatments affect the tryptic susceptibility of all protein components of the food to the same extent. As mentioned above this criterion was practically fulfilled in the cases studied.

The results are summarized in Table 4 columns 5 and 6. Comparison with the data in columns 1 and 2 demonstrates that the pH-drop assay gives, within experimental error, the same results as the other two methods applied, if the appropriate RDR calculations are used.

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Volatile Components of an Unflavored Textured Soy Protein

JENNIFER M. AMES and GLESNI MACLEOD

ABSTRACT

Aroma volatiles of textured soy protein (TSP) were extracted and concentrated to a valid isolate using established techniques. By GC-MS mainly, 146 positive identifications and 26 partial characterizations were made. The majority have not been published previously as volatiles of TSP or relevant raw materials (raw soybeans, flakes, flours, concentrates, isolates). Some are probably natural soybean metabolites – β-damascenone and bovolide being reported here for the first time in soy. Many result from lipid oxidation/degradation and from heating sugars and/or amino acids. However, carotenoid degradation and aromatic ring fusion are also indicated, and could play a hitherto undetermined role in soy processing technology.

INTRODUCTION

PREVIOUS PUBLICATIONS on the volatile components of unflavored textured soy protein (TSP), or any relevant TSP raw materials, have included analysis of (a) the raw bean (Fujimaki et al., 1965; Arai et al., 1966a; Arai et al., 1967; Wang, 1972; Greuell, 1974; Honig and Rackis, 1975; Honig et al., 1979; Moll et al., 1979; Doi et al., 1980; Kato et al., 1981; Batinić-Haberle et al., 1981); (b) soy flakes or flours (Teeter et al., 1955; Fujimaki et al., 1965; Arai et al., 1966b; Sessa et al., 1969; Maga and Lorenz, 1974; Maga, 1977; Honig et al., 1979; Hsieh et al., 1982; Melton et al., 1981; How and Morr, 1982); (c) soy concentrate (Honig et al., 1979); (d) soy isolate (Qvist and von Sydow, 1974; Honig et al., 1979; Jackson, 1981; How and Morr, 1982); and (e) TSP (Palkert, 1980). From all these analyses, about 200 volatile components have been identified.

It is noteworthy that only one publication (Palkert, 1980) has reported the analysis of textured (extruded) soy protein volatiles, and in that study 55 identifications were made, 20 of which had not been reported previously in the soy products subjected to less severe heat treatment.

The evolution and use of TSP as a basis for meat anologs has been one of the most significant food technology developments of recent years. In an attempt to create authentic meat substitutes, the flavor industry has responded with a notable growth of improved simulated meat flavors. However, even if perfect meat flavors existed, the successful flavoring of soy protein is limited by off-flavors inherent in soy (or developed during processing) and the absence of a good, attractive positive e.g. meat-like flavor (Rackis et al., 1979; Schutte and van den Ouweland, 1979). It follows therefore that a bland TSP is a useful pre-requisite, and a knowledge of the volatile components which derive from TSP should help achieve this goal. The work described here was initiated in this context.

MATERIALS & METHODS

Isolation of volatile components

Isolate 1. Commercially produced TSP (150g), manufactured from defatted soy flour, was mixed with distilled water (700mL) in a 10L flask and extracted for 1 hr in a Likens and Nickerson (1964) apparatus, as modified by MacLeod and Cave (1975), using triple-distilled 2-methylbutane (20mL) as the solvent. The extract was concentrated to 200μL by a low temperature/high vacuum distillation procedure (MacLeod and Coppock, 1976) using a reduced pressure of approx. 0.02 mm Hg.

Isolate 2. A more concentrated isolate was obtained as above but using TSP (300 g), distilled water (1500mL) and 2-methylbutane (20mL). Three such extracts were combined during concentration to 200μL.

Blank isolate. A blank isolate was obtained, as for isolate 2, but using distilled water only in the 10L flask during solvent extraction.

Gas chromatography

Isolates were analyzed by gas chromatography (GC) on packed and capillary columns using, respectively, Pye-Unicam Series 104 (Model 64) and Perkin Elmer Sigma 2B instruments, both equipped with a heated (250°C) FID. For the fused silica capillary column (50m x 0.32mm i.d.) coated with 0.5μm film of BP20 bonded stationary phase (equivalent to PEG 20M), helium carrier gas (2mL/min) was used in conjunction with a nitrogen make-up gas (30mL/min) and the temperature program was 60°C for 3 min, followed by an increase of 2°C/min to 200°C for the remainder of the run. The injection point heater was at 250°C, and typically 1-4μL were injected using split ratios varying from 5:1 to 20:1 at an attenuation of 1×10^{2} (5×10^{-10} A fsd). Retention times and peak areas were recorded by a Hewlett Packard integrator (Model 3370B); for isolate 1, peak areas were expressed as a percentage of the total peak area i.e. relative percentage abundance (RPA). Packed column analyses were performed using a glass column (5.5m x 4mm i.d.) packed with 10% PEG20M coated on acid-washed 100-120 BSS mesh Diatomite C, with nitrogen (30 mL/min) as the carrier gas and a temperature program of 60°C for 10 min, followed by an increase of 12°C/min to 175°C for the remainder of the run. Typically 4 – 10 μL were injected at an attenuation of 1×10^{3} (1×10^{-9} A fsd).

Gas chromatography-mass spectrometry

Components were identified as far as possible by gas chromatography-mass spectrometry (GC-MS) analysis of isolate 2, using a Perkin Elmer Sigma 3 GC interfaced via a single-stage all glass jet separator at 250°C to a Kratos MS 25 instrument linked online to a Kratos DS 50S data processing system and equipped with a computer-controlled multipeak monitoring (MPM) unit. The same GC conditions as described above were employed but using Grade A helium (40 mL/min) as the carrier gas for the packed column. Significant operating parameters of the mass spectrometer during electron impact ionization work were as follows: ionization voltage 70eV; ionization current 100 μA; source temperature 200°C; accelerating voltage 1.3 kV (packed), 4 kV (capillary); resolution 600; scan speed 3 sec/decade (packed), 1 sec/decade (capillary), repetitive throughout the run. Identical conditions were employed during chemical ionization mass spectrometry except for the following: reagent gas, methane; ionization potential 110eV; emission current 5mA.

Gas chromatography/microwave plasma detection (GC-MPD)

Selective detection of carbon, hydrogen, oxygen, nitrogen and sulfur in the volatile components separated by GC was achieved by a microwave plasma detector (MPD), the operation and reliability of which has been discussed (Brenner, 1978). An MPD (Applied Chromatography Systems) was coupled to a Pye Unicam Series 104 (Model 84) GC. The GC conditions described above for the packed

Authors Ames and MacLeod are affiliated with the Dept. of Food Science & Nutrition, Queen Elizabeth College, Univ. of London, Campden Hill Road, London W8 7AH, England.

column were employed, but using, as carrier gas, Grade A helium purified by a rare gas purifier (British Oxygen Co., RGP MK3). Significant operating parameters of the MPD were as follows: tube current 100mA (oxygen scavenge), 50mA (nitrogen scavenge); HV attenuators C15, H14, N13, O13, S13, Cl 12; ghost corrections variable. Analyses were performed in triplicate using both scavenge gases.

Empirical formulae were calculated by relating elemental GC peak heights to those of compounds positively identified by GC-MS:

$$\frac{C}{X} = \frac{\text{Ht of C pk in UK}}{\text{Ht of X pk in UK}} \times \frac{\text{Ht of X pk in Std}}{\text{Ht of C pk in Std}} \times \frac{\text{No. of C atoms in Std}}{\text{No. of X atoms in Std}}$$

where X is an element other than carbon (C) which occurs in the unkonwn (UK), and C/X is the ratio of carbon atoms to X atoms in the unknown. The internal standards used were toluene, 2-butylfuran, 2-pentylfuran, benzaldehyde and naphthalene.

Odor assessment

Aromas of components separated by GC using 10μL isolate 2 on the packed column in the Pye Unicam 104 instrument, were described at an external GC odor port by three experienced assessors. An outlet splitter (10:1) diverted the major fraction of the eluate through a heated (200°C) line to the odor port.

RESULTS & DISCUSSION

VALID AROMA ISOLATES of the TSP were prepared using well-established procedures that have been reported previously (MacLeod and Coppock, 1976). Aroma isolation conditions were less severe than those used during processing of soybeans to TSP (Pagington, 1975; Kinsella, 1978), and the heat treatment was fairly representative of normal cooking conditions. The creation of artifacts during isolation was therefore unlikely, and this is supported by the fact that an aroma which was characteristic and highly representative of the reconstituted and heated (1 hr) TSP was associated with isolate 1; these aromas were described by three experienced assessors as cereal-like (not roasted), slightly green/beany and slightly musty/moldy. The qualitative GC peak patterns of the two isolates were identical, but isolate 2 was more concentrated than isolate 1.

Isolates were analyzed by GC, GC-MPD and GC-MS (using both electron impact and chemical ionization mass spectrometry) and the results are presented in Table 1. Use of triple-distilled solvent gave a blank isolate chromatogram showing only the 2-methylbutane peak, closely followed by two small impurity peaks, and these are not included in Table 1. The retention data given in the table were obtained using the 50m fused silica column coated with BP20. Kovats retention indices (Jennings and Shibamoto, 1980) of many components (on PEG 20M) are also included, and they confirm the general elution sequence. Select element detection and the calculation of empirical formulae by GC-MPD analysis also confirmed mass spectral interpretations in several instances. The qualitative data in Table 1 were obtained using packed and capillary columns; some components were best identified by GC-MS using one type of column. In all instances where positive identities are given, the mass spectra obtained on GC-MS agreed with those in the literature (e.g. Lardelli et al., 1966; Demole et al., 1970; Eight Peak Index of Mass Spectra, 1974; Jennings and Shibamoto, 1980; Kennet et al., 1977–1982). The polar PEG 20M (and equivalent) columns were selected for analysis because they gave far superior resolution of the total isolate than similar nonpolar OV101 (and equivalent) columns. However, the minimum temperature requirement of about 60°C for PEG 20M did preclude the identity of low boiling components such as pentane.

The complex chemical composition of the volatile fraction is immediately obvious from Table 1. A total of 146 aroma components was positively identified together with a further 26 partial characterizations comprising, in all, about 85% (w/w) of the volatiles. Most of the unidentified components were present in extremely small concentrations and gave rise to poor mass spectra, but three sizeable GC peaks (representing 2.0, 1.5 and 1.5% of the total volatiles) could not be characterized. One hundred three components reported here have not been reported previously as volatiles of any relevant TSP raw materials i.e. raw soybeans, flakes, flours, concentrates or isolates; an additional 35 compounds were not reported by Palkert (1980) in the only previous study on TSP volatiles. Such newly identified components are clearly labelled in Table 1.

A wide range of chemical classes is represented in the TSP aroma; these are mainly aliphatic hydrocarbons, alcohols, aldehydes, ketones, sulfur compounds, esters, benzenoids, terpenes, furans, lactones, thiophens, pyrazines and thiazoles. Some possible precursors and formation pathways for several of the components identified are presented in Table 2, which shows that many can be accounted for by lipid oxidation/degradation and/or the effect of heat on sugars and/or amino acids. Thermal degradations, if any, during chromatography were deemed insignificant, as evidenced by the fact that a chromatogram obtained using the capillary column and lower GC temperature conditions (final column temperature 150°C; detector and injector 175°C) could be correlated with the original chromatogram. These temperatures approximate those used during extrusion of soy protein when considerably higher pressures are also used.

It is well established that autoxidation and/or the action of lipoxygenase is of paramount importance in soy flour (Heinze et al., 1978). In addition, during certain stages of soybean processing and especially during soaking, lipid degradation may be catalyzed by other enzymes including lipases, phospholipases, "hydroperoxidases" and isomerases (Gardner, 1980). Furthermore thermally induced oxidation, as well as nonoxidative thermal degradation of lipids, can occur in any product – such as TSP – subjected to heat. Some residual oil remains even in defatted products (Heinze et al., 1978) and the polyunsaturated fatty acid content of soy lipids is 63% (Masson, 1981). Linoleic and linolenic acids are believed to be the most common oxidation substrates (Smouse and Chang, 1967; Litman and Numrych, 1978; Kinsella and Damodaran, 1980) and their oxidation is facilitated by maceration of the bean and/or the presence of moisture (Kinsella and Damodaran, 1980). The predominant oxidation products are alkanals, alk-2-enals, alka-2,4-dienals, the corresponding alcohols and both saturated and unsaturated ketones (Hoffman, 1962; Forss, 1972; Tressl et al., 1972; Frankel, 1983), and these are also the major volatile components of reverted soybean oil (Smouse and Change, 1967; Selke et al., 1970). Gremli (1974) concluded that aldehydes represent the major fraction of soy protein volatiles, and in agreement with this, aliphatic aldehydes were present in the highest concentration (~32% of the total volatiles) in our TSP aroma – largely due to hexanal at 15.5% of the volatiles; other major aldehydes present were pentanal, hept-2-enal, oct-2-enal and deca-2,4-dienal (see Table 1). The latter compound is the major volatile carbonyl component produced on degradation of linoleic acid at elevated temperatures (Swoboda and Lea, 1965; Kimoto and Gaddis, 1969) and has been associated with the flavor of fried foods (Forss, 1972). It has been identified in a variety of soy protein products, and its formation is likely to be enhanced during toasting and extrusion. Two of its isomers, present at a combined level of 5.0% in our TSP aroma, have been associated previously with an undesirable fatty, oily note in soy (Heinze et al., 1978). Aldehydes generally are believed to affect the flavor of soy products to the greatest extent, due not only

Table 1—Volatile components of an unflavored textured soy protein

Component	New[a,b] identification	t_R (min)	Kováts index (lit.)	RPA	Odor quality
3-methylpentane	a	4.41		0.02	
2-methylpentane	a	4.55		0.25	
methylcyclopentane[d]	b	4.62		0.50	
acetaldehyde[d]	b	4.82	690	1.00	
cyclohexane[d]	a	5.47	765	0.10	
octane[d]	a		800	0.10	oily, fatty, sweaty
methylpropanal[d]	b		800	0.20	caramel, burnt
carbonyl sulfide	a	5.52		0.02	burnt
acetone		5.82	810	0.75	
butanal[c]		6.56		0.01	
2-methylfuran[d]	b		866	–	
2-methyloctane[d]	a	6.73		0.10	green
butanone	b	7.12	908	0.05	
2-methylbutanal	b	7.38		0.20	
3-methylbutanal[d]	b	7.38	937	0.50	fresh green, chem. solvent
2,2,4-trimethylheptane	a	7.70		0.10	
3-methylnonane[c]	a	7.78		0.01	
2-ethylfuran[d]	b	8.08	951	0.50	buttery, caramel
butanedione (diacetyl)	b	8.78	963	0.10	buttery
butenone[d] (methyl vinyl ketone)	a		995	–	green, stale,
pentanal[d]		8.88	1002	1.75[f]	grassy
decane[d]	a		1000	–	caramel, buttery
2-methylbutan-2-ol	a	9.38		1.25	
trichloromethane	b	9.76		0.05	
thiophen	b	10.12	1035	3.25	fruity, stored apples
2-ethyl-5-methylfuran	b	10.25	1024	0.02	
2-propylfuran	b	10.25	1083	0.02	
ethyl butanoate	a	10.52	1025	0.10	
toluene[d]		10.93		0.10	chem. solvent
pentane-2,3-dione	a	11.57	1044	0.25	buttery, caramel
dimethyl disulfide	b	12.25	1081	0.10	
pentan-2-ol	b	12.25	1091		
hexanal		12.65	1084	15.50	green
2-methylthiophen[c]		13.05		0.01	
2-methylbut-2-enal	b	13.36		0.02	
β-pinene	a	13.56	1124	0.05	
2-methylbutyl acetate[d]	a	14.26	1110	0.25	estery, pear-drops
ethylbenzene[c]		14.58		0.20	
2-butylfuran	b	14.77	1130	0.50	wet hay
p-xylene[c]		15.12	1140	0.05	stale, cold meat fat
m-xylene[c,d]		15.28	1147	0.10	unpleasant, rancid
heptane-2,3-dione	a	15.57	1138	0.25	
car-3-ene	a	15.85	1144	0.05	
myrcene	a	16.20	1156	0.10	
*iso*propylbenzene[c,d] (cumene)	a	17.05		0.05	chem. solvent
heptan-2-one[d]		17.63	1172	2.25	slightly green, nutty
heptanal		17.88	1186	1.00	green, nutty
o-xylene[c]		17.88	1191	0.10	
limonene	a	18.32	1206	1.75	fruity, fragrant
hexan-2-ol		18.99	1192	0.20	
propylbenzene[c]	a	19.41		0.05	
hex-2-enal	b	19.68	1207	0.05	
1-ethyl-3-methylbenzene[c]	a	19.77		0.10	
2-pentylfuran	b	20.13	1229	3.50	
pentan-1-ol[d]		20.70	1213	2.75	yeast-like
thiazole	a	21.08	1246	0.02	
β-ocimene	a	21.08	1250(*t*)	–	
1,3,5-trimethylbenzene[c]	a	21.20		0.01	
styrene		21.46		0.02	
octan-3-one	b	21.59	1190	0.01	
2-methylbutyl butanoate	a	21.85	1259	0.10	
1-ethyl-2-methylbenzene[c]	a	21.85		0.02	
methylpyrazine		22.20	1251	0.05	
a methyl *iso*propylbenzene (a cymene)	a	22.29		0.05	
a methyl *iso*propylbenzene (a cymene)	a	22.62		0.10	
1,2,4-trimethylbenzene[c]	a	23.48		0.20	
octan-2-one		23.78	1275	0.20	fragrant
octanal		23.93	1278	0.20	oily, nut oil
a diethylbenzene	a	24.50		0.05	nutty, green sap-like
a methylpropylbenzene	a	24.72		0.10	
a butylbenzene	a	25.26		0.02	
2,5-dimethylpyrazine[c,d]		26.23	1306	0.25	
hept-2-enal[d]	b	26.47		2.0	oily, stale, rancid, linseed oil
a C_4-benzene	a	26.54		0.02	
ethylpyrazine	b	27.12		0.20	burnt, iron scorch
hexan-1-ol[d]		27.30	1316	5.0	green, earthy
cyclohexyl acetate	a	27.90	1343	0.10	
a dimethylethylbenzene	a	28.01		0.02	
2-butylthiophen	b	28.08		0.10	
a dimethylethylbenzene	a	28.44		0.10	
1,2-dimethyl-3-ethylbenzene	a	28.91		0.20	
o-methylstyrene	a	29.20		0.02	
nonan-2-one		30.60	1420	0.05	fragrant, slightly fruity
nonanal[d]	b	30.73	1382	0.50	sweet, fragrant
2-ethyl-5-methylpyrazine		30.87		0.50	
hex-2-en-1-ol[d]	b	30.97	1368(*t*)	2.75	salad vegetable, rancid, blue cheese
trimethylpyrazine		31.82	1387	0.05	
oct-3-en-2-one	b	31.95		1.50	flat, dull, green
a tetramethylbenzene	a	32.96		0.01	
oct-2-enal[d]	b	33.38	1427	2.50	dandelion/dock leaves
oct-1-en-3-ol	b	33.70	1420	2.25	oily, fatty, cucumber, stale nuts
a dimethylethylpyrazine[d]		35.35		0.20	nutty
2-acetylfuran	a	35.91	1491	0.01	
2-pentylthiophen		36.00	1462	0.01	
a C_4-pyrazine		36.34		0.02	
2-furaldehyde		36.43	1449	0.25	roasted cereal, roasted lamb fat, rancid
hepta-2,4-dienal[d]	b	36.51		0.05	unpleasant, green, sap-like
2,6-dimethylstyrene	a	36.68		0.02	
indene	a	37.01		0.02	
1,3-dimethyl-4-ethylbenzene	a	37.06		0.10	
decan-2-one		37.39	1480	0.10	
hepta-2,4-dienal[d]	b	37.60		1.00	green, cucumber
decanal	b	37.75	1485	0.01	slightly green, fragrant
a dimethylindan	a	38.09		0.01	
non-3-en-2-one	a	38.82		0.25	
benzaldehyde		39.43	1502	6.00	almonds, nutty
1,2,3,4-tetrahydronaphthalene	a	39.83		0.20	
dec-4-enal	a	39.97	1523(c)	0.05	
non-2-enal	a	40.30	1540	0.75	
octan-1-ol	b	40.72	1519	0.25	floral, hyacinth, oily, fatty
2-methylpyrrole	a	41.05		0.01	
2-methyl-1,2,3,4-tetrahydronaphthalene	a	41.94		0.01	
2,4 (or2,5)-dimethyl-*iso*propylbenzene	a	42.25		0.02	
octa 3,5-dien-2-one	a	42.65		1.75	
2-propionylfuran[d]	a	43.04	1563	0.02	green, nutty
octa 2,4-dienal[d]	a	43.26		0.02	
3-methylindan-1-one (?)	a	43.45		0.02	
octa-2,4-dienal[d]	a	43.85		0.05	oily, walnuts
1,6-dimethylindan	a	45.51		0.10	
phenylacetaldehyde[d]		47.41	1646	0.02	floral, fragrant
acetophenone		47.69	1627	0.10	slightly meaty
nona-2,4-dienal[d]		48.33		0.01	
a trimethylindan	a	48.33		–	
thiophen-3-carboxaldehyde	a	49.15		0.01	
o-hydroxybenzaldehyde (salicylaldehyde)	a	49.40	1668	0.10	
heptadecane	a	49.64	1700	0.02	
thiophen-2-carboxaldehyde	a	50.15		0.20	
a terpenoid	a	50.15		0.05	
6-methyl-1,2,3,4-tetrahydronaphthalene	a	50.43		0.02	
nona-2,4-dienal[d]		50.83		0.25	unpleasant, cereal-like, wet wool
terephthaldehyde[d]	a	51.25		0.05	
naphthalene		52.95		0.50	
undec-2-enal	a	53.82		0.05	
deca-2,4-dienal[d]	b	54.62		0.50	
p-methylacetophenone[c]	a	55.59	1750	0.05	
deca-2,4-dienal[d]	b	57.47		4.50	
2,5-dimethylthiophen-3-carboxaldehyde	a	57.47		0.20	
2-butanoyl-5-methylfuran	a	57.79	1748	0.05	
1-*trans*-but-2-enoyl-2,6,6-trimethylcyclohexa-1,3-diene[e] (β-damascenone)	a	58.15	1801[g]	0.20	
2-methylnaphthalene[c]	a	59.81		0.05	floral, fragrant
2-acetyl-4-methyl-cyclopentane-1,3-dione	a	61.11		0.01	
1-methylnaphthalene[c]	a	61.67		0.10	hay
a monoterpene	a	62.05		0.05	
2-phenylethanol[c]	a	62.28	1859	0.01	floral, fragrant
2,6-di-*t*-butyl-*p*-hydroxytoluene (BHT)		62.80		0.10	toasted cereal
undeca-2,4-dienal	a	63.49		0.02	
2-methyl-5-pentanoylfuran	a	63.69		0.05	
2-pentanoylthiophen	a	63.97	1993	0.02	
2-phenylbut-2-enal	a	64.31		0.02	
2-ethylnaphthalene[c]	a	64.80		0.05	
benzothiazole	a	65.20		0.20	
1-ethylnaphthalene[c]	a	65.51		0.10	
a dimethylnaphthalene	a	65.71		0.25	
10-methylnonadecane	a	66.88		0.01	
a dimethylnaphthalene	a	67.32		0.05	
a dimethylnaphthalene	a	67.67		0.20	
1,5-di*iso*propyl-3-ethylbenzene	a	68.05		0.05	
pantadecan-2-one	a	68.50		0.05	
a dimethylnaphthalene	a	69.65		0.02	
4-hydroxydecanoic acid, lactone (γ-decalactone)	a	69.86	2101	0.10	
an *iso*propylnaphthalene	a	70.39		0.10	
1,4-di*iso*propyl-2,5-dimethylbenzene(?)	a	72.52		0.50	
diphenylmethane	a	72.97		0.05	
an *iso*propylnaphthalene	a	73.47		0.02	
a trimethylnaphthalene	a	73.66		–	
a trimethylnaphthalene	a	73.88		0.05	
a trimethylnaphthalene	a	74.66		0.02	
2,3-dimethyl-4-hydroxynona-2,4-dienoic acid, lactone[f] (bovolide)	a	77.04	2133[h]	0.05	
a trimethylbenzaldehyde	a	77.44		0.05	
2-methoxy-4-vinylphenol (*p*-vinylguaiacol)	b	78.35		1.75	
2-benzylideneheptanal (α-pentylcinnamaldehyde)	a	84.63	2211	0.10	
dibenzofuran	a	85.39		0.20	

RPA values relate to isolate 1 and have been corrected as follows: >5% quoted to nearest 0.5%; 0.25-5% quoted to nearest 0.25%; <0.25% quoted as 0.25, 0.20, 0.10, 0.05, 0.02 and 0.01%; '–' indicates no accurate peak area measurement possible.

[a] Denotes compounds which have not been reported previously as volatiles of any relevant TSP raw materials, i.e. raw soybeans, soy flakes, soy flours, soy concentrate or soy isolate (see Introduction)

[b] Denotes compounds which have not been reported previously as volatiles of TSP (Palkert, 1980)

[c] Denotes identification by t_R measurements on reference compounds as well as by GC-MS i.e. when mass spectra could only partially characterize components

[d] Denotes confirmation of identity by GC-MPD.

[e] MS lit. Demole et al. (1970); Nursten and Woolfe (1972); Tressl et al. (1978)

[f] MS lit. Lardelli et al. (1966)

[g] Tressl et al. (1978)

[h] Patel (1983)

to their low odor threshold values (Kinsella and Damodaran, 1980), but also to their ability to bind to soy protein (Gremli, 1974). Components of Table 2, other than aliphatic aldehydes, which could have derived from lipid degradation and which were present in relatively large concentrations in the TSP aroma were heptan-2-one, oct-3-en-2-one, pentan-1-ol, hexan-1-ol, hex-2-en-1-ol, oct-1-en-3-ol, benzaldehyde and 2-pentylfuran. Several of the aliphatic hydrocarbons of Table 1 have not been identified previously in relevant soy raw materials and probably arise from thermal nonoxidative degradation of lipids e.g. during extrusion (Nawar, 1969; Litman and Numrych, 1978).

Table 2 shows that a significant impact of heat is to produce certain short chain aliphatic aldehydes, ketones and sulfur compounds, certain benzenoids, furanoids, thiophens and pyrazines. Thermal degradation of sugars, pyrolysis of amino acids, Strecker degradations and Maillard reactions will certainly occur during extrusion – a process involving temperatures of about 150°C and pressures of about 1000 psi (Pagington, 1975). Thiamin degradation by heat may also contribute to the presence of 2-methylfuran and 2-methylthiophen (van der Linde et al., 1979) while thermal decarboxylation of ferulic acid is the likely explanation for the presence of *p*-vinylguaiacol (Greuell, 1974). Thiophen is present in the TSP aroma at the relatively high concentration of 3.25% of the total volatiles, and previous studies reporting the presence of thiophens in soy protein products have also involved the application of heat at 121°C or above (Qvist and von Sydow, 1974; Palkert, 1980). Similarly, the only soy protein product in which dimethyl disulfide has been reported previously was a heated soy isolate (Qvist and von Sydow, 1974). Also, pyrazines have been identified only in heated soy products (Maga, 1977; Doi et al., 1980; Palkert, 1980).

Certain compounds are renowned for their contribution to soy off-flavors. For example, green, beany off-odor has been attributed to several aliphatic alcohols, aldehydes, ketones (including pent-1-en-3-one i.e. ethyl vinyl ketone) and 2-pentylfuran (e.g. Mattick and Hand, 1969; Heinze et al., 1978; van den Ouweland and Schutte, 1978; Rackis et al., 1979; Kinsella and Damodaran, 1980). Oxidized, oily, rancid and paint-like odors have been attributed to the higher alka-2,4-dienals (Rackis et al., 1979). Musty, moldy quality has been associated with geosmin and acetophenone (Rackis et al., 1979; Kinsella and Damodaran, 1980) while a cooked off-flavor is reported to develop as a result of the presence of *p*-vinylphenol and *p*-vinylguaiacol (van den Ouweland and Schutte, 1978). In the present study, specific attempts were made to detect these compounds, including use of the MPM unit of the GC-MS, but no evidence of the presence of either ethyl vinyl ketone (I 1047, reference compound) or *p*-vinylphenol or geosmin could be obtained. The absence of ethyl vinyl ketone was also noted by Palkert (1980) in his study of TSP. It derives from lipoxygenase action, and other oxidation products were certainly present in our TSP volatiles. Both *p*-coumaric acid and ferulic acid, the precursors of *p*-vinylphenol and *p*-vinylguaiacol respectively (Rackis et al., 1979), are reportedly extracted from soy flour with polar solvents e.g. aqueous ethanol (Rackis et al., 1979; Kinsella, 1978) such that the resultant product does not develop the offensive cooked flavor; however if this explains the absence of *p*-vinylphenol in the TSP volatiles, it would appear that ferulic acid is the more likely of the two precursors to survive such treatment.

Some of the compounds identified here for the first time in TSP (or its relevant raw materials) deserve special mention. The monoterpenes (C_{10}) are probably naturally occurring metabolites of the bean. However, an interesting C_{13} terpenoid-related compound, β-damascenone, was also present (see Fig. 1). This compound was first identified by Demole et al. (1970) in Bulgarian rose oil, and it has since been reported in Burley tobacco (Demole and Berthet, 1971) and in several plant foods e.g. cooked apples (Nursten and Wolffe, 1972) and tea (Renold et al., 1974). It derives in nature from oxidative degradation of the caro-

Table 2—Possible origins of some of the TSP aroma components

Lipid oxidation/ degradation	Heated sugars and/or amino acids	Other
octane[abcde]	acetaldehyde[tuvw]	cyclohexane[k']
decane[afg]	butanal[k]	methylcyclo-pentane[k']
heptadecane	pentanal[kw]	toluene[l']
penten-1-ol[abcdfh]	hexanal[x]	*m*-xylene[l']
hexan-1-ol[dfh]	methylpropanal[tyza']	BHT[m']
octan-1-ol[bcdel]	2-methylbutanal[tvb']	*p*-vinylguaiacol[n']
pentan-2-ol[f]	3-methylbutanal[vz]	2,6-dimethyl-naphthalene[l']
hexan-2-ol	acetone[tu]	naphthalenes[l'o']
hex-2-en-1-ol	butanone[tux]	monoterpenes[p']
oct-1-en-3-ol[abcef]	butanedione[tx]	β-damascenone[p'q']
butanal[bcfg]	pentane-2,3-dione[tx]	2-methylfuran[r']
pentanal[bcdef]	butenone[x]	bovolide[p']
hexanal[abcdef]	toluene[txc'd']	2-methylthiophen[r']
heptanal[bcdf]	ethylbenzene[tc']	chloroform[s']
octanal[abcdef]	propylbenzene[c']	
nonanal[abcdef]	*o*-xylene[t]	
decanal[abcde]	*m*-xylene[tx]	
2-methylbut-2-enal	*p*-xylene[tx]	
hex-2-enal[cg]	1,2,4-trimethylbenzene[t]	
hept-2-enal[abce]	1,3,5-trimethylbenzene[t]	
oct-2-enal[abcdfg]	benzaldehyde[xc']	
non-2-enal[bcdfj]	phenylactaldehyde[vc']	
undec-2-enal[abcde]	acetophenone[e']	
dec-4-enal[l]	sytrene[c']	
hepta-2,4-dienal[fgk]	indene[c']	
octa-2,4-dienals	carbonyl sulfide[f']	
nona-2,4-dienal[abc]	dimethyl disulfide[va']	
deca-2,4-dienals[abce]	2-methylfuran[t]	
undeca-2,4-dienal	2-ethylfuran[t]	
heptan-2-one[dfglmn]	2-propylfuran[t]	
octan-2-one[dfglmn]	2-ethyl-5-methylfuran[t]	
nonan-2-one[fgmn]	2-furaldehyde[twg']	
decan-2-one[gmn]	2-acetylfuran[twxg'h']	
pentadecan-2-one[gmn]	thiophen[xf'g']	
octan-3-one[g]	2-methylthiophen[xf']	
butanedione[a]	2-butylthiophen	
pentane-2,3-dione[a]	2-pentylthiophen	
heptane-2,3-dione	thiophen-2-carboxaldehyde[xh']	
oct-3-en-2-one[g]	thiophen-3-carboxaldehyde	
non-3-en-2-one	2,5-dimethylthiophen-3-carboxaldehyde	
octa-3,5-dien-2-one[j]	2-methylpyrrole[i']	
p-xylene[o]	methylpyrazine[g'i']	
alkylbenzenes[p]	ethylpyrazine[i'j']	
benzaldehyde[afq]	2,5-dimethylpyrazine[g'j']	
acetophenone[af]	2,6-dimethylpyrazine[j']	
naphthalene[o]	trimethylpyrazine[wg'i']	
2-butylfuran[o]	2-ethyl-5-methylpyrazine	
2-pentylfuran[bceflor]	thiazole[g']	
γ-decalactone[s]		

[a] Frankel (1980)
[b] Frankel et al. (1981)
[c] Frankel (1983)
[d] Selke et al. (1977)
[e] Selke et al. (1980)
[f] Smouse and Chang (1967)
[g] Chang et al. (1978)
[h] Tressl et al. (1977)
[i] Selke et al. (1978)
[j] Badings (1970)
[k] Sessa (1979)
[l] Nawar et al. (1978)
[m] Nawar (1969)
[n] Litman and Numrych (1978)
[o] Forss (1972)
[p] Min et al. (1977)
[q] Kawada et al. (1967)
[r] Chang et al. (1966)
[s] Watanabe and Sato (1971)
[t] Thermal degradation of glucose (Sugisawa, 1966; Heynes et al., 1966; Walter and Fagerson, 1968)
[u] Pyrolysis of various amino acids (MacLeod and Seyyedain-Ardebili, 1981)
[v] Strecker degradation (MacLeod and Seyyedain-Ardebili, 1981)
[w] Heated glucose (or fructose)/glycine (van den Ouweland et al., 1978)
[x] Heated glucose/cysteine (Scanlan et al., 1973)
[y] Pyrolysis of valine (Merritt and Robertson, 1967)
[z] Pyrolysis of leucine (Lien and Nawar, 1974)
[a'] Pyrolysis of methionine (Fujimaki et al., 1969)
[b'] Pyrolysis of isoleucine (Lien and Nawar, 1974)
[c'] Pyrolysis of phenylalanine (MacLeod and Seyyedain-Ardebili, 1981)
[d'] Pyrolysis of tyrosine (Merritt and Robertson, 1967)
[e'] Decomposition of tryptophan (Kinsella and Damodaran, 1980)
[f'] Pyrolysis of cysteine (Kato et al., 1973a; Merritt and Robertson, 1967)
[g'] Heated cysteine (or cystine)/carbonyl compound (Kato et al., 1973b)
[h'] Heated cysteine/cystine-ribose (Mulders, 1973)
[i'] Pyrolysis of serine or threonine (Wang and Odell, 1973; Kato et al., 1970)
[j'] Heated glucose/H_2S/NH_3 (Shibamoto and Russell, 1977)
[k'] Possible impurity in defatting solvent (Honig et al., 1979)
[l'] Thermal degradation of carotenoids (Finar, 1959; Drawert et al., 1981)
[m'] Lipid antioxidant (Frankel, 1980)
[n'] Thermal decarboxylation of ferulic acid (Greuell, 1974)
[o'] Fused ring formation from other benzenoids (Vitzthum et al., 1975)
[p'] Naturally-occurring metabolite
[q'] Oxidative degradation of carotenoids (Isoe et al., 1973)
[r'] Th amin degradation (van der Linde et al., 1979)
[s'] Possible contaminant deriving from pesticide residues (Ho and Coleman, 1981)

tenoid neoxanthin (Isoe et al., 1973) and, although it is present in green tobacco, curing ageing increases its concentration considerably (Enzell, 1981); a similar increased production may occur TSP during processing and storage.

Thermal degradation of carotenoids is known to occur and, for example, β-carotene forms toluene, *m*-xylene and 2,6-dimethylnaphthalene (Finar, 1959), especially in the absence of air (Drawert et al., 1981). These products are formed by cyclization of fragments of the ployene chain, without the β-ionone rings being involved (Finar, 1959). This route may well explain the above products and also some of the very many other benzenoid derivatives listed in Table 1, in addition to the alternative feasible formation routes summarised in Table 2. In particular, the several naphthalene derivatives identified may form, as described, by thermal degradation of carotenoids. Some naphthalene derivatives have been reported previously in heated foods e.g. coffee (Stoll et al., 1967), cocoa (Vitzthum et al., 1975) and roasted peanuts (Walradt et al., 1971). They have also been identified in the volatiles produced on heating the benzenoid porous polymer, Porapak Q (Vitzthum et al., 1975) and so a fused ring system can form on heating, – possibly from simple substituted benzenes e.g. xylenes, ethylbenzene, styrene. The high temperatures and pressures of extrusion may well, therefore, be envisaged as suitable conditions for the formation of such fused aromatic compounds in TSP. The soybean itself is also a natural source of many nonvolatile benzenoids (Heinze et al., 1978) which could act as precursors of volatile derivatives. For example, the identified 2-benzylideneheptanal or α-pentylcinnamaldehyde (Table 1) probably arises as a reaction product of a naturally occurring cinnamic acid.

A final compound worth mentioning is bovolide (see Fig. 1). It was first identified as a new γ-lactone in butter and has a strong celery-like odor (Boldingh and Taylor, 1962; Lardelli et al., 1966); it was so-named because of its bovine origin. It has since been identified in other products e.g. tobacco (Demole and Berthet, 1972). The original authors have suggested that, in the absence of other homologs, it is not connected with normal biological fatty acid synthesis, and they showed that its origin in butter is from grass in the cow fodder. It is probably also naturally occurring in soybeans.

CONCLUSION

THIS STUDY SHOWS that the aroma of TSP is due to a large number of volatile components, representing a wide range of chemical classes. Some are likely to be naturally occurring metabolites of the soybean, and in this context two components – β-damascenone and bovolide – are reported here for the first time in soy. Others are accounted for by lipid oxidation/degradation and by the effect of heat on sugars and/or amino acids. However, this work indicates that carotenoid degradation also probably occurs significantly, and this could play a hitherto undetermined role in soy processing technology. Its significance to the flavor of processed tomato products is already realized (Drawert et al., 1981). Additionally, as evidenced by the identification of several naphthalene derivatives, aromatic ring fusion or cyclization may also result under extrusion conditions.

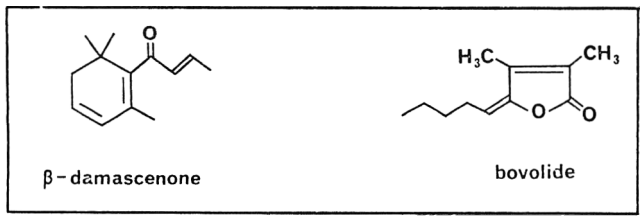

Fig. 1—Chemical structure of β-damascenone and bovolide.

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Relative Bioavailability of Dietary Iron from Three Processed Soy Products

MARY FRANCES PICCIANO, KARL E. WEINGARTNER, and JOHN W. ERDMAN, JR.

ABSTRACT

The relative bioavailability of iron from soy flour (SF), freeze-dried soy beverage (SB) and soy concentrate (SC) was determined utilizing a hemoglobin repletion bioassay. Weanling male rats were fed a low iron depletion diet (3.5 ppm Fe) for 4 wk. For the next 2 wk groups of rats were fed repletion diets containing 0, 6, 12, or 18 ppm added iron from ferrous sulfate, SF, SB, or SC. Slope ratio analysis revealed that the relative iron bioavailabilities from SC (92%) and SF (81%) were not different from the reference standard, ferrous sulfate added to a casein-based diet, whereas that from SB (66%) was significantly less ($P<0.01$) than the inorganic source of iron. Analysis of results at individual iron levels suggested an iron bioavailability of SC>SF>SB.

INTRODUCTION

Recent human studies suggesting that soy products are inhibitory to nonheme iron absorption (Cook et al., 1981, Morck et al., 1982) are in reasonable agreement with some previous human studies (Bjorn-Rasmussen et al., 1973; Ashworth et al., 1973) but not with others (Cook et al., 1972; Layrisse et al., 1969). Bodwell (1983) most recently found no adverse affects on iron status due to feeding humans moderate quantities of soy products daily for 6 months.

Rat feeding studies employing hemoglobin repletion methodology (Steinke and Hopkins, 1978; Rotruck and Luhrsen, 1979) or radiolabeled test meals (Schricker et al., 1983) show relative iron availability from various soy products to be 60-90% that of $FeSO_4$ added to casein-based diets.

Previous publications from our laboratory (Forbes and Parker, 1977; Forbes et al., 1979) described the bioavailability of zinc, magnesium, and calcium from three soy products: full fat soy flour (SF), freeze-dried soy beverage (SB), and a commercial soy concentrate (SC). It was determined that zinc was poorly available from the three soy products, especially SC. Magnesium was highly available from SF and SB; magnesium utilization from SC was good but was less than from the other soy products. Calcium added to all soy products was highly available. Zinc added to SF was highly available, while zinc added to SC was not fully available. From these and other studies (Erdman et al., 1980; Ketelsen et al., 1984), we can conclude that conditions during processing influence the bioavailability of zinc from soy products.

Since common types of food processing techniques have been reported to cause changes in available iron (Lee and Clydesdale, 1979; Anon, 1982), the present study was designed to determine if the relative iron availability, as indicated by the rat hemoglobin repletion assay, differed between these same three soy products.

Author Picciano is affiliated with the Dept. of Food & Nutrition, 457 Bevier Hall, and Authors Weingartner and Erdman are with the Dept. of Food Science, 567 Bevier Hall, Univ. of Illinois, Urbana, IL 61801.

MATERIALS & METHODS

Production of soy products

Three soybean products, full-fat soy flour (SF), freeze-dried soy beverage (SB) and a commercial soy concentrate (SC) were utilized for the current studies. The soy flour and soy beverage were prepared in an identical manner to those products used in the previous work, and the concentrate was derived from the same lot of commercial soy product (Forbes and Parker, 1977; Forbes et al., 1979). The composition of the soy products is shown in Table 1. The soy flour and beverage were processed in a manner similar to published procedures (Erdman et al., 1977; Nelson et al., 1976).

For full fat soy flour, whole soybeans (Bonus variety) were dry-cleaned and heated in a variable air dryer (Proctor and Schwartz, Inc., Philadelphia PA) at 93°C for 20 min and quickly passed through a spinning drum-plate apparatus in order to split and separate the hulls and cotyledons. The cotyledons were blanched by stirring them into boiling distilled water (5:1, water:bean ratio) for 20 min. The blanch water was discarded and the cotyledons were immediately milled with an equal weight of distilled water in a Rietz Disintegrator (Rietz Co., Westchester, PA) utilizing a 0.023 inch mesh screen. The resulting slurry was dried on an 8-inch double drum dryer and flaker (Blow-Knox Company, Buffalo, NY). The dryer was operated at 40 psi steam with spacing between the drums set at 0.01 inch. The resultant flake was passed through a 40 mesh sieve with gentle rubbing and was stored at 1°C until use.

To produce the freeze-dried soy beverage, Bonus soybeans were cleaned, dehulled and blanched as described above. The cotyledons were ground with enough distilled water added to the drained, blanched beans to make a final 10% slurry. The slurry was reground, heated in a steam jacketed kettle to 85°C and immediately homogenized at 82°C with a Gaulin model 15M-8TA single piston two stage homogenizer (Gaulin Corp., Everett, MA). The second stage was set at 500 psig and the first at 3500 psig. This soy beverage was diluted to 6% soy solids with distilled water, reheated to 85°C and rehomogenized as above. The soy beverage was then freeze dried (Stokes Process Equipment Co., Philadelphia, PA) and passed through a screen and stored at 1°C until used.

The soy concentrate was obtained directly from the manufacturer (GL 301, Griffith Labs, Chicago, IL). It was produced by the following general procedure. To a defatted soy flour with high NSI (Nitrogen Solubility Index) was added tap water, $NaHSO_3$ and

Table 1—Composition of soy products

	Flour	Beverage	Concentrate
Solids[a] (%)	95.3	91.4	95.7
Protein[a] (%)	42.9	42.5	68.3
Ether extract[a] (%)	22.5	21.3	0.3
Calcium[b] (%)	0.17	0.18	0.22
Magnesium[b] (%)	0.19	0.19	0.10
Iron[b] (ppm)	55.8	63.0	103.8
Zinc[a] (ppm)	44	44	32
Total phosphorus[c] (%)	0.58	0.63	0.60
Phytate phosphorus[d] (%)	0.36	0.32	0.47
Phytic acid[d] (%)	1.24	1.17	1.67

[a] Analyses performed using AOAC (1975) methods. Solids, section 14.076; Protein (N x 6.25), section 2.049; Ether Extract, section 14.080; Zinc, section 25.146

[b] Atomic absorption spectrophotometric analysis of wet ashed (nitric acid-sulfuric acid:4-1) or dry ashed samples (550°C for 5 hr)

[c] Bartlett (1959)

[d] Earley and DeTurk (1944), assuming 28.2% phosphorus in phytic acid

enough HCl to reach the isoelectric point of the protein (pH 4.2). After filtering, the cake was washed with tap water, filtered, and the second cake was neutralized to pH 6.7 wiith NaOH solution. This neutralized product was heated to 50-60°C and spray dried.

Analytical methods

Procedures used to analyze the soy products and the diets are indicated in footnotes in Table 1. Hemoglobin was determined from tail blood of rats by the cyanomethemoglobin method (Hycel 116C, Hycel Inc., Houston, TX).

Experimental design

One hundred four weanling male Sprague-Dawley rats (Harlan Industry, Inc., Cumberland, IN) weighing about 50g were individually housed in stainless steel cages in a controlled-temperature environment. For 4 wk the rats were fed a low iron depletion diet (3.5 ppm Fe) (Table 2) to reduce their body stores of iron. Depletion was ascertained by a mean hemoglobin concentration of ≤ 6.0 g/100 mL. The rats then were divided into 13 groups of similar hemoglobin concentration (Average Hb = 5.3 g/mL; range 5.1-5.6 g/dL) and body weight (Average weight = 160g; range = 150-169g). No significant differences were noted among groups after the 4-wk depletion period when rat weight was multiplied by Hb concentration for each rat. During the 2-wk regeneration period test diets were fed. Relative iron bioavailability was determined by the hemoglobin repletion assay according to AOAC procedure (Fritz et al., 1975). The control diets (Table 2) were casein-based and contained either 0, 6, 12 or 18 ppm iron as ferrous sulfate. Experimental diets were formulated to contain 6, 12 or 18 ppm iron from one of the soy products. Soy replaced casein on an isonitrogenous basis and corn oil was added to equalize total fat in all diets at 7.27%. DL-methionine was added to all diets at levels of 0.1%. In addition, to help equalize protein quality between casein and soy protein, L-methionine was supplemented to all soy diets at a level of 1.74% of soy protein in the diet. Iron content of diets was verified by analysis.

Statistical analysis

The data obtained were statistically analyzed by regression analysis to compare the slopes of the hemoglobin regeneration lines relating hemoglobin gain per unit of iron content of the diet (Fritz et al., 1975). In addition, standard analysis of variance and least significant difference tests were performed on data derived from each dietary iron level (Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE RESULTS of the hemoglobin repletion assay are shown in Tables 3 and 4 and Fig. 1. Slope ratio analysis of final hemoglobin concentration regressed against added dietary iron (Fig. 1) indicates that although relative iron bioavailability from all three soy products fell below the standard $FeSO_4$ (SC = 92, SF = 81, and SB = 66%), only the bioavailability of iron from the soy beverage was significantly lower than the standard. The processing procedures utilized to produce the SF and SB were quite similar as were the iron and phytate contents. Therefore, it was surprising that the relative iron bioavailability from the beverage was significantly lower than the casein control while iron from SF was not.

When analysis of variance and LSD analysis are performed upon data at each iron level (Tables 3 and 4), statistical differences between iron sources are noted only in final nemoglobin concentration and for total Hb gain. The latter parameter shows differences only at the 18 ppm iron level. No differences in a given iron level are noted for 2 wk gain, feed intake or feed efficiency (Table 3). Nor are differences noted in total hemoglobin gain at 6 or 12 ppm, total iron intake or total body hemoglobin gain per iron intake (Table 4). It is of interest to note that for each iron level the final hemoglobin concentration for both soy flour and soy beverage are significantly lower ($P<0.05$) than for the casein control. The soy concentrate is lower than the control for only the lowest (6 ppm added iron) iron level. A general ranking of SC>SF>SB related to iron availability can be made.

The phytate-to-zinc molar ratios of SF, SB, and SC are 28, 26, and 52, respectively. In the previous work with zinc (Forbes and Parker, 1977; Forbes et al., 1979) the soy concentrate demonstrated the lowest zinc bioavailability and the highest phytate-to-zinc molar ratio. For zinc this ratio is generally thought to be inversely proportional to zinc bioavailability (Oberleas, 1975). Nevertheless, we have clearly shown that adequate zinc bioavailability can be obtained from some soy concentrates and isolates with high phytate-to-zinc molar ratios (Erdman et al., 1980; 1983) or from soybean curd with low dietary levels of calcium (Forbes et al., 1983). Phytic acid is clearly a factor that strongly influences zinc bioavailability, but it is only one factor.

In this present work, rating soy proteins empirically for iron bioavailability would not suggest that high phytic acid concentration is detrimental to iron absorption. Literature reports concerning the role of phytic acid on iron bioavailability are not consistent (Anon, 1982). Most studies that have utilized sodium phytate addition to diets to simulate the effect of endogenous phytic acid have demonstrated inhibition of iron absorption (Rotruck and Luhrsen, 1979; Morris, 1983), but the sodium phytate addition may not be

Table 2—Composition of depletion, control and test diets

	Depletion[a,b]	Control	Test
		(%)	
Soy product[c]	–	–	5.8 – 17.3
Casein	20	20	4.8 – 15.7
Corn oil	5.0	7.27	0 – 7.27
Calcium carbonate	2.0	2.0	2.0
Monosodium phosphate	2.0	2.0	2.0
Potassium chloride	0.5	0.5	0.5
Iodized salt	0.5	0.5	0.5
Fe-Free trace mineral premix[d]	0.27	0.27	0.27
Chloine chloride[a]	0.15	0.15	0.15
Vitamin premix[d]	0.10	0.10	0.10
DL-Methionine	0.10	0.10	0.10
L-Methionine	–	–	0.03 – 0.24
Cerelose[e]	69.38	67.11	57.0 – 64.0
Added Fe (Soy product or $FeSO_4$)[f]	0.0	variable	variable

[a] Fritz et al. (1975)
[b] Iron content by analysis, 3.5 ppm
[c] Soy flour, soy beverage or soy concentrate
[d] Fritz et al. (1974)
[e] Added to bring diets up to 100 parts
[f] Added to provide 0, 6, 12 or 18 ppm iron

Table 3—Mean weight gain, feed intake and feed efficiency of iron depleted rats fed test diets during the repletion period[a]

Diet group	Added dietary iron level (ppm) 0	6	12	18
		2 wk Weight grain (g)		
Casein	26.0 ± 6.8	49.8 ± 6.5	64.3 ± 12.1	76.1 ± 12.0
Soy flour	—	61.3 ± 9.9	65.6 ± 15.2	78.1 ± 11.8
Soy concentrate	—	56.8 ± 10.3	63.5 ± 6.5	85.6 ± 13.4
Soy beverage	—	58.9 ± 15.4	73.3 ± 12.9	75.0 ± 9.4
		2 wk Feed intake (g)		
Casein	278.4 ± 46.1	273.5 ± 45.6	279.3 ± 48.6	339.0 ± 26.3
Soy flour	—	295.8 ± 43.7	281.9 ± 61.3	307.6 ± 63.5
Soy concentrate	—	291.6 ± 68.5	306.4 ± 57.4	320.1 ± 45.8
Soy beverage	—	290.8 ± 64.4	279.6 ± 46.7	278.8 ± 27.5
		Feed efficiency (g gain/g feed intake)		
Casein	0.10 ± 0.03	0.19 ± 0.04	0.24 ± 0.05	0.23 ± 0.04
Soy flour	—	0.22 ± 0.06	0.24 ± 0.05	0.26 ± 0.06
Soy concentrate	—	0.21 ± 0.07	0.22 ± 0.05	0.27 ± 0.04
Soy beverage	—	0.21 ± 0.08	0.27 ± 0.06	0.27 ± 0.03

[a] Rats fed an iron depletion diet for four weeks were switched to test diets and were maintained on these diets ad libitum for 2 wk. Data points represent mean of 8 animals ± S.D. Statistical analysis of diet groups at single dietary iron levels revealed no differences in gain, intake or feed efficiency due to diet type at any iron intake level ($P < 0.05$).

Table 4—Mean hemoglobin concentration, total body hemoglobin gain, iron intake and total body hemoglobin gain per mg iron intake of iron depleted rats fed repletion test diets[a]

Diet group	Added dietary iron level (ppm) 0	6	12	18
		Final hemoglobin concentration (g/100 mL)		
Casein	3.8 ± 0.7	5.8 ± 0.7[a]	7.5 ± 1.2[a]	9.3 ± 0.9[a]
Soy flour	—	5.1 ± 0.6[b]	6.6 ± 0.7[bc]	8.2 ± 0.6[bc]
Soy concentrate	—	5.3 ± 0.5[b]	7.0 ± 0.8[ab]	8.8 ± 0.9[ab]
Soy beverage	—	4.9 ± 0.4[b]	6.0 ± 0.8[c]	7.4 ± 1.1[c]
		Total body hemoglobin gain (g)[b,c]		
Casein	−0.09 ± 0.04	0.21 ± 0.07	0.51 ± 0.18	0.93 ± 0.18[a]
Soy flour	—	0.18 ± 0.05	0.44 ± 0.07	0.68 ± 0.13[b]
Soy concentrate	—	0.20 ± 0.07	0.48 ± 0.11	0.85 ± 0.13[a]
Soy beverage	—	0.18 ± 0.07	0.37 ± 0.10	0.55 ± 0.15[b]
		Total iron intake (mg)[b]		
Casein	—	1.64 ± 0.27	3.35 ± 0.58	6.10 ± 0.47
Soy flour	—	1.77 ± 0.26	3.38 ± 0.74	5.54 ± 1.14
Soy concentrate	—	1.75 ± 0.41	3.67 ± 0.68	5.76 ± 0.83
Soy beverage	—	1.75 ± 0.39	3.36 ± 0.56	5.02 ± 0.50
		Total body Hb gain/mg Fe intake[b,c]		
Casein	—	0.13 ± 0.03	0.15 ± 0.06	0.15 ± 0.03
Soy flour	—	0.10 ± 0.03	0.14 ± 0.03	0.13 ± 0.04
Soy concentrate	—	0.12 ± 0.05	0.13 ± 0.03	0.15 ± 0.03
Soy beverage	—	0.11 ± 0.05	0.11 ± 0.03	0.11 ± 0.03

[a] The treatment of the rats is described in footnote 1 of Table 3. Within each column, means with differing superscript letters are significantly different at the $P < 0.05$ level.
[b] Calculated during the two week repletion period only
[c] Assuming rat blood volume to be 5.46 percent of body weight (Lombardi and Oler, 1967 Lab. Invest. 17: 308).

representative of food phytate (Morris, 1983). Monoferric phytate, the major fraction of iron in wheat bran, has good bioavailability for humans and rats (Morris, 1983; Morris and Ellis, 1976). Although, only a portion of the iron in soy is reported to be in the form of monoferric phytate (Ellis and Morris, 1981), results from this present study and previous studies (Welch and VanCampen, 1975; Rotruck and Luhrsen, 1979) suggest that endogenous phytic acid in soy products apparently does not play a primary role in iron utilization.

The purpose of the present study was to utilize the rat hemoglobin repletion assay to determine if three soy products differed in their iron bioavailability. Although it absorbs a much higher percentage of iron from foods than humans, the rat has often been utilized to attempt to rank soy products. This model may predict differences in iron bioavailability for humans. Recently, Schricker et al. (1983) studied the relative iron availability from a soy flour, a soy protein concentrate, and a soy protein isolate with the purpose of ranking the three soy protein products in rats with differing iron status. They found that iron from the casein-based diets was more available for absorption by rats than any of the soy products, being significantly ($P < 0.05$) higher in most instances. In general all of the soy products performed equally as well in their study (relatively bioavailability from 70-90%) regardless of the iron status of the rat. In the present work the relative iron bioavailability by hemoglobin repletion assay ranged from 66-92% for the three soy products tested. This range is similar to that found by Schricker and coworkers (1983). However, we found that only the soy beverage diet produce significantly lower hemoglobin repletion than the casein diet.

Cook et al. (1981) reported that for humans iron was less available from soy protein isolate than from soy flour, yet Shricker et al. (1983) showed that for the rat availability of iron from these products was similar. However, these two studies utilized soy products produced from two different companies. Processing techniques vary considerably be-

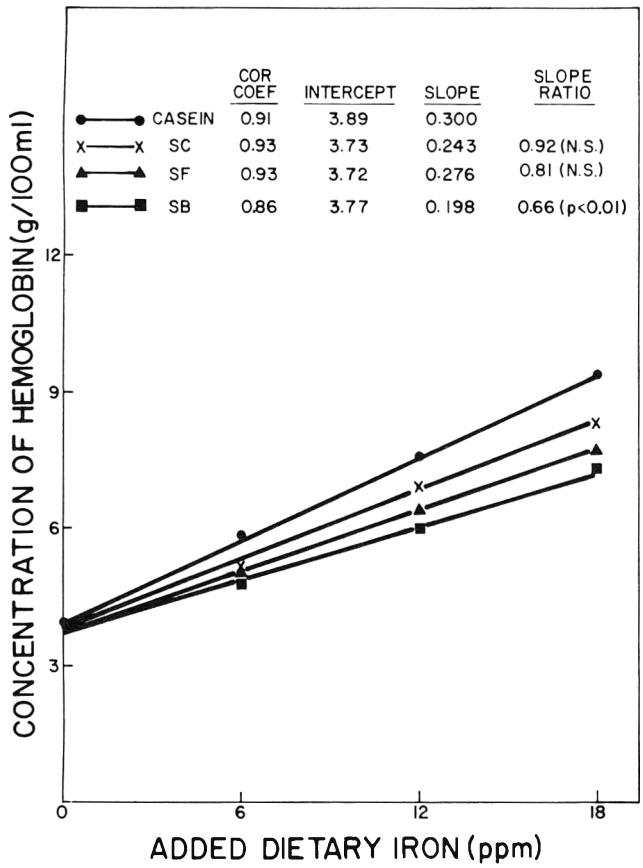

Fig. 1—Regressions of concentration of hemoglobin upon iron added to casein-based diet in the form of $FeSO_4$ (●—●) or added as soy concentrate (x—x), soy flour (▲—▲), or soy beverage (■—■) substituted for an isonitrogenous amount of casein protein. Each data point represents the average response of eight rats.

tween companies. In fact, some companies produce a number of types of soy protein isolates for commercial sale which differ greatly in their method of production. Therefore, to adequately determine if the rat model can rank iron bioavailability from soy products or from other plant foods in a similar manner to humans will require testing the same product in both species.

Because the iron concentration in the soy concentrate was high, less soy concentrate, and therefore more casein, was utilized to formulate the soy concentrate diets. The increased casein/soy products ratio could contribute to enhanced iron absorption from the soy concentrate diets relative to soy flour and soy beverage diets. Alternately, the high iron content of soy concentrate may be due to contamination during processing and this iron may be of high availability to the rat.

In conclusion, for the rat was can generally rank the bioavailability of iron from three soy products as soy concentrate (92%) > soy flour (81%) > soy beverage (66%). These values agree with ranges reported by other workers (Steinke and Hopkins, 1978; Rotruck and Luhrsen, 1979; Schricker et al., 1983). Based upon the results with iron-depleted rats, all three soy products should be considered as good plant sources of iron. The differences between relative iron availability from products cannot be ascribed to phytic acid content. Since zinc bioavailability has been shown to be influenced by techniques in production of soy products (Forbes et al., 1979, Erdman et al., 1980, 1983; Ketelson et al., 1984) processing may have contributed to the differences in iron bioavailability seen in this study.

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In Vitro Evaluation of the Effects of Ortho-, Tripoly- and Hexametaphosphate on Zinc, Iron and Calcium Bioavailability

MICHAEL B. ZEMEL

ABSTRACT

Effects of orthophosphate, tripolyphosphate and hexametaphosphate on zinc, iron and calcium availability were evaluated following in vitro peptic and peptic-pancreatic digestions. Solubility was used as an index of potential availability for zinc and calcium; both total soluble and ionized levels were considered for iron. Orthophosphate depressed zinc solubility while both polyphosphates enhanced it. All three phosphates depressed ionized, but not total, soluble iron, with the greatest effect exerted by tripolyphosphate. Results indicate that effects of phosphates on zinc and iron availability may be predicted by their effects on soluble zinc and soluble ionic iron levels following in vitro digestions. Effects on calcium availability were not explained by this method.

INTRODUCTION

PHOSPHATES have been widely reported to affect mineral bioavailability. Increasing dietary phosphate, in the form of orthophosphate, has been reported to decrease the absorption of zinc and iron in animals (Hegsted et al., 1949; Cabell and Earle, 1964; Mahoney and Hendricks, 1978; Zemel and Bidari, 1983), to decrease iron absorption in man (Snedeker et al., 1983; Zemel et al., 1983) and to exert little or no effect on zinc and calcium utilization in man (Spencer et al., 1979; Greger and Snedeker, 1980; Snedeker et al., 1982; Zemel et al., 1983). Polyphosphates, however, have substantially more pronounced effects on mineral utilization than orthophosphates. Hexametaphosphate has been found to reduce calcium (Zemel and Linkswiler, 1981; Zemel et al., 1983) and iron (Zemel and Bidari, 1983; Zemel et al., 1983, 1984) absorption, and tripolyphosphate to reduce the absorption of iron (Mahoney and Hendricks, 1978; Zemel et al., 1984). In contrast, recent data (Zemel and Bidari, 1983; Zemel et al., 1984) indicates that both tripoly- and hexametaphosphates cause increases in the absorption and utilization of zinc.

The effects of ortho- and polyphosphates on mineral utilization may be explained, in part, by their effects on food mineral solubility in the gastric and/or intestinal environments. Results of several studies (Rao and Prabhavathi, 1978; Lock and Bender, 1980; Hallberg and Bjorn-Rasmussen, 1981; Miller et al., 1981; Schricker et al., 1982) indicate agreement between iron solubility following in vitro peptic or peptic-pancreatic digestions and iron bioavailability in vivo. Schwartz et al. (1982) and Wien and Schwartz (1983) reported agreement between dietary calcium exchangeability with an extrinsic isotope following an in vitro peptic-pancreatic digestion and calcium absorption, although some discrepancies were noted.

The objectives of the present study were to: (1) evaluate the effects of hexametaphosphate on the binding of calcium, iron and zinc to phytate and wheat bran at gastric and intestinal pH values; and (2) evaluate the effects of ortho-, tripoly- and hexametaphosphates added to ground beef or soy protein on zinc, calcium and iron solubility and on soluble ionic iron (Fe^{+2} + Fe^{+3}) following in vitro peptic and peptic-pancreatic digestions.

Author Zemel is affiliated with the Div. of Nutrition & Food Science, Dept. of Family & Consumer Resources, Wayne State Univ., Detroit, MI 48202.

MATERIALS & METHODS

THE EFFECTS of sodium hexametaphosphate on the binding of calcium, zinc and iron by sodium phytate or wheat bran were evaluated at pH's 1.0 and 6.5 and in sequential treatments of pH's 1.0 and 6.5 or 1.0, 6.5 or 8.0. Zero, one or two grams sodium phytate were incubated while shaking for 2 hr at 37°C with 0.0, 0.5, 1.0 or 2.0g sodium hexametaphosphate in either 100 mL 0.2N HCl (pH 1.0) or 100 mL 5% phosphate buffer (pH 6.5). Both solutions contained 10 ppm calcium (as $CaCO_3$), zinc (as ZnO) and iron (as $FeCl_3$). Following incubation, all solutions were centrifuged at 5000 x *g* at 5°C for 30 min and the supernatants were filtered through Whatman #1 paper. The filtrates were subsequently diluted and analyzed for calcium, zinc and iron by atomic absorption spectrophotometry.

For sequential treatments, 2g sodium phytate were incubated at 37°C for 1 hr with either 0 or 2g sodium hexametaphosphate in 100 mL 0.2N HCl containing 10 ppm calcium, zinc and iron. The samples were then brought to pH 6.5 with NaOH (6N, 1N and 0.1N) incubated for an additional hour, and then either left at pH 6.5 or raised to pH 8.0 with NaOH and incubated for a third hour. All samples were then centrifuged, filtered, diluted and analyzed as described above. These experiments were then repeated with identical amounts of wheat bran being substituted for sodium phytate.

In a second series of experiments, the effects of 1% orthophosphate, tripolyphosphate or hexametaphosphate added to ground beef or soy protein concentrate on soluble calcium, zinc, iron and ionic iron were evaluated following in vitro gastric and intestinal digestions based on the method of Miller et al. (1981). Tubes containing 5g of either lean ground beef (locally purchased) or a soy protein concentrate (Central Soya, Fort Wayne, IN) and 0 or 50 mg sodium orthophosphate (monobasic), sodium tripolyphosphate or sodium hexametaphosphate were subjected to a pepsin-HCl digestion, as described by Miller et al. (1981), for 2 hr on a shaker in a 37°C incubator. Samples were then either continued in an "intestinal" digestion (described below) or were immediately centrifuged at 5,000 x *g* for 30 min at 5°C; the supernatants were then recentrifuged at 25,000 x *g* for 1 hr and the resulting supernatants frozen for later analysis (designated as "gastric digests").

The pH of those tubes subjected to simulated intestinal digestions was raised to 7.0 using 1.0N and 0.1N $NaHCO_3$. In order to determine the precise quantity of $NaHCO_3$ to add, additional pepsin-digested tubes were titrated and time was allowed for the pH to rise. Once the desired pH was reached, each tube was immediately sealed to prevent further CO_2 loss and pH changes. Using this method, it was possible to consistently achieve a pH of 7.00 ± 0.10.

Prior to sealing each tube, a pancreatin-bile mixture suspended in 0.1N $NaHCO_3$, described by Miller et al. (1981), was added. The samples were then digested for an additional 2 hr while shaking in a 37°C incubator and centrifuged as described above; the resulting supernatants were designated as "gastrointestinal" digests. All samples were subjected to eight replications each of the gastric and gastrointestinal digestions.

Supernatants from gastric and gastrointestinal digests were analyzed for calcium, zinc and iron by atomic absorption spectrophotometry, and for ionic iron by a spectrophotometric bathophenanthroline method (Anon., 1980). All glass and plastic ware used in these experiments were cleaned with nitric acid (50%) and distilled-deionized water prior to use. Only deionized, glass distilled water was used in reagent preparation and analysis. All data were evaluated statistically by analysis of variance and Duncan's mul-

tiple range test was used to separate significantly different group means.

RESULTS

Calcium

Hexametaphosphate caused significant decreases in calcium solubility in the presence of phytate at pH's 1 and 6.5 (Table 1) and in both sequential treatments (Table 2). Addition of 1.0g wheat bran caused an approximate doubling of the soluble calcium concentration at pH 1 and 6.5, while the addition of 2.0g wheat bran increased soluble calcium by approximately 2.5 fold (Table 3). Progressively increasing the hexametaphosphate content above 0.5 g in the tubes containing the wheat bran, however, resulted in a progressive decrease in soluble calcium levels at both pH values (Table 2). In contrast, hexametaphosphate addition caused an increase in calcium solubility from wheat bran in both sequential treatments, with the greater effect occurring in the pH 1.0 → 6.5 → 8.0 treatment (Table 4).

Addition of 1.0% orthophosphate, tripolyphosphate or hexametaphosphate to the soy protein concentrate all caused similar (ca. 26%) decreases in soluble calcium levels following the simulated gastric digestion (Table 5). A greater decrease (ca. 59%) was observed when the phosphates were added to ground beef (Table 6), but there wre no significant differences among the phosphates. In contrast, only orthophosphate caused a significant decrease in calcium solubility from soy protein concentrate following the simulated gastrointestinal digestion, and hexametaphosphate caused an increase (Table 5). When added to the ground beef, all three phosphates caused an increase in calcium solubility following the simulated gastrointestinal digestion, and the two polyphosphates both exerted a significantly greater effect than the orthophosphate (Table 6).

Zinc

Addition of phytate caused a slight decrease in zinc solubility at pH 1.0, but not at 6.5 (Table 1). At pH 6.5, addition of hexametaphosphate to tubes containing phytate caused substantial increases in zinc solubility, while much smaller increases were found at pH 1.0 (Table 1). Hexametaphosphate in the presence of phytate caused slight increases in soluble zinc levels following both sequential pH treatments (Table 2). Substitution of wheat bran for the phytate yielded similar results at pH 1.0 and 6.5 (Table 3), but effected much larger increases in zinc solubility following the sequential pH treatments.

Table 1—Effects of hexametaphosphate and phytate on Ca, Zn and Fe solubility at pH 1.0 and 6.5[a]

pH	Hexametaphosphate (g)	Phytate (g)	Soluble Ca	Soluble Zn	Soluble Fe
			% of control		
1.0 (control)	0	0	100.0[a]	100.0[a]	100.0[a]
1.0	0	1.0	79.1[a]	92.1[b]	100.0[a]
1.0	0.5	1.0	56.9[c]	103.7[c]	108.3[b]
1.0	1.0	1.0	48.6[d]	109.8[d]	112.5[b]
1.0	2.0	1.0	48.6[d]	114.1[d]	104.1[a]
1.0	0	2.0	94.1[a]	91.3[b]	105.3[a]
1.0	0.5	2.0	48.6[d]	104.7[c]	116.6[b]
1.0	1.0	2.0	50.0[d]	100.5[a]	104.1[a]
1.0	2.0	2.0	73.7[b]	109.8[d]	104.1[a]
6.5 (control)	0	0	100.0[a]	100.0[a]	100.0[a]
6.5	0	1.0	91.2[b]	162.6[b]	519.8[b]
6.5	0.5	1.0	73.3[c]	177.4[c]	618.8[c]
6.5	1.0	1.0	77.8[c]	186.0[d]	599.0[c]
6.5	2.0	1.0	84.5[c]	210.3[e]	599.0[c]
6.5	0	2.0	79.0[c]	171.2[f]	555.7[b]
6.5	0.5	2.0	66.7[d]	170.3[f]	539.1[b]
6.5	1.0	2.0	64.5[d]	180.7[c]	499.0[b]
6.5	2.0	2.0	97.4[a]	200.2[g]	526.0[b]

[a] Nonmatching superscripts in each column represent significant ($p < 0.05$) differences for each pH.

Table 2—Effects of hexametaphosphate and phytate on Ca, Zn and Fe solubility in sequential pH treatments[a]

pH treatment	Hexametaphosphate (g)	Phytate (g)	Soluble Ca	Soluble Zn	Soluble Fe
			% of control		
1.0 → 6.5	0 (control)	2.0	100.0[a]	100.0[a]	100.0[a]
1.0 → 6.5	2.0	2.0	95.4[b]	110.8[b]	101.9[a]
1.0 → 6.5 → 8.0	0 (control)	2.0	100.0[a]	100.0[a]	100.0[a]
1.0 → 6.5 → 8.0	2.0	2.0	86.5[b]	110.8[b]	93.6[b]

[a] Nonmatching superscripts in each column represent significant differences for each pH treatment.

Table 3—Effects of hexametaphosphate and wheat bran on Ca, Zn and Fe solubility at pH 1.0 and 6.5[a]

pH	Hexametaphosphate (g)	Wheat bran (g)	Soluble Ca	Soluble Zn	Soluble Fe
			% of control		
1.0 (control)	0	0	100.0[a]	100.0[a]	100.0[a]
1.0	0	1.0	203.3[b]	129.0[b]	119.4[b]
1.0	0.5	1.0	186.6[c]	126.0[b]	116.1[b]
1.0	1.0	1.0	150.0[d]	134.8[b]	138.7[c]
1.0	2.0	1.0	143.3[d]	151.8[c]	148.4[c]
1.0	0	2.0	253.2[e]	145.7[c]	145.2[c]
1.0	0.5	2.0	233.3[f]	141.0[c]	151.6[c]
1.0	1.0	2.0	233.3[f]	155.0[c]	238.7[d]
1.0	2.0	2.0	203.3[b]	167.7[d]	174.2[e]
6.5 (control)	0	0	100.0[a]	100.0[a]	100.0[a]
6.5	0	1.0	200.0[b]	116.3[b]	144.7[b]
6.5	0.5	1.0	242.9[c]	175.9[c]	600.3[c]
6.5	1.0	1.0	209.6[b]	215.8[d]	533.7[d]
6.5	2.0	1.0	181.0[d]	239.9[e]	622.8[c]
6.5	0	2.0	269.1[e]	130.0[f]	200.0[e]
6.5	0.5	2.0	271.5[e]	220.0[d]	622.8[c]
6.5	1.0	2.0	276.2[e]	246.0[e]	600.3[c]
6.5	2.0	2.0	247.7[c]	262.8[g]	655.9[f]

[a] Nonmatching superscripts in each column denote significant ($p < 0.05$) differences for each pH.

Table 4—Effects of hexametaphosphate and wheat bran on Ca, Zn and Fe solubility in sequential pH treatments[a]

pH treatment	Hexametaphosphate (g)	Phytate (g)	Soluble Ca	Soluble Zn	Soluble Fe
			% of control		
1.0 → 6.5	0 (control)	2.0	100.0[a]	100.0[a]	100.0[a]
1.0 → 6.5	2.0	2.0	144.3[b]	728.8[b]	344.0[b]
1.0 → 6.5 → 8.0	0 (control)	2.0	100.0[c]	100.0[c]	100.0[c]
1.0 → 6.5 → 8.0	2.0	2.0	179.4[d]	287.3[d]	211.9[d]

[a] Nonmatching superscripts in each column represent significant differences for each pH treatment.

The addition of ortho-, tripoly-, or hexametaphosphate to the soy protein concentrate had no effect on zinc solubility following the gastric digestion (Table 5), while both ortho- and hexametaphosphate caused reductions in zinc solubility from the beef following gastric digestion (Table 6). In contrast, tripoly- and hexametaphosphates caused increases in zinc solubility from the soy (Table 5) and beef (Table 6) following the complete gastrointestinal digestion, and orthophosphate caused a decrease (Table 6).

Iron

Addition of hexametaphosphate to either phytate (Table 1) or wheat bran (Table 3) caused increases in iron solubility at pH 1.0 and 6.5. Hexametaphosphate also caused an increase in iron solubility in the presence of wheat bran, but not phytate, following the sequential pH treatments.

When added to the soy protein concentrate (Table 5), orthophosphate caused a decrease and both polyphosphates caused increases in iron solubility following gastric and gastrointestinal digestions. In contrast, all three phosphates caused decreases in soluble ionic iron concentrations, with significantly greater decreases being effected by the polyphosphates. When added to the ground beef (Table 6), however, all three phosphates caused increases in total soluble iron following the gastric digestion. All three phosphates were without effect on iron solubility from the ground beef following the gastrointestinal digestion, while ionic iron was depressed by all three; the two polyphosphates exerted a significantly greater effect than the orthophosphate.

DISCUSSION

ORTHOPHOSPHATE caused a moderate decrease in zinc solubility from the ground beef following the complete (i.e. "gastrointestinal") in vitro digestion, and was without significant effect on zinc solubility from the soy protein concentrate. In contrast, tripolyphosphate and hexametaphosphate both significantly enhanced zinc solubility from the soy and the beef. These results are consistent with the observed effects of ortho- and polyphosphates on zinc bioavailability in vivo. Orthophosphates has been found either to have no effect on (Pond et al., 1975; Pond et al., 1978; Spencer et al., 1979; Greger and Snedeker, 1980; Snedeker et al., 1982; Zemel et al., 1983) or to depress (Cabell and Earle, 1964; Zemel and Bidari, 1983) zinc utilization. In contrast, polyphosphates have been found to enhance zinc bioavailability in rats (Zemel and Bidari, 1983; Zemel et al., 1984). However, Zemel et al. (1984) found tripolyphosphate to exert a greater effect than hexametaphosphate, while the opposite would be predicted from the solubility considerations presented here.

Results of this study indicate that the effects of hexametaphosphate on calcium bioavailability in vivo may also be predicted from solubility considerations at either pH 1.0 or 6.5, but not following either sequential pH treatment. In addition, the effects of the three phosphates on calcium solubility following the simulated gastric and gastrointestinal digestions were not entirely consistent with the observed effects of these compounds on calcium bioavailability. Wien and Schwartz (1983) reported that the exchangeability of an extrinsic tracer with food calcium following an in vitro peptic digestion similar to that used here agreed closely with in vivo measurements of calcium exchangeability in rats consuming the same foods, but in vitro calcium exchangeability following a peptic-pancreatic digestion did not agree with in vivo measurements. In the present study, all three phosphates depressed calcium solubility in the gastric digests; similarly hexametaphosphate has been reported to cause a slight decrease in calcium utilization in man (Zemel and Linkswiler, 1981), and orthophosphate has been found to interfere with calcium absorption and utilization in rats (Mahoney and Hendricks, 1978). In humans, however, orthophosphate has been reported to either have no effect on (Kim and Linkswiler, 1979; Hegsted et al., 1981) or to enhance (Zemel and Linkswiler, 1981; Zemel et al., 1983) calcium absorption. Furthermore, recent data indicates that neither tripolyphosphate nor hexametaphosphate exert significant effects on calcium bioavailablity in rats (Zemel et al., 1984). This discrepancy between in vitro calcium solubility and in vivo bioavailability measurements may result from the formation of very stable soluble calcium complexes which are not absorbed. Measuring soluble ionic calcium instead of total soluble calcium in peptic-pancreatic digests may be expected to provide a more satisfactory index of calcium availability. This possibility is now being evaluated.

The effects of ortho-, tripoly- and hexametaphosphate on iron solubility following the in vitro gastric and gastrointestinal digestions are in disagreement with the observed effects of these compounds on iron bioavailability in vivo. Orthophosphate has been reported to decrease (Hegsted et al., 1949; Monsen and Cook, 1976, Mahoney and Hendricks, 1978; Snedeker et al., 1982; Zemel et al., 1983)

Table 5—Effects of 1.0% orthophosphate, tripolyphosphate and hexametaphosphate added to soy protein concentrate on soluble zinc, calcium, iron and ionic iron following simulated gastric and gastrointestinal digestions[a]

	Soluble calcium	Soluble zinc	Soluble iron	Ionic iron
	% of Control			
Gastric digestion				
Control	100.0[a]	100.0[a]	100.0[a]	100.0[a]
+Orthophosphate	73.4[b]	100.3[a]	81.6[b]	94.4[b]
+Tripolyphosphate	73.2[b]	101.4[a]	173.7[c]	47.2[c]
+Hexametaphosphate	72.9[b]	96.3[a]	122.5[d]	80.6[d]
Gastrointestinal digestion				
Control	100.0[a]	100.0[a]	100.0[a]	100.0[a]
+Orthophosphate	85.0[b]	98.2[a]	95.9[b]	65.1[b]
+Tripolyphosphate	97.4[a]	115.7[b]	243.9[c]	77.9[c]
+Hexametaphosphate	127.8[c]	124.1[c]	155.1[d]	57.0[d]

[a] Nonmatching superscripts in each column denote significant ($p < 0.05$) differences for each treatment. Ca, Zn and Fe levels in the soy protein concentrate were 4.398, 0.461, and 0.106 ppm, respectively.

Table 6—Effects of 1.0% orthophosphate, tripolyphosphate and hexametaphosphate added to ground beef on soluble zinc, calcium, iron and ionic iron following simulated gastric and gastrointestinal digestions[a]

	Soluble calcium	Soluble zinc	Soluble iron	Ionic iron
	% of Control			
Gastric digestion				
Control	100.0[a]	100.0[a]	100.0[a]	100.0[a]
+Orthophosphate	41.0[b]	82.5[b]	154.5[b]	138.4[b]
+Tripolyphosphate	43.6[b]	100.7[a]	127.3[c]	* [c]
+Hexametaphosphate	38.5[b]	86.5[b]	204.5[d]	13.7[d]
Gastrointestinal digestion				
Control	100.0[a]	100.0[a]	100.0[a]	100.0[a]
+Orthophosphate	145.5[b]	80.0[b]	91.8[a]	86.0[b]
+Tripolyphosphate	427.7[c]	153.6[c]	91.8[a]	60.7[c]
+Hexametaphosphate	400.0[c]	180.0[d]	100.0[a]	50.7[d]

[a] Nonmatching superscripts in each column denote significant ($p < 0.05$) differences for each treatment. Ca, Zn and Fe levels in the beef were 0.629, 0.224, and 0.187 ppm, respectively.
* Undetectable

iron absorption and utilization, and polyphosphates have been found to cause significantly greater decreases in iron utilization than orthophosphate (Mahoney and Hendricks, 1978; Zemel and Bidari, 1983). In the present study, however, these effects were not consistently found; consequently, iron solubility following peptic or peptic-pancreatic digestion does not appear to be indicative of iron bioavailability. In contrast, the effects of these phosphates on soluble ionic iron measurements following the gastric digestion appear to reflect the effects of these compounds on iron bioavailability in vivo. Both polyphosphates caused decreases in ionic iron levels, with the greatest effect being exerted by tripolyphosphate. Similarly, hexametaphosphate has been found to reduce iron absorption in man (Zemel et al., 1983); both tripolyphosphate (Mahoney and Hendricks, 1978; Zemel et al., 1984) and hexametaphosphate (Zemel and Bidari, 1983) have been reported to do so in rats, with tripolyphosphate exerting a substantially greater effect. Results of other investigations also indicate that ionic iron in in vitro gastric digests is indicative of iron availability. Rao and Prabhavathi (1978) reported that ionic iron, as measured with α,α-dipyridyl, in pepsin-HCl digests of foodstuffs correlated well with iron absorption measurements in human subjects. More recently, Miller et al. (1981) found that dialyzable iron following a pepsin-pancreatic digestion was indicative of food iron availability; furthermore, the addition of substances which affect food iron availability in vivo, such as ascorbic acid, tea and whole wheat bread, produced similar changes in dialyzable iron. However, these determinations of dialyzable iron measured the total low molecular weight soluble forms of iron and did not distinguish between the ionic and complexed forms of iron. Data from the present study indicate that ionic, but not total soluble iron, is indicative of iron availability.

Results of this investigation indicate that the effects of ortho- and polyphosphates on zinc utilization may be explained by their effects on zinc solubility following an in vitro gastrointestinal digestion. Thus, the solubility of zinc in food subjected to in vitro gastric and intestinal digestions may prove to be a useful indicator of zinc bioavailability. Similarly, soluble ionic iron levels in foodstuffs subjected to these digestions appear to reflect iron bioavailability. However, the effects of phosphates on calcium solubility do not appear to be predictive of calcium availability.

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Kinetics of Tannin Deactivation during Anaerobic Storage and Boiling Treatments of High Tannin Sorghums

BARNABAS N. MITARU, ROBERT D. REICHERT, and ROBERT BLAIR

ABSTRACT

Grain from three high tannin sorghums had their moisture contents raised by adding distilled water at 15, 20 and 25% W/W, respectively, and were stored under CO_2 atmosphere at 25, 35 and 45°C, respectively, for 1–20 days. In another trial, grain from the same high tannin sorghum sources was boiled in water at a ratio of 1g to 3 mL for periods of 10–60 min. Both anaerobic storage and boiling treatments deactivated the tannins with time. Moisture content and temperature levels influenced the rate of tannin deactivation during anaerobic storage. The process of tannin deactivation for both anaerobic storage and boiling treatments followed first order kinetics.

INTRODUCTION

THE LOWER NUTRITIONAL QUALITY of high tannin sorghums is attributed to the ability of the tannins to bind proteins in the gut and hence reduce their availability. Anaerobic storage of moist high tannin sorghum grain deactivates tannins and improves the nutritive value of these sorghums (Reichert et al., 1980). Boiling of high tannin sorghum grain also reduces the assayable tannin content of high tannin sorghums but does not improve the nutritional quality of these sorghums for rats (Price et al., 1980). The mechanisms involved in tannin deactivation during anaerobic storage or boiling treatments are not known. It is probable that during anaerobic storage the tannins either polymerize to higher oligomers which are insoluble in methanol or they are bound to protein and other constituents of the grain. Since anaerobic storage improves the nutritional quality of the high tannin sorghum, it is unlikely that during anaerobic storage the tannins are bound to protein. The polymerization of the tannins to higher oligomers which are insoluble and have lost their ability to bind proteins may be the mechanism occurring during anaerobic storage (Reichert et al., 1980). Protein binding activity seems to increase with size of tannin polymers and peaks somewhere between 3 and 10 monomers (Goldstein and Swain, 1963; Roux, 1972). The high polymerized tannins (>10 flavan monomers) either are too insoluble, have too few reactive sites or are too large to fit the protein orientation for cross linking (Joslyn and Goldstein, 1964). The tannin deactivation mechanism during the boiling treatment is probably different from that occurring during the anaerobic storage and may involve reactions between tannins and proteins of the sorghum grain.

Information on the kinetics of tannin deactivation during anaerobic storage and boiling treatments of sorghums is needed for practical application of these methods. Therefore, the following study was conducted to examine the rates of tannin deactivation in three high tannin sorghums during anaerobic storage at different time periods and three moisture and temperature levels, and during boiling for different time periods.

Author Mitaru is affiliated with the Dept. of Animal & Poultry Science, Univ. of Saskatchewan, Saskatoon, Canada, S7N 0W0. Author Reichert is with the Plant Biotechnology Institute of the National Research Council of Canada, Saskatoon, Saskatchewan, Canada, S7N 0W9. Author Blair, formerly with the Dept. of Animal Science, Univ. of Saskatchewan, is now affiliated with the Dept. of Animal Science, Univ. of British Columbia, Vancouver, British Columbia, Canada V6T 2A2.

MATERIALS & METHODS

Sorghum grain

The high tannin sorghum cultivars, P570, AKS614 and AR3003 x TX430 with tannin contents of 3.9, 3.2 and 2.3%, respectively, and an average moisture content of 7.8% were used in the experiment. The cultivar P570 was obtained from King Grain Ltd. (Chatham, Ontario), while the latter two were obtained from the Univ. of Arkansas (Fayetteville, AR).

Effect of anaerobic storage on tannin deactivation

Sorghum grain (25g) was weighed into 100 mL plastic, air tight containers. Distilled water was added at 15, 20, and 25%, respectively, by weight and immediately purged with CO_2 to remove air. The containers were sealed and shaken until all the distilled water was imbibed by the grain as determined visually. The samples were then placed in ovens set at 25°, 35°, and 45°C, respectively, and stored for 0, 1, 3, 5, 10, 15 or 20 days.

The samples were removed from the ovens after the appropriate period and stored in a freezer. The samples were then ground in a UD 1092 Cyclotec sample mill and stored in the same containers and in the freezer until required for tannin and moisture analysis.

Effect of boiling on tannin deactivation

Distilled water (60 mL) was preheated to boiling, then sorghum grain (20g) was added to the flasks. At the end of the 10, 20, 30, 40, 50 or 60 min time periods, the appropriate flask was removed from the heater and placed in dry ice in acetone to cool for 10 min. All samples were then removed, frozen and freeze-dried. The samples were then ground in a UD 1092 Cyclotec sample mill from UD Corporation (Boulder, CO), and stored in the freezer for chemical analyses later.

Chemical analysis

Tannin content was analyzed by the vanillin-HCl method, with reagent blanks as suggested by Price et al. (1978) and expressed as percentage of dry matter. Moisture content of the grain was determined by the standard method (AOAC, 1980).

Statistical analysis

Analysis of variance was conducted to test the effects of cultivar, temperature, moisture, and storage time on tannin deactivation. Since tannin analysis was done in duplicate and there was no replication, the higher order interaction means of squares term was used as the error term in the calculation of F values.

Determination of the rate of tannin deactivation

The data were transformed to natural logarithms and subjected to regression analysis to determine the rates of tannin deactivation during anaerobic storage and boiling treatments. It is assumed that in anaerobic storage and boiling some chemical mechanisms deactivate tannin such that it becomes insoluble in methanol. Hence determination of extractable tannin at different times indirectly measures the extent of deactivation of tannin.

The general equation for a first order reaction is:

$$dx/dt = k\,(a - x) \qquad \text{[Eq. 1]}$$

where dx/dt = rate of disappearance of MeOH soluble tannin; a = initial concentration of MeOH soluble tannin; x = concentration of MeOH soluble tannin at time t; $(a - x)$ = concentration of MeOH insoluble tannin at time t; and k = rate constant.

Separating the variables and integrating Eq. (1) yields

$$-\ln (a - x) + \ln a = kt. \qquad \text{[Eq. 2]}$$

and rearranging yields the following equation

$$\ln (a - x) = \ln a - kt. \qquad [Eq. 3]$$

If a series of measurements of x at different times, t, are made and ln (a – x) is plotted, as ordinate, against t, a straight line of slope –k, and an intercept of ln a at time = 0 should result. If the straight line is obtained it is considered that the reaction obeys the first order law, the slope being the first order rate constant whose units are reciprocal time. The concentration units are not involved in k since it is expressed in terms of a ratio of concentrations.

RESULTS & DISCUSSION

Water imbibition

The average time taken by the grain to imbibe water is shown in Table 1. For the three water levels, P570 absorbed water fastest, followed by AR3003 x TX430 and then AKS614. This indicates differences in water imbibing capacity of sorghum cultivars.

Influence of cultivar, temperature, moisture and storage time on tannin deactivation during anaerobic storage

Anaerobic storage of the high tannin sorghums reduced the extractable tannin content with time. The decrease was

Table 1—Time taken by sorghum grain to imbibe distilled water

Water added[a]	Sorghums		
	P570	AKS614	AR3003xTX430
	min		
15%	15	45	45
20%	30	105	90
25%	60	150	120

[a] Percent of the grain sample weight.

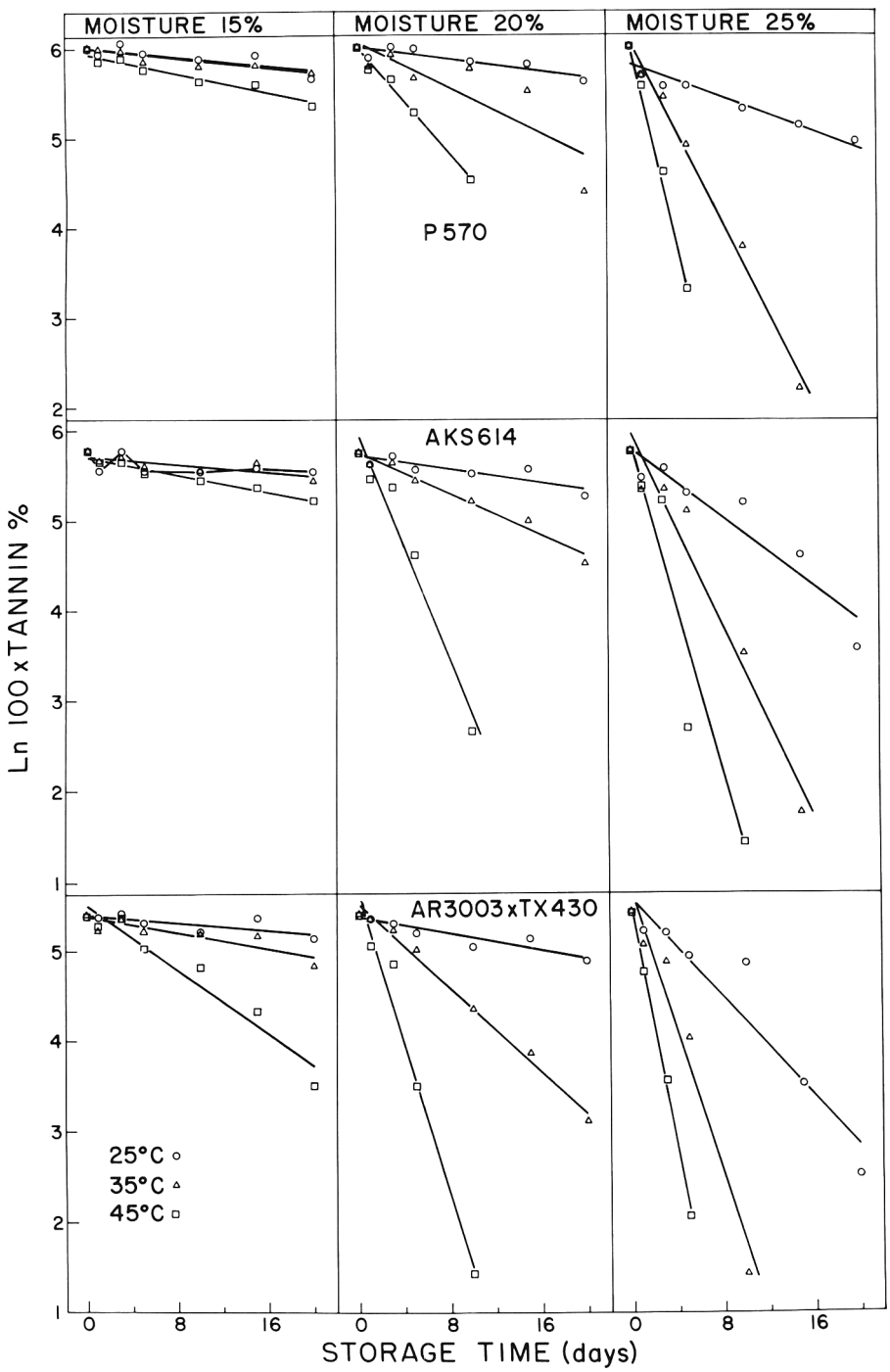

Fig. 1—Effect of moisture and temperature on the rate of tannin deactivation during anaerobic storage.

Table 2—Analysis of variance for tannin deactivation during anaerobic storage of three high tannin sorghums at three temperatures and three moisture levels

Source of variation	DF	SS	MS	F
Main effects				
Cultivars (C)	2	114.47	57.24	301.26**
Temperature (T)	2	51.27	25.64	
Linear	1	51.18	51.18	269.37**
Quadratic	1	0.09	0.09	0.47^{ns}
Moisture (M)	2	104.35	52.18	
Linear	1	104.04	104.04	547.58**
Quadratic	1	0.31	0.31	1.63^{ns}
Days (D)	6	171.91	28.65	150.80**
Interactions				
C X T	4	1.79	0.44	2.36^{ns}
C X M	4	8.22	2.05	10.79**
C X D	12	3.35	0.28	1.47^{ns}
T X M	4	7.89	1.97	10.37**
T X D	12	17.03	1.42	7.47**
M X D	12	29.95	2.50	13.16**
Error (higher order interactions)	128	23.64	0.19	

**P < 0.01
^{ns}P > 0.05

Table 4—Kinetic parameters of tannin deactivation during the boiling of high tannin sorghum grains

Sorghum	ln a (in 10 x tannin %)	k (min^{-1})	r
P570	3.57	0.034	0.98**
AKS614	3.51	0.031	0.98**
AR3003 x TX430	2.96	0.035	0.99**

**P < 0.01

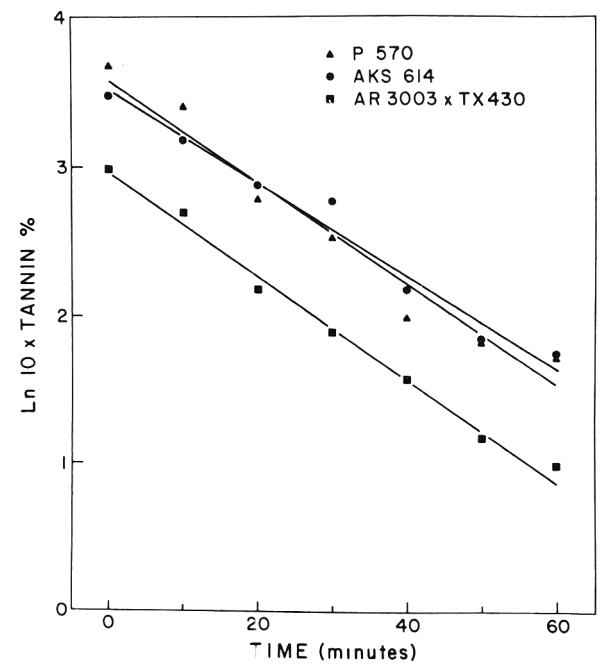

Fig. 2—Effect of boiling on the rate of tannin deactivation in three high tannin sorghums.

shown to be exponential and was more pronounced at higher moisture and temperature levels.

The analysis of variance results (Table 2) showed that

Table 3—Kinetic parameters of deactivation during anaerobic storage of high tannin sorghums

Sorghum	Temp °C	Moisture %	ln a (ln 100 x tannin %)	k (days^{-1})	r
P570	25	15	6.01	0.013	0.76*
		20	5.99	0.017	0.90**
		25	5.80	0.045	0.96**
	35	15	5.98	0.012	0.89**
		20	6.04	0.062	0.85*
		25	6.06	0.247	0.99**
	45	15	5.93	0.027	0.96**
		20	5.98	0.143	0.99**
		25	6.06	0.535	0.99**
AKS614	25	15	5.67	0.007	0.50^{ns}
		20	5.73	0.019	0.88**
		25	5.81	0.095	0.95**
	35	15	5.72	0.011	0.79*
		20	5.77	0.057	0.99**
		25	5.98	0.259	0.98**
	45	15	5.71	0.025	0.97**
		20	5.96	0.311	0.98**
		25	5.90	0.460	0.97**
AR3003xTX430	25	15	5.41	0.011	0.76*
		20	5.37	0.023	0.93**
		25	5.57	0.137	0.96**
	35	15	5.39	0.023	0.88**
		20	5.51	0.116	0.99**
		25	5.66	0.396	0.98**
	45	15	5.50	0.089	0.97**
		20	5.60	0.408	0.99**
		25	5.44	0.666	0.99**

**P < 0.01
*P < 0.05
^{ns}P > 0.05

cultivar, temperature, moisture and time of storage had highly significant effects on tannin deactivation. Temperature and moisture had linear effects (Table 2) on tannin deactivation while the time of storage had a curvilinear effect. All the first order interactions except cultivar x temperature and cultivar x days were highly significant.

The cultivar x temperature and cultivar x days interactions were not significant (P > 0.05). This indicates that the three cultivars were affected by temperature and storage time (days), respectively, in a similar manner. The significant (P < 0.01) interaction between moisture content and cultivars (Table 2) is interpreted to mean that the change in moisture level produced different rates of tannin deactivation in the three sorghum cultivars (Fig. 1). Temperature and moisture levels affected the rate of tannin deactivation jointly as shown by their highly significant interaction (Table 2). Storage time showed highly significant interactions with both temperature change and moisture content.

Rates of tannin deactivation during anaerobic storage and boiling treatments

In the anaerobic storage experiment, the plots of ln(a − x) against time for the three sorghum cultivars, three temperatures and three moisture levels were shown to yield straight lines (Fig. 1), suggesting first order reactions. The regression and correlation coefficients for the lines in Fig. 1 are shown in Table 3. All the correlation coefficients except one were significant at (P < 0.05) and (P < 0.01), respectively.

The slopes of the lines (k) could be regarded as the rate

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Predicting Steady Shear and Dynamic Viscoelastic Properties of Guar and Carrageenan Using the Bird-Carreau Constitutive Model

J. L. KOKINI, K. L. BISTANY, and P. L. MILLS

ABSTRACT

The Bird-Carreau constitutive model, a five parameter semi-empirical set of equations, was used to predict the steady shear viscosity, η, the dynamic viscosity, η', and the out-of-phase component of the complex viscosity divided by frequency, η''/ω. For most of the frequency/shear rate region in the range 0.1 - 100 sec^{-1} for 1% and 1.25% guar, the models accurately predicted the experimental data. η''/ω data for 2.0% and 2.5% carrageenan dispersions were predicted accurately in the high frequency region but were not successfully simulated in the low shear rate/frequency region.

INTRODUCTION

FOOD POLYSACCHARIDES are used to improve the textural characteristics of many fluid and semi-solid food material. Most are not metabolized and their caloric value is quite low making them useful in the development of diet foods. Small amounts of these macromolecules can drastically affect the rheological properties of the food medium in which they are used (Blanshard and Mitchell, 1979).

Studies of the rheology of polysaccharides has been limited to dilute solution measurements to characterize their conformation in solution (Mitchell, 1979; Whitcomb and Macosko, 1978; Anderson and Rahman, 1967, Morris and Ross-Murphy, 1981) and to modeling their steady flow behavior at different concentrations using relatively simple models such as the power-law model and the Ree-Eyring model (Kassem and Mattha, 1969, Doublier and Launay, 1981). Concentrated solutions of polysaccharides in the concentration range of value of the food industry have been shown to be non-Newtonian in steady shear flows at large enough shear rates where the actual shear rate at the onset of non-Newtonian behavior is a function of concentration (Schurz, 1976; Glicksman, 1982). In transient flows, such as small amplitude oscillatory shear flows, they show both elasticity and viscosity and are linear viscoelastic at small strains. It was recently shown that there is a close correlation between their steady and dynamic viscosities at small and large shear rates when their dispersions do not undergo structure decay (Mills and Kokini, 1983).

Rheological modeling is ideal when a single model can explain data obtained in several different kinds of experiments. Then data obtained from one kind of experiment can be used to predict data which would have been obtained from another kind of experiment.

In the past several constitutive equations have been developed to simulate the flow behavior of solutions of polymeric materials or melts (Spriggs, 1965; Chen and Bogue, 1972; Bird and Carreau, 1968) and have been tested with several polymeric solutions by Christiansen and Leppard (1975) and Baird (1980). Of all models tested, the Bird-Carreau model was most successful for a number of polymeric materials over a large range of conditions.

Authors Kokini, Bistany, and Mills are affiliated with the Food Science Dept., New Jersey Agricultural Experiment Station, Cook College – Rutgers Univ., New Brunswick, NJ 08903.

The Bird-Carreau model is a nonlinear extension of the generalized Maxwell model (Leppard, 1975). The model uses four empirical constants and the zero shear rate limiting viscosity η_o of the solutions. Two constants, α_1 and λ_1 are typically obtained from a logarithmic plot of η vs. $\dot{\gamma}$ and the other two constants α_2 and λ_2 are obtained from a logarithmic plot of η' vs. ω (Fig. 1) (Bird et al., 1977). The five parameters are then used to predict η, η', and η''/ω.

The Bird-Carreau prediction for η is (Bird et al., 1977):

$$\eta = \sum_{\rho=1}^{\infty} \frac{\eta_\rho}{1 + (\lambda_{1\rho}\dot{\gamma})^2}$$

At large shear rates the above equation is approximated by:

$$\eta = \frac{\pi\eta_o}{Z(\alpha_1)-1} \cdot \frac{(2^{\alpha_1}\lambda_1\dot{\gamma})^{(1-\alpha_1)/\alpha_1}}{2\alpha_1 \sin\left(\frac{1+\alpha_1}{2\alpha_1} \cdot \pi\right)}$$

where,

$$\lambda_{1\rho} = \lambda_1 \cdot \left(\frac{2}{\rho+1}\right)^{\alpha_1}$$

$$\eta_\rho = \eta_o \cdot \frac{\lambda_{1\rho}}{\Sigma \lambda_{1\rho}}$$

$$Z(\alpha_1) = \sum_{k=1}^{\infty} \kappa^{-\alpha_1}$$

The Bird-Carreau prediction for η' is:

$$\eta' = \sum_{\rho=1}^{\infty} \frac{\eta_\rho}{1 + (\lambda_{2\rho}\omega)^2}$$

and at high frequencies η' is approximated by:

$$\eta' = \frac{\pi\eta_o}{Z(\alpha_1)-1} \cdot \frac{(2\alpha_2\lambda_2\omega)^{(1-\alpha_1)/\alpha_2}}{2\alpha_2 \sin\left(\frac{1+2\alpha_2-\alpha_1}{2\alpha_2} \cdot \pi\right)}$$

and finally the prediction for η''/ω is:

$$\frac{\eta''}{\omega} = \sum_{\rho=1}^{\infty} \frac{\eta_\rho \cdot \lambda_{2\rho}}{1 + (\lambda_2 \rho\, \omega)^2}$$

and this equation converges to the following equation at high frequencies:

$$\frac{\eta''}{\omega} = \frac{2^{\alpha_2}\lambda_2\pi\eta_o}{Z(\alpha_1)-1} \cdot \frac{(2^{\alpha_2}\lambda_2\omega)^{(1-\alpha_1-\alpha_2)/\alpha_2}}{2\alpha_2 \sin\left(\frac{1+\alpha_2-\alpha_1}{2\alpha_2} \cdot \pi\right)}$$

where $\lambda_{2\rho} = \lambda_2 \left(\frac{2}{p+1}\right)^{\alpha_2}$

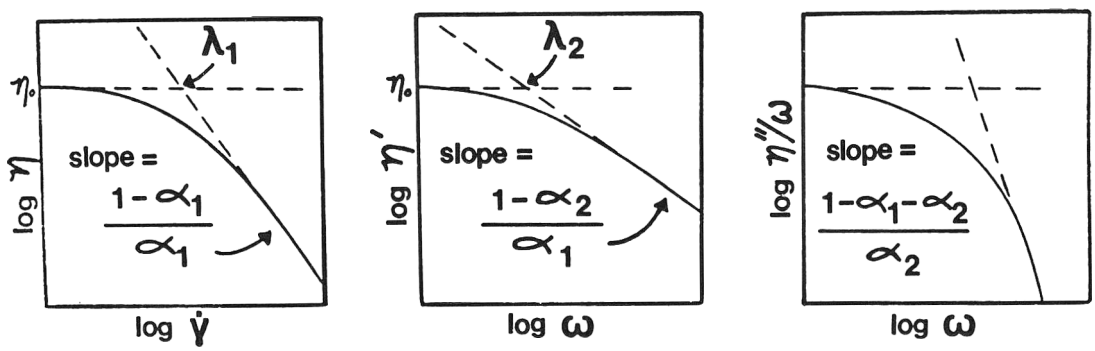

Fig. 1—Graphical determination of empirical constants for use in the Bird-Carreau model.

In this paper 1% and 1.25% guar gum suspensions and 2.0% and 2.5% carrageenan dispersions will be used to test the ability of the Bird-Carreau model to predict η, η' and η''/ω.

MATERIALS & METHODS

GUAR GUM was obtained from Sigma Chemical Company, No. G-4129 and experimental lambda-carrageenan was obtained from the Marine Colloids Division of FMC Corporation. Guar dispersions were prepared at concentrations of 1% and 1.25% and carrageenan dispersions were prepared at concentrations of 2.0% and 2.5%. Both gum dispersions were prepared in a similar manner. A known quantity of gum was introduced to a pre-measured amount of distilled water in a blender. Sprinkling the sample into the center of the vortex insured adequate wetting of the powder and proper dispersion of the gum. The dispersions were mixed at room temperature which varied between 23 and 25°C. They were blended at a low speed for 15 sec to insure proper dispersion and shorten the experiment time. The samples were then put on a shaker at 150 rpm for 12 hr. When the viscosity of dispersions obtained without the use of blending were compared to the viscosity of those obtained by blending it was seen that the final solution viscosity of guar or carrageenan dispersions were the same.

The rheological measurements were performed on the Rheometrics Fluids Rheometer, Model RFR-7800. The 10 g-cm transducer was used with the less concnetrated dispersions and the 100 g-cm transducer was used with the more concentrated solutions. The tests were performed at room temperature which varied between 23 and 25°C. The cone and plate geometry of the rheometer was used with a cone angle of 0.0196 radians and plate radius of 2.5 cm. All measurements were performed in triplicate and the geometric average of these values was calculated. In order to help insure test reproducibility, a uniform sample size of 2.5 mL was used.

Dynamic rheological tests were conducted under the aforementioned experimental conditions at a strain level of 20% for guar and 30% for carrageenen. Strain sweeps were conducted to insure that the solutions were in the linear region. Other details of these experiments have been summarized in a previous paper (Mills and Kokini, 1984).

RESULTS & DISCUSSION

EMPIRICAL CONSTANTS determined from η' vs ω and η vs. $\dot{\gamma}$ data for use with the Bird-Carreau model are tabulated on Table 1. The first important parameter is the zero shear limiting viscosity equal to 20 Pa•s for 1% guar and 70 Pa•s for 1.25% guar. This value was determined by extrapolating the viscosity η data to low shear rates. α_1 and α_2 obtained from the high shear rate slopes of log η vs. log $\dot{\gamma}$ and the high frequency slopes of log η' vs. log ω are close in magnitude but are not equal. This shows that slopes in the high frequency/shear rate region of η' and η are not equal. This shows that slopes in the high frequency/shear rate region of η' and η are not equal. λ_1 and λ_2 values are equal to 1.33 and 2.50 sec, respectively, for 1% guar and 1.56 and 3.57 sec, respectively, for 1.25% guar dispersions. These values reflect the onset of nonlinear behavior in the η' and η curves. The fact that λ_1 associated with η is greater than λ_2 associated with η' in both cases is the result of the fact that the onset in nonlinear behavior for η' is faster than that of η.

Similarly for lambda-carrageenan the zero shear limiting viscosities obtained through extrapolation of the data to low shear rates are equal to 4.8 Pa•sec for the 2% dispersion and to 13.5 Pa•s for the 2.5% dispersion. α_1 and α_2 values are again close but not equal. These values as well as λ_1 and λ_2 values are shown in Table 2.

It should be noted at this point that the ability to predict two different slopes for η vs. $\dot{\gamma}$ and η' vs ω shows the flexibility of the Bird-Carreau model. This is indeed what is most commonly observed with many polysaccharide molecules (Mills and Kokini, 1984). Also with most carbohydrate polymers the characteristic times for the onset of non-Newtonian flow are different. The Bird-Carreau model provides that flexibility as well.

The actual prediction of the steady shear viscosity η, the dynamic viscosity η' and η''/ω are shown in Fig. 2 to 5 for 1%, 1.25% guar and 2% and 2.5% carrageenan dispersions.

In Fig. 2, the Bird-Carreau model is compared with experimental data of the 1.0% guar solution in the frequency/shear rate range of 0.1 - 100 sec^{-1}. For η''/ω the model very accurately predicted the high frequency region. The coefficient of determination (R^2) in this region was 0.99. The low frequency region was somewhat less accurately predicted. The R^2 in this region was 0.97. This could be due to the fact that parameters λ_1, λ_2, α_1 and α_2 were determined using the procedure of Bird et al. (1977) previously described. This procedure was selected because of its simplicity. An alternative procedure could be to curve-fit the η vs. $\dot{\gamma}$ and η' vs ω data to obtain the four parameters, as was used by Baird (1980). Similarly, experimental values of η' vs. ω and η vs. $\dot{\gamma}$ were fitted quite accurately by the model as demonstrated by R^2 values of 0.99 in high frequency region and R^2 values of 0.98 in the low frequency region.

Table 1—Guar: Empirical constants for use with Bird-Carreau model

Conc. %	η_o (pa • s)	α_1	α_2	λ_1 (s)	λ_2 (s)	d_2/α_1	λ_1/λ_2
1	20.0	3.33	3.19	1.33	2.50	0.96	0.53
1.25	70.0	5.85	5.67	1.56	3.57	0.97	0.44

Results for 1.25% guar are shown in Fig. 3. The Bird-Carreau model predicts η''/ω vs. ω quite well at both high and low frequency regions. The R^2 of the fit is 0.98 in both regions. Similarly the low and high shear rate/frequency regions of η and η' are well simulated with R^2 values of 0.98.

The 2.0% and 2.5% dispersions of carrageenan were modeled in the same manner as the guar dispersions (Fig. 4 and 5). η''/ω values are closely predicted in the moderate to high frequency region for both concentrations with R^2 values of 0.99 in all cases. At low frequencies the model predicts a plateau region which was not observed experimentally at either concentration. When the low frequency region was included in goodness of fit estimates the R^2 dropped to 0.93 for 2% carrageenan and to 0.95 for 2.5% carrageenan. Experimental values of η vs. $\dot{\gamma}$ and η' vs. ω were also fairly well predicted for the 2.0% carrageenan dispersions. The overall R^2 was 0.98 for both η and η'. For the 2.5% carrageenan dispersions η and η' were also predicted well with R^2 of 0.98 in each case.

The model predicted that η' was approximately equal to η at small shear rate and frequencies. This is due to the fact that all small values of $\dot{\gamma}$ and ω both $(\lambda_{1\rho}\dot{\gamma})^2$ are $(\lambda_{2\rho}\omega)^2$ were small compared to 1. This reduces both quantities to

$$\eta' = \eta = \sum_{\rho=1}^{\infty} \eta_\rho$$

which is precisely what was observed experimentally for all dispersions.

The complete test of the model necessitates prediction of the primary normal stress coefficient ψ_1 given by:

$$\psi_1 = 2 \sum_{\rho=1}^{\infty} \frac{\eta_\rho \lambda_{2\rho}}{1 + (\lambda_{1\rho}\dot{\gamma})^2}$$

A test of ψ_1 could not be achieved because reproducible ψ_1 values in a wide range of shear rates were not available. It is, however, important to note that at small $\dot{\gamma}$ and ω:

$$\psi_1 = 2 \sum_{\rho=1}^{\infty} \eta_\rho \lambda_{2\rho}$$

and

$$\frac{\eta''}{\omega} = \sum_{\rho=1}^{\infty} \eta_\rho \lambda_{2\rho}$$

This suggests that

Table 2—Carrageenan: Empirical constants for use with Bird-Carreau model

Conc. %	η_o (pa • s)	α_1	α_2	λ_1 (s)	λ_2 (s)	d_2/α_1	λ_1/λ_2
2.0	4.80	1.88	1.81	0.426	0.444	0.96	0.96
2.5	13.5	2.43	2.24	0.585	0.763	0.92	0.77

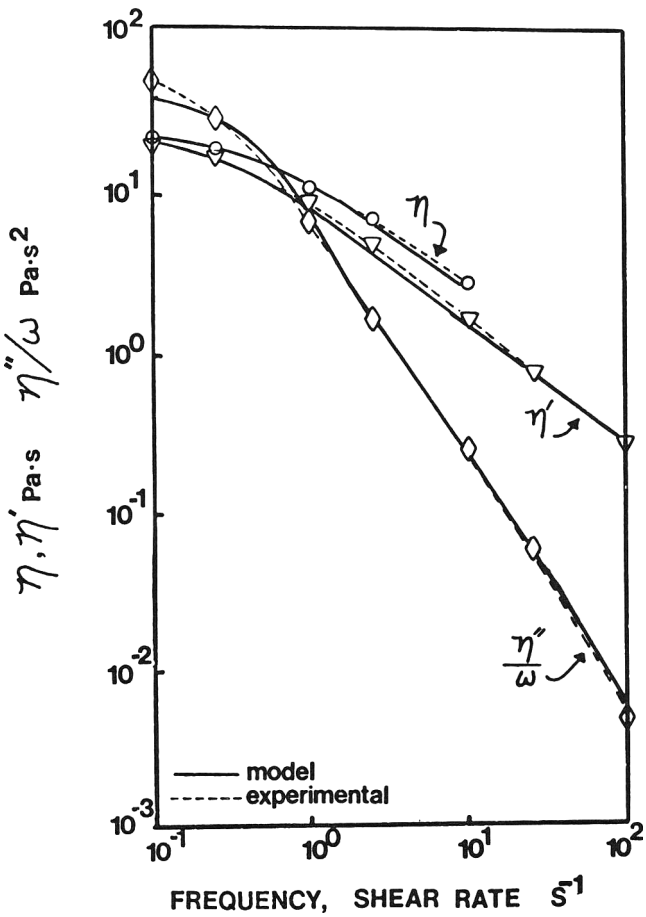

Fig. 2—Predictions of the Bird-Carreau model for 1.0% guar: solid lines represent experimental data and dotted lines represent predictions of the Bird-Carreau model.

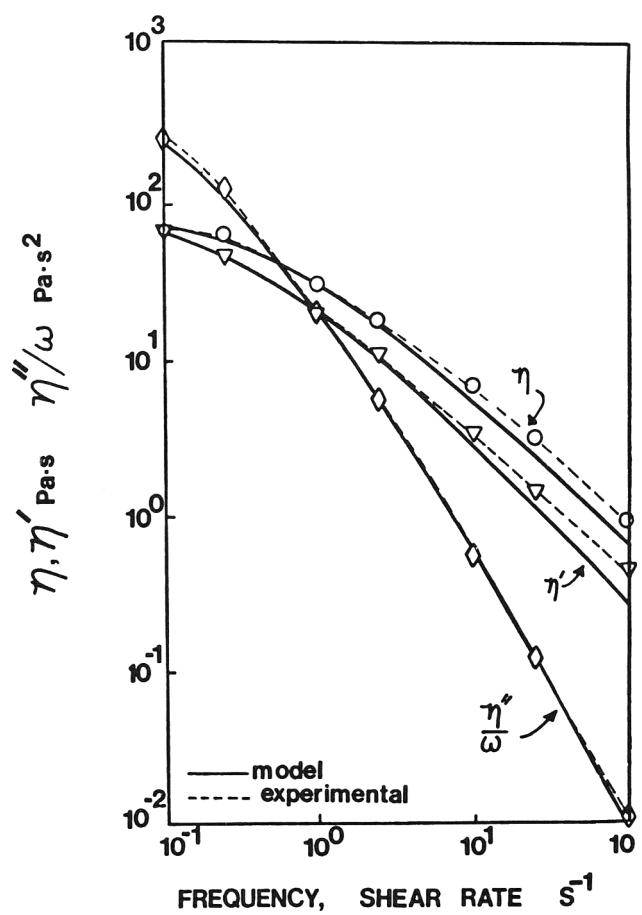

Fig. 3—Predictions of the Bird-Carreau model for 1.25% guar: solid lines represent experimental data and dotted lines represent predictions of the Bird-Carreau model.

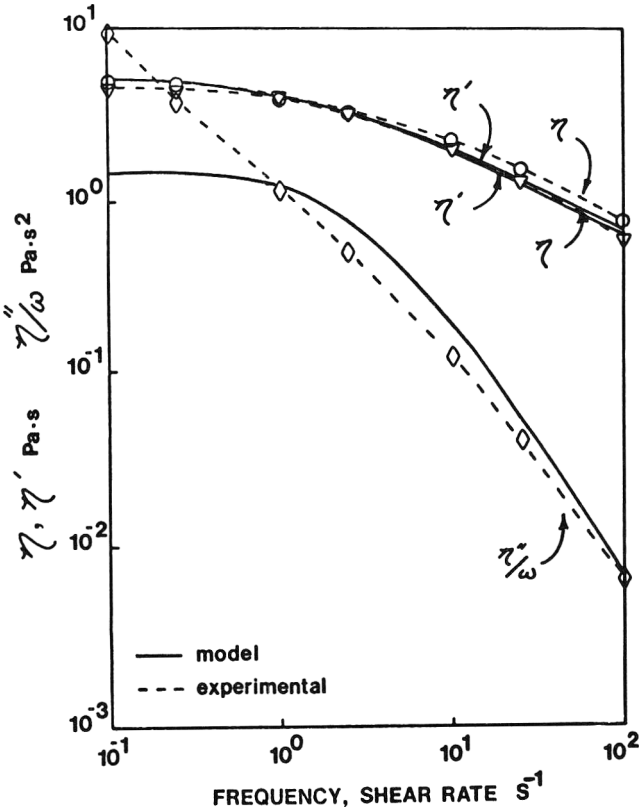

Fig. 4—Predictions of the Bird-Carreau Model for 2.0% carrageenan: solid lines represent experimental data nd dotted lines represent predictons of the Bird-Carreau model.

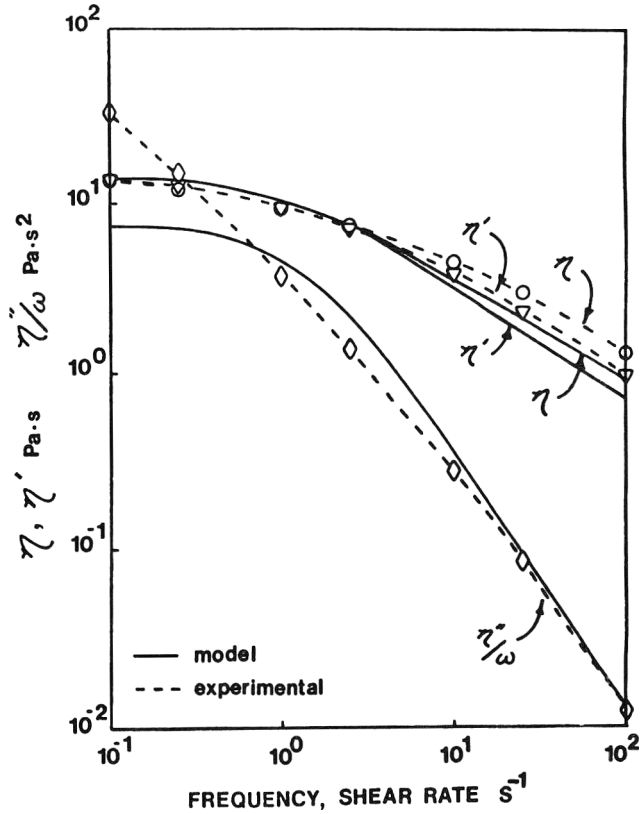

Fig. 5—Predictions of the Bird-Carreau model for 2.5% carrageenan: solid lines represent experimental data and dotted lines represent predictions of the Bird-Carreau model.

$$\psi_{10} = \frac{2\eta''}{\omega}\bigg|_{\substack{\text{small}\\ \dot{\gamma}=\omega}}$$

Consequently, at small shear rates one would expect ψ_1 and η''/ω to be related. However, the fact that experimental data for η''/ω did not tend to a limiting value at small frequencies and shear rates suggests that the prediction of the Bird-Carreau model might not hold for the systems studied. However, a firm conclusion can only be obtained after normal force data are generated.

In the past it was shown that the steady viscosity function and the primary normal stress coefficient are important rheological parameters in fluid and semi-solid texture (Dickie and Kokini, 1983). If a rheological model is able to relate material properties obtained from steady shear flows to those obtained from oscillatory flows, then small amplitude dynamic viscoelastic properties can be used in texture studies.

In conclusion, the Bird-Carreau model was shown to predict relatively well small amplitude dynamic viscoelastic properties and steady shear viscosity functions of 1.0 and 1.25% guar dispersions and 2.0 and 2.5% lambda carrageenan dispersions in water.

LIST OF SYMBOLS

α_1, α_2	Constants in the Bird-Carreau model (dimensionless)
$\lambda_{1\rho}, \lambda_{2\rho}$	Time constants (sec)
η_ρ	Viscosity Constants (Pa sec)
η_o	Zero shear limiting viscosity (Pa sec)
η	Viscosity function (Pa sec)
η'	Dynamic viscosity (Pa sec)
η''	Out of phase component of the complex viscosity (Pa sec)
ψ_1	Primary normal stress coefficient (Pa sec^2)
ψ_{10}	Zero shear primary normal stress coefficient (Pa sec^2)
$\dot{\gamma}$	Shear rate (sec^{-1})
ω	Frequency (sec^{-1})
$Z(\alpha_1)$	Zeta-Riemann function (dimensionless)
ρ	Index

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Factors Affecting Catalase Activity in *Staphylococcus aureus* MF-31

PATRICK M. GALLIGAN, WILLIAM A. BARRIER, and SCOTT E. MARTIN

ABSTRACT

Selected conditions were examined for their possible roles in the regulation of catalase activity in *Staphylococcus aureus* MF-31. The addition of the heme precursor 5-aminolevulinic acid resulted in increased catalase activity whereas the addition of exogenous hydrogen peroxide did not. Catalase activity decreased when *S. aureus* MF-31 cells were grown in media containing glucose. Cells grown in media with succinate or citrate substituted for glucose exhibited increased catalase activity. Cells grown in tryptic soy broth (0.5% NaCl) supplemented with NaCl showed increased catalase activity at salt concentrations up to 3%.

INTRODUCTION

NUMEROUS STUDIES have been undertaken on a variety of organisms in attempting to determine factors affecting catalase activity in microbial cells. The results of these experiments have been varied and often contradictory. If there exists a single mechanism for the control of catalase activity in prokaryotic organisms, it has yet to be elucidated. The following are some conditions which have previously been examined for their possible roles in the regulation of catalase activity: (1) phase of growth of the cells (Lin, 1963; Kwiek et al., 1970a, b; Finn and Condon, 1975; Yoshpe-Purer et al., 1977); (2) pH of the growth medium (Finn and Condon, 1975); (3) oxidation-reduction potential of the medium (Hassan and Fridovich, 1978); (4) type of growth medium with particular interest in the effect of glucose (Gregory et al., 1977; Cross and Ruis, 1978; Hassan and Fridovich, 1978; Marie and Parak, 1980); (5) degree of aeration or anaerobiosis (Hassan and Fridovich, 1970; Rodriquez-Bravo and Pionetti, 1981); (6) presence of hydrogen peroxide [H_2O_2] in the culture medium (Clayton, 1960; Finn and Condon, 1975; Yoshpe-Purer et al., 1977; Marie and Parak, 1980; Rodriquez-Bravo and Pionetti, 1981); (7) presence of a heme precursor in the culture medium (Ishida, 1976); and (8) presence of certain ions in the medium thought to affect catalase activity (Hassan and Fridovich, 1978). There have been several reports of increased catalase activity caused by addition of exogenous H_2O_2. This was seen with *Rhodopseudomonas spheroides* (Clayton, 1960), *Escherichia coli* (Udou and Ichikawa, 1979) and *Salmonella typhimurium* (Finn and Condon, 1975). However, Hassan and Fridovich (1978) were able to elevate catalase activity in the absence of H_2O_2 in *E. coli* by adding nitrate to the medium of anaerobically growing cells. In addition, Marie and Parak (1980) did not show an increase in catalase activity by addition of H_2O_2 to growing *Micrococcus luteus* cells.

Glucose has been shown to repress catalase activity in *M. luteus* (Marie and Parak, 1980), *E. coli* (Hassan and Fridovich, 1978), *Saccharomyces cerevisiae* (Cross and Ruis, 1978) and *Bacteroides fragilis* (Gregory et al., 1977). In the case of *E. coli*, growing the cells in the presence of 8 mM adenosine 3′,5′-cyclic monophosphoric acid (cAMP) largely overcame the effect of glucose. Thus, it appears that the control of catalase biosynthesis in *E. coli* is an example of catabolite repression. The addition of heme precursors also has been shown to increase catalase activity in *E. coli* (Hassan and Fridovich, 1978) and in *M. luteus* (Marie and Parak, 1980). Some chemical compounds and ions have an inhibitory effect upon catalase activity. Litchfield (1977) showed that mammalian catalase was inactivated at a low pH by the chloride anion. This effect resulted from the formation of a stable catalase-chloride compound with a stoichiometry of one chloride per catalase heme. In this work we investigated the effects of salt, hydrogen peroxide, and carbohydrates on the regulation catalase activity in *Staphylococcus aureus* MF-31.

Authors Barrier and Martin are affiliated with the Dept. of Food Science, 905 S. Goodwin Ave., 567 Bevier Hall, Univ. of Illinois, Urbana, IL 61801. Author Galligan, formerly with the Univ. of Illinois, is now affiliated with Packard Instrument Company, Downers Grove, IL 60515.

MATERIALS & METHODS

Media and culture preparation

The test organism was *Staphylococcus aureus* MF-31. Broth media utilized for the growth of *S. aureus* were: tryptic soy broth (TSB) and a semi-defined medium (SDM). The SDM was similar to that of Pariza and Iandolo (1969), and contained per liter of distilled water: vitamin free casamino acids, 10g; glucose, 2g; tryptophan, 0.2g; potassium phosphate dibasic, 5g; and MEM vitamins, 1 mL. The MEM vitamin mixture (Gibco Labs., Grand Island, NY) contained per liter: D-Ca pantothenate, 100 mg; choline chloride, 100 mg; folic acid, 100 mg; i-inositol, 200 mg; nicotinamide, 100 mg; pyridoxal HCl, 100 mg; riboflavin, 10 mg; thiamin HCl, 100 mg. In cases where succinate and citrate were substituted for glucose in SDM, equimolar amounts were used.

Growth was monitored by Klett-Summerson colorimetry using a blue filter (380-430 nm).

Culture division

In tests where H_2O_2 or 5-aminolevulinic acid hydrochloride (5-ALA) were examined for their effects upon catalase activity, the following protocol was performed. Cultures of *S. aureus* were allowed to grow to early exponential phase. At this time, one-half of the culture was aseptically transferred to a sterile flask and designated as the control. The remaining half of the culture was transferred to a separate flask which contained the test compound. Subsequent growth and catalase activities were then compared between the control culture and the test culture. Cells for catalase assays were removed at appropriate intervals and collected on membrane filters (0.45 μm; Millipore Corp., Bedford, MA). The cells were then washed from the filter to a centrifuge tube using 10 mL of 100 mM potassium phosphate buffer (PPB), pH 7.2, pelleted by centrifugation, and the pellets stored frozen (−20°C).

Cell lysis procedure

Lysostaphin (Sigma Chemical Co., St. Louis, MO) and deoxyribonuclease I (Sigma) were prepared separately in TM_4 buffer [10 mM tris (hydroxymethyl) amino methane, pH 7.6, 0.5 mM $MgCl_2$] to concentrations of 0.5 mg/mL and 200 μg/mL, respectively. Both solutions were stored frozen and thawed as needed. Frozen cell pellets of *S. aureus* were thawed and suspended in 1 mL of TM_4 buffer. For lysis, 0.4 mL of the lysostaphin solution was added to the cell suspension. The suspensions were mixed and then incubated at 35°C for 15 min. Following incubation, 0.1 mL of DNase solution was added. After incubation at room temperature for 1 min,

the suspensions were centrifuged at 10,000 x *g* for 20 min. The supernatant was retained and stored in an ice bath.

Catalase assay

The colorimetric assay of Sinha (1972) was used. This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2. The absorbance at 260 nm (A_{260}) of the cell lysates was measured, and a volume of lysate equivalent to $1/A_{260}$ was determined. This volume of lysate was added to the assay mixture of H_2O_2 and 10 mM PPB, pH 7.0. Samples were taken periodically and mixed with the dichromate-acetic acid mixture, stopping the reaction. The quantity of H_2O_2 remaining was determined from a standard curve. From this value, the amount of catalase activity at that time was determined. The initial catalase activity (K_o) was calculated by the extrapolation to zero time. Activity was reported as K_o per A_{260} unit of lysate, or Kat_f (Eq. 1).

$$Kat_f = \frac{K_o}{\text{gram protein used in assay}} \quad (1)$$

Protein determination

The protein content of lysates was determined according to Lowry et al. (1951).

Experimental conditions

5-Aminolevulinic acid. One volume of a stationary phase TSB culture (12 hr-ca. 10^9 cells/mL) was transferred to 1800 volumes of TSB and incubated at 35°C. After 5 hr growth, the culture was divided, and 5-aminolevulinic acid hydrocholoride (Sigma) was added to one portion to a final concentration of 250 mg/L. The other portion was retained as the control. Catalase activity was determined as K_o/A_{260} and calculated to give the activity as percent activity. The catalase activity of the heme precursor-treated cells after 10 hr of growth was defined as 100%.

In addition, a stationary phase culture of *S. aureus* MF-31 was split into four portions and treated as follows: flask 1 – TSB (control), flask 2 – puromycin added (100 μg/mL); flask 3 – 5-ALA added (0.25 mg/mL), and flask 4 – puromycin (100 μg/mL) and 5-ALA (0.25 mg/mL) added. The flasks were incubated 6 hr (post stationary phase) and sampled for catalase activity.

Heme. Stock cultures of *S. aureus* MF-31 were inoculated in 200 mL of TSB with and without glucose. Hemin (Sigma) was added to give a final concentration of 0.05 mg/mL. The culture was incubated at 35°C with rotary shaking. Samples were taken at stationary phase.

Carbohydrates. Stock cultures of *S. aureus* were inoculated into TSB, a glucose-containing medium, or SDM. Succinate, citrate, or no carbohydrate were substituted for glucose in SDM for some tests. Cultures were incubated at 35°C with rotary shaking. As each culture reached stationary phase, a sample was removed and centrifuged. Catalase activity was determined as Kat_f and calculated to give the activity as percent activity. The catalase activity of stationary phase cells grown in TSB was defined as 100%.

Hydrogen peroxide. One volume of a stationary phase TSB culture was transferred to 1800 volumes of TSB and incubated at 35°C. After 5 hr of growth, the culture was split into three equal parts. Hydrogen peroxide was added to the first portion to obtain a final concentration of 0.075 mM. Hydrogen peroxide was added to the second portion to a final concentration of 0.25 mM. The final portion of the split culture was retained as the control. Samples were removed at appropriate intervals. Cells were lysed as described previously. Catalase activity was determined as K_o/A_{260} and calculated to give the activity as percent activity. Catalase activity of the control cells after 8 hr was defined as 100%.

NaCl. Stock cultures of *S. aureus* were inoculated into TSB containing varying amounts of added NaCl. Cultures were incubated at 35°C with rotary shaking. As each culture reached stationary phase, a 10 ml sample was removed and centrifuged. Catalase activity was determined as K_o/A_{260} in the cell lysates.

RESULTS

THE EFFECTS of adding the heme precursor 5-aminolevulinic acid hydrochloride to TSB containing log phase *S. aureus* cells are presented in Fig. 1. Cell growth was not affected by exposure to the heme precursor. However, catalase activity was stimulated. Cells exposed to the heme precursor had twice the activity of control cells when the cells entered stationary phase.

In order to determine if the stimulatory effect of 5-ALA was due to an activation of a pre-existing apoprotein or due to *de novo* protein synthesis, cells were grown in the presence of 5-ALA and 5-ALA plus puromycin. The catalase activity of cells grown in the presence of puromycin – 5-ALA was 19% lower than the control and 64% lower than cells grown with 5-ALA alone (Table 1).

To determine if the formation of the porphyrin backbone was a limiting step in the synthesis of catalase, *S. aureus* cells were grown in TSB or TSB without glucose in the presence or absence of heme. The catalase activity of cells grown in TSB without glucose was more than twice that of cells grown in TSB with glucose (Table 2). Cells

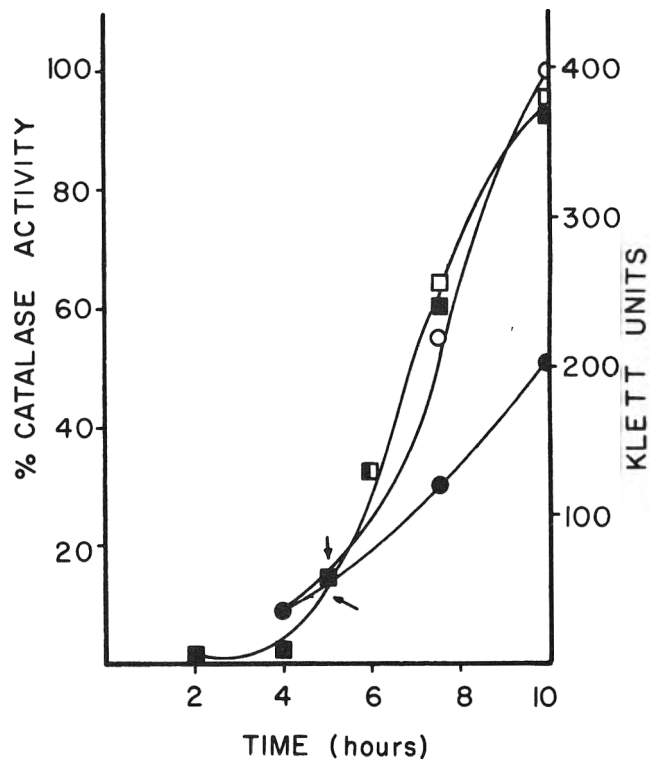

Fig. 1—Effects of addition of 5-aminolevulinic acid hydrochloride on catalase activity of Staphylococcus aureus: ■ *Klett Units in TSB;* □ *Klett Units in TSB + 5-ALA;* ● *Catalase activity in TSB;* ○ *Catalase activity in TSB + 5-ALA.*

Table 1—Effects of 5-aminolevulinic acid on catalase activity

	Kat_f	% Kat_f
TSB (with glucose)	1735	100
TSB + puromycin	1425	82
TSB + 5-ALA	2521	145
TSB + puromycin + 5-ALA	1400	81

Table 2—Influence of glucose and heme on catalase activity in Staphylococcus aureus *MF-31*

	Kat_f	% Kat_f
TSB	2594	100
TSB without glucose	5864	226
TSB + heme	3129	121
TSB without glucose + heme	1324	51

grown in TSB with glucose and heme had catalase activities similar to cells grown in TSB with glucose. However, cells grown in TSB without glucose plus heme had lower catalase activities than those grown in TSB without glucose.

The effect of carbohydrates on catalase activity in *S. aureus* cells is shown in Table 3. Growth in the two glucose-containing media, TSB and SDM with glucose, resulted in comparatively low levels of catalase activity. When citrate or succinate were substituted for glucose in SDM, or when no carbohydrate source was added, cellular catalase activity increased.

The effect on catalase activity when exogenous H_2O_2 was added to TSB containing log phase *S. aureus* cells is shown in Fig. 2. Two different quantities of H_2O_2 were examined (0.075 mM and 0.25 mM) but neither resulted in increased catalase levels. The control cells and the cells exposed to 0.075 mM H_2O_2 maintained approximately the same levels of activity. The cells exposed to 0.25 mM H_2O_2 exhibited slightly less activity than the control cells, probably due to death that was incurred by the cells at this high level of H_2O_2. Similar results were obtained using 0.1 mM, 0.5 mM, and 1.0 mM H_2O_2 (data not presented).

Fig. 3 shows the effects on catalase activity when *S. aureus* cells were grown to stationary phase in TSB containing differing levels of NaCl. Cells grown in TSB with little or no added NaCl (0.5-3.0%) showed relatively high levels of catalase activity. Cells cultured in 4.5 or 7.5% NaCl showed lower levels of catalase activity. Activities appeared to decrease as the NaCl concentration increased above 3.0%.

Table 3—Effect of various carbohydrates on catalase activity of Staphylococcus aureus *MF-31 cells*

Medium	Kat_f	% Kat_f
TSB (with glucose)	1504	100
SDM (with glucose)	688	46
SDM no carbohydrate	2446	163
SDM + succinate	1953	130
SDM + citrate	1763	117

DISCUSSION

CATALASE ACTIVITY was stimulated by exposure to the heme precursor 5-aminolevulinic acid hydrochloride in the presence of glucose. It may be that the synthesis of the heme group, and not the protein moiety, is the rate limiting step for producing high catalase activities in *S. aureus*. It is also possible that glucose or a glucose catabolite may exert its inhibitory effect on catalase production at the point of synthesis of the heme precursor, since the addition of the heme precursor resulted in much higher catalase levels in the presence of glucose.

The catalase activity of *S. aureus* cells grown in TSB without glucose, but with heme, was lower than cells grown in TSB without glucose. The catalase activity of cells grown in TSB with heme was slightly higher than those grown in TSB without added heme. In the former case, heme might be acting as an end product inhibitor or repressor. Lascelles (1964), investigating the heme biosynthetic chain of *Rhodopseudomonas spheroides*, found evidence of end product inhibition and end product repression. Burnham and Lascelles (1963) showed that the end product heme inhibited the first enzyme (Δ-aminolevulinate synthetase) of the chain. When heme was added to the culture medium the formation of aminolevulinate was repressed three- to fourfold. In the latter case, the lack of a difference of catalase activities between *S. aureus* cells grown in TSB with glucose and TSB with glucose and heme may be explained by the evidence which shows that the specific activity of Δ-aminolevulinate synthetase can be increased by the presence of glucose (Poulson, 1976).

Catalase activity was enhanced when *S. aureus* cells were grown using citrate or succinate, in place of glucose. Citrate

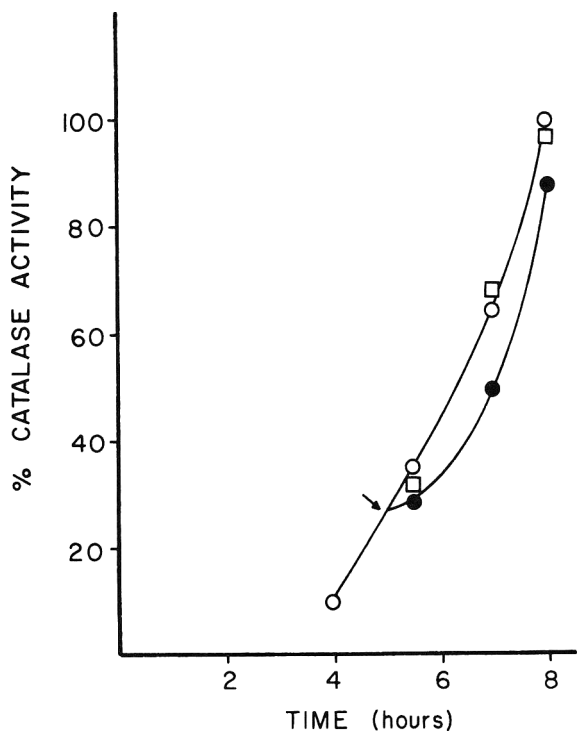

Fig. 2—Effects of addition of hydrogen peroxide on catalase activity of Staphylococcus aureus: ○ *TSB;* □ *TSB + 0.075 mM* H_2O_2; ● *TSB + 0.25 mM* H_2O_2.

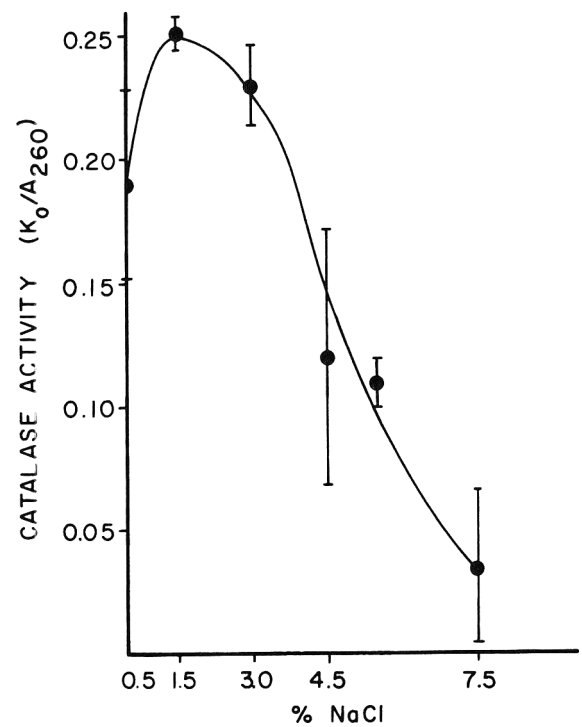

Fig. 3—Catalase activity of Staphylococcus aureus *cells grown to stationary phase in varying NaCl concentrations.*

and succinate are Krebs cycle intermediates and their utilization as energy sources likely involves enhanced respiratory activity with concomitant H_2O_2 production. Glucose, however, may be catabolized fermentatively, greatly reducing H_2O_2 formation in the cell. A possible catalase control mechanism, and one which does not preclude the first, is that catalase is co-induced with the components of the electron transport chain. Facultatively anaerobic, fermentative bacteria such as *S. aureus,* when presented with a rich medium containing glucose, derive most of their energy by fermentation (Paigen and Williams, 1970). In this case, the need for defenses against H_2O_2 is diminished. However, with a supply of substrates whose utilization depends upon the electron transport chain, more H_2O_2 is likely to be produced and higher levels of catalase might be necessary for survival.

Exposure of *S. aureus* cells to exogenous H_2O_2 did not result in increased catalase activity. Exogenous addition of H_2O_2 also failed to increase catalase activities in *E. coli* (Hassan and Fridovich, 1978) and *M. luteus* (Marie and Parak, 1980). In these experiments there was only a slight loss in cell viability subsequent to the H_2O_2 addition. Thus, it appears that H_2O_2 *per se* does not induce higher catalase levels in *S. aureus.*

Catalase activities were adversely affected when cells were grown in higher levels of NaCl. This experiment provided evidence that catalase activities are decreased when *S. aureus* cells are grown in high levels of NaCl. It was evident that cells grown in TSB with 1.5% or 3.0% NaCl had higher catalase activities than cells grown in the absence of added NaCl (0.5%). Udou and Ichikawa (1979) reported that *S. aureus* cells grown in a glucose medium showed enhanced oxygen consumption in the presence of 0.6% or 3% NaCl. However, O_2 consumption was suppressed at a concentration of 6%. This increased O_2 consumption indicated increased aerobic respiration. Furthermore, catalase activity might be expected to increase in response to these events and in particular to the formation of H_2O_2. Therefore, the increased catalase activities found in cells grown in 1.5% and 3.0% NaCl may be in response to increased respiratory activity.

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Relationships Between the Hard-to-Cook Phenomenon in Red Kidney Beans and Water Absorption, Puncture Force, Pectin, Phytic Acid, and Minerals

WILFREDO MOSCOSO, M. C. BOURNE, and L. F. HOOD

ABSTRACT

The effect of high temperature, high humidity storage on cooking quality and physicochemical properties of dry, mature red kidney beans was evaluated over a storage period of 9 months. The rate of softening of beans during cooking, and the rate of dissolution of pectin during cooking followed apparent first-order kinetics and their apparent rate constants correlated highly with each other. The apparent softening rate constants decreased with increasing time of storage. The loss of cookability in mature bean seeds stored under high temperature-high humidity conditions probably results from a decrease in phytic acid phosphorus and alterations in the ratio of monovalent to divalent cations in the tissue.

INTRODUCTION

THE TERM "cookability" as applied to legume seeds refers to the condition by which they achieve a degree of tenderness during cooking acceptable to the consumers. Normally, the time required to soften the freshly harvested seeds is taken as a basis for comparison.

Certain storage conditions can result in an increase in the cooking time required to properly soften legumes (Burr et al., 1968; Morris, 1963; Muneta, 1964). Storing legumes at high temperatures and relative humidities, conditions normally encountered in the humid tropics, accelerate the problem. This suggests that chemical or biochemical factors are responsible. Even though considerable efforts have been devoted to studying this problem, the exact mechanism by which stored beans become hard-to-cook remains obscure. Factors that may be implicated in the loss of cookability are reviewed by Moscoso (1981).

This study was designed to measure some chemical and biochemical changes in legume seeds during storage under humid tropical conditions and to relate these changes to ease-of-cooking of the beans.

MATERIALS & METHODS

Storage conditions

Red kidney beans of the variety Redkloud, were purchased locally soon after harvest. The beans were size graded on a sieve containing slits 12/64" wide x 3/4" long. The minus 12/64" fraction consisting of small, broken and halved beans, was discarded and the plus 12/64" fraction comprising about 90% of the beans was used.

The beans were divided into three lots of 9 kg each. One lot was placed immediately in a 5 gal plastic pail provided with an air tight removable lid to maintain the original moisture content of 17.9%. The remaining two lots were dried by spreading on the laboratory bench. After drying for approximately 7 and 12 hr, the moisture contents of the two lots were 14.9 and 12.5%, respectively. Each lot was then transferred to a 5 gal plastic pail with lid. All lots were treated with 35g of "Thylate" (active ingredient, tetramethyl thiuran disulfide, E.I. DuPont de Nemours Co.) in order to prevent mold growth during storage. The moisture contents were measured throughout the storage period and no changes were found.

Authors Bourne and Hood are affiliated with the Institute of Food Science, Cornell Univ., NYS Agricultural Experiment Station, Geneva, NY 14456. Author Moscoso, formerly with Cornell Univ., is now with Instituto Superior de Agricultura, Apartado #166, Santiago, Republica Dominicana.

The lots containing 17.9% and 14.9% moisture were stored in a constant temperature room at 32°C, while the 12.5% moisture lot was stored at 2°C. All lots were aerated and mixed twice a month by removing the lids for 2 min, closing, inverting the container several times and aerating again for 2 min. Aliquots were withdrawn at 3, 6, and 9 months and subjected to the tests described below.

Water absorption

The procedure described by Sefa-Dedeh et al. (1978) was used. The determinations were done in triplicate, and no corrections for lost solutes were made.

Cooking conditions

Several 113g aliquots of dry beans were placed in 303 x 406 tin cans. The cans were filled with distilled water and the beans soaked at room temperature for 18 hr. The cans were sealed and placed in an open kettle containing boiling water for 15, 30 and 45 min. A maximum of 45 min cooking time was selected because deviations from first order kinetics occur with longer cooking times (Huang and Bourne, 1983). At the end of each cooking time, the cans were removed from the boiling water and quickly cooled to room temperature by immersing in cold tap water. Beans which were soaked for 18 hr and not cooked were taken as 0 cooking time. The seed coats and cotyledons were peeled apart by hand prior to the chemical analysis.

The canned beans were stored overnight at 3°C. The texture measurements and preparation of the samples for the chemical analyses were performed the day after canning. This procedure was designed to control the bean-water ratio during cooking and to eliminate the mechanical effects of beans rubbing against each other when boiled in an open kettle. It was not intended to duplicate conventional canning procedures.

Texture determination

A flat faced 3/32" diameter circular steel punch was used to measure the firmness. It was mounted in an Instron Universal Testing Machine which was operated at a crosshead speed of 30 cm/min and a chart speed of 5 cm/min. The maximum puncture force was taken as an index of bean firmness (Bourne, 1972). A total of 500 individual beans were punched for each treatment and the mean peak force calculated.

Marc preparation

A procedure similar to that described by Kon (1968) was used to prepare the cotyledon and seed coat marcs. A 50-g sample of cotyledons was blended with 150 mL 95% ethyl alcohol in a Waring Blendor at full speed for 5 min. The mixture was centrifuged at 4,000 x *g* for 10 min and the alcoholic supernatant discarded. The extraction was repeated twice with 150 mL 95% ethyl alcohol and once with 150 mL absolute ethanol. The seed coats were first freeze-dried, and then ground in a Ultracentrifugal mill (Brinkman Instruments, Inc.) to pass a 60 mesh sieve. An aliquot was weighed into a centrifuge tube and extracted three times with three volumes of 95% ethyl alcohol, once with three volumes of absolute alcohol. The wet cotyledon and seed coat marcs were transferred to petri dishes and dried under vacuum at room temperature.

Pectic substances

The colorimetric procedure described by Blumenkrantz and Asboe-Hansen (1973) was used to determine anhydrogalacturonic acid (AGA). The procedure developed by McCready and McComb (1952) was used to determine total pectic substances. A modification of the procedure of Dietz and Rouse (1953) was used to determine water insoluble pectic substances. One gram of dry marc was

weighed into a conical 50 mL centrifuge tube. Two milliliters 95% ethyl alcohol and 40 mL distilled water were added. The mixture was stirred, allowed to stand for 10 min at room temperature, stirred and centrifuged for 10 min at 1,000 x *g*. The supernatant was decanted into a 100 mL volumetric flask and the extraction was repeated on the residue with 40 mL distilled water. The second extract was added to the same volumetric flask. After diluting to the 100 mL mark with distilled water, the extract was filtered through Whatman #41 ashless filter paper. The AGA content in the filtrate was determined as described above. The water insoluble pectic substances were determined by subtracting the water soluble pectic substances from the total pectic substances content in the samples.

Chemical analyses

Phytic acid phosphorous was determined by using a procedure similar to the one described by Chang et al. (1977). The absorbance of the phosphomolybdate was read at 744 nm. The calcium, magnesium and potassium contents were determined by atomic absorption spectrophotometry after ashing the marcs at 550°C overnight, dissolving in 20% HCl, filtering and making up to standard volume.

RESULTS & DISCUSSION

Water absorption

The water absorption patterns for all samples were characterized by rapid water uptake during the first 6 hr after which the rate slowed down as saturation point was reached (Fig. 1). Similar curves were obtained for the samples stored for 3, 6, and 9 months. A positive correlation (r = 0.94) was observed between the initial moisture content of the beans and their rate of water uptake during the first hour of the soaking process. This agrees with results reported by Crean and Haisman (1963a) and Gloyer (1921) who found that impermeability to water in dry beans was induced by storing seed at low moisture content; and by Sefa-Dedeh and Stanley (1979) who state that the seed coat is the main factor affecting the initial water uptake in dry legumes.

The maximum water absorbed by the beans during soaking is affected by both the initial moisture content and the storage temperature. Water uptake by beans of 12.5% moisture stored at 2°C was virtually unaffected by storage time up to 9 months, while those stored at 32°C showed a decrease of about 10% in the maximum water absorbed during the storage period (Fig. 2).

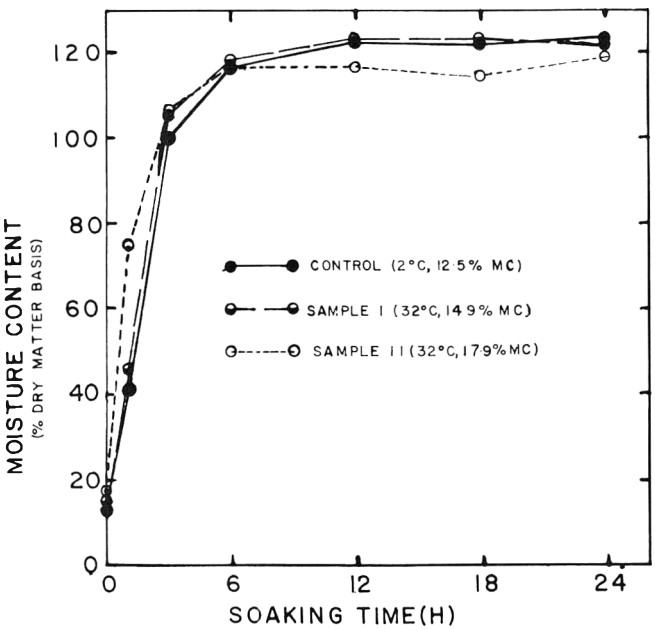

Fig. 1—Water absorption in dry beans after 3 months storage.

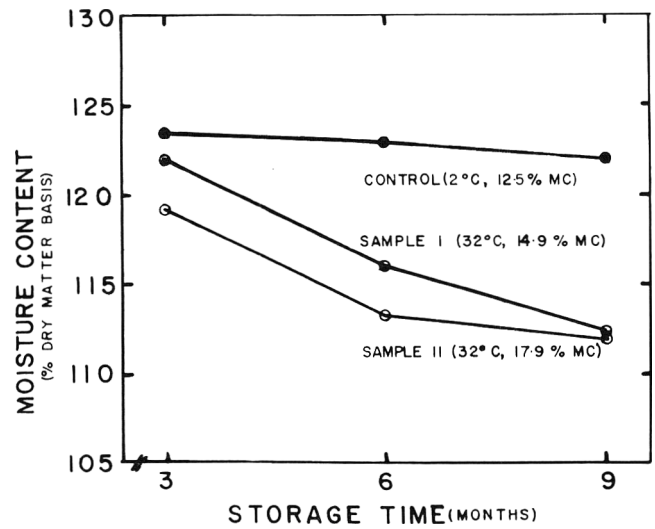

Fig. 2—Water absorption in stored dry beans after soaking for 24 hr.

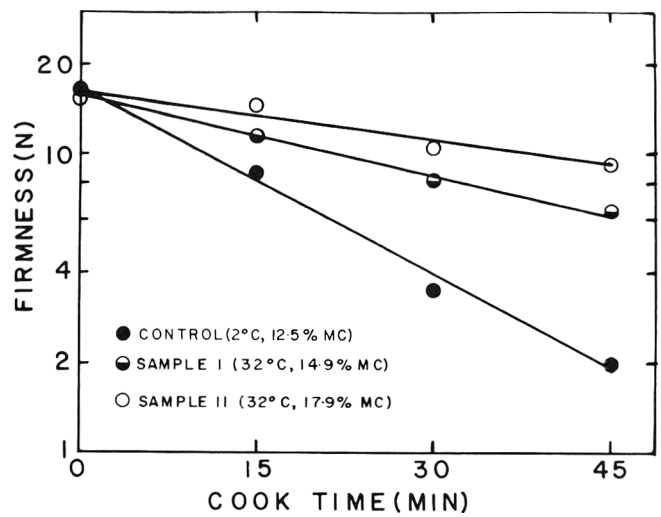

Fig. 3—Softening of dry beans stored for 6 months.

Table 1—Apparent softening rate constants and correlation coefficients for stored dry beans

Storage time (months)	Control (2°C, 12.5% H_2O)		Sample I (32°C, 14.9% H_2O)		Sample II (32°C, 17.9% H_2O)	
	Rate constant (min^{-1})	Correlation coefficient	Rate constant (min^{-1})	Correlation coefficient	Rate constant (min^{-1})	Correlation coefficient
3	0.020	−0.991	0.013	−0.997	0.0084	−0.930
6	0.021	−0.996	0.009	−0.996	0.0055	−0.935
9	0.021	−0.994	0.007	−0.998	0.0055	−0.931

Cookability

The firmness of beans cooked for 0, 15, 30, 45 min after 6 months storage is shown in Fig. 3. Similar plots were obtained for beans stored for 3 months and 9 months. The rectilinearity of these semilog plots indicates that the softening process follows apparent first order kinetics. The apparent softening rate constants derived from the slopes of these semilog plots are given in Table 1 together with the correlation coefficients of the lines whose slopes were used to calculate the apparent rate constants. The high correlation coefficients show a good fit to apparent first order kinetics for all samples. The apparent rate constant for the control sample did not change during storage, but it declined substantially for both samples stored at 32°C. These results confirm previous reports that high temperature and high moisture content during storage increase the time required to soften legume seeds during cooking (Burr et al., 1968; Jackson and Varriano-Marston (1981); Mattson et al., 1950; Morris, 1963).

Total pectic substance

Tables 2 and 3 show respectively the total pectic substances in seed coat and cotyledon marcs prepared from stored dry beans after cooking for 0, 15, 30 and 45 min. The sample stored at 2°C lost pectic substances at a faster rate than the samples stored at 32°C. The differences among the total pectic substances content at the beginning of the cooking process might be due to the presence of non-pectic materials that were not extracted by the ethanol.

Cooking resulted in losses of pectic substances from the seed coats for all the samples. Even though the results were not entirely consistent during the storage period, the beans stored at 2°C experienced a larger decrease in the total pectic substances content than the beans stored at 32°C after cooking for 45 min (Table 2). A similar pattern was observed for the total pectic substances content in the cotyledons; more pectic substances were lost from the cotyledons stored at 2°C after cooking for 45 min than from the two treatments (Table 3).

Water insoluble pectic substances

Tables 4 and 5 show the water insoluble pectic substance content of seed coat and cotyledon marcs for stored beans cooked for 0, 15, 30 and 45 min. Plots of the logarithm of the insoluble pectic substances from both the seed coats and cotyledons versus the cooking time are rectilinear suggesting that the dissolution of the pectic substances during cooking follows first-order kinetics. The apparent rate constants calculated from the slope of the line are given in Table 6. In all cases the correlation coefficients were better than −0.80 and in most cases better than −0.94 (Table 6) indicating a good fit to first order kinetics.

The apparent rate constants for the dissolution of the pectic substances in both the seed coats and cotyledons were highest for the control sample (2°). A consistent decrease in the apparent rate constants was observed during storage of beans at 32°C.

The apparent softening rate constants were highly

Table 2—Total pectic substances content of dry seed coat marcs

Storage time (months)	Cook time (min)	Control (2°C, 12.5% H_2O) mg/AGA/g Dry marc	Sample I (32°C, 14.9% H_2O) mg/AGA/g Dry marc	Sample II (32°C, 17.9% H_2O) mg/AGA/g Dry marc
3	0	119.2	120.8	104.9
	15	91.5	91.0	86.2
	30	78.8	92.7	90.9
	45	76.2	91.7	87.1
6	0	125.3	114.6	109.2
	15	102.6	107.2	111.0
	30	99.5	102.5	93.2
	45	80.8	97.7	95.2
9	0	127.8	109.1	100.4
	15	101.6	105.6	97.7
	30	93.7	105.9	99.6
	45	86.3	103.7	97.8

AGA = anhydrogalacturonic acid

Table 3—Total pectic substances content of dry cotyledon marcs

Storage time (months)	Cook time (min)	Control (2°C, 12.5% H_2O) mg/AGA/g Dry marc	Sample I (32°C, 14.9% H_2O) mg/AGA/g Dry marc	Sample II (32°C, 17.9% H_2O) mg/AGA/g Dry marc
3	0	28.8	27.2	26.8
	15	25.4	26.2	25.7
	30	24.7	25.9	24.3
	45	24.8	26.6	25.2
6	0	30.5	29.9	30.8
	15	27.9	28.4	28.3
	30	29.2	29.2	27.8
	45	27.8	28.9	28.7
9	0	29.5	28.1	27.3
	15	26.7	27.7	26.8
	30	26.3	27.5	26.1
	45	25.9	27.6	26.3

AGA = anhydrogalacturonic acid

correlated with the apparent rate constants for the dissolution of the pectic substances in the seed coats and cotyledons (Fig. 4 and 5). the correlation coefficients being respectively r = 0.96 for the seed coat and r = 0.97 for the cotyledon. This evidence supports the theory that changes in the pectic substances are responsible for the changes in the cooking properties of the dry beans (Rockland and Jones, 1974; Sefa-Dedeh et al., 1979).

Phytic acid phosphorous

Beans stored at 2°C showed no significant change in phytic acid phosphorous content during the 9 months of storage (Fig. 6). The beans stored at 32°C showed a consistent decrease in the phytic acid phosphorous content during storage, with the higher moisture samples showing the greatest decrease.

The phytic acid phosphorous content of the soaked beans correlated well with the softening rates of the beans (r = 0.96), and the rate constants for the dissolution of the pectic substances in the seed coats (r = 0.95) and cotyledon (r = 0.92). This suggests that high phytic acid phosphorous content in the beans favors a rapid rate of softening and dissolution of the pectic substances making the beans more cookable. This point is illustrated by plotting the firmness of the beans after cooking for 45 min versus the phytic acid phosphorous content of the soaked beans (Fig. 7). These findings confirm the results reported by Kon (1979),

Table 4—Water insoluble pectic substances of dry seed coat marcs

Storage time (months)	Cook time (min)	Insoluble pectic substances (% of total) Control (2°C, 12.5% H_2O)	Sample I (32°C, 14.9% H_2O)	Sample II (32°C, 17.9% H_2O)
3	0	98.2	97.2	95.8
	15	88.7	91.8	92.1
	30	89.0	90.5	91.9
	45	86.7	89.8	89.5
6	0	97.4	95.7	96.0
	15	93.4	92.0	94.1
	30	91.3	91.8	92.0
	45	88.6	90.4	92.0
9	0	98.2	96.2	95.8
	15	92.1	93.2	93.8
	30	90.1	92.2	93.2
	45	88.8	91.4	92.1

Table 5—Water insoluble pectic substances of dry cotyledon marcs

Storage time (months)	Cook time (min)	Insoluble pectic substances (% of total) Control (2°C, 12.5% H_2O)	Sample I (32°C, 14.9% H_2O)	Sample II (32°C, 17.9% H_2O)
3	0	84.4	90.0	88.4
	15	79.4	87.7	88.5
	30	77.4	86.8	87.0
	45	74.2	85.2	86.1
6	0	83.7	88.8	88.5
	15	79.0	87.3	87.6
	30	78.3	86.5	87.5
	45	76.1	86.4	87.4
9	0	84.4	89.3	87.9
	15	78.3	87.7	88.1
	30	77.9	87.3	87.7
	45	74.5	86.6	87.5

Table 6—Apparent rate constants and correlation coefficient for the dissolution of the pectic substances in seed coats and cotyledons

Storage time (months)	Control (2°C, 12.5% H_2O) Rate constant (10^{-3} x min^{-1})	Correlation coefficient	Sample I (32°C, 14.9% H_2O) Rate constant (10^{-3} x min^{-1})	Correlation coefficient	Sample II (32°C, 17.9% H_2O) Rate constant (10^{-3} x min^{-1})	Correlation coefficient
			Seed coats			
3	1.07	−0.864	0.73	−0.907	0.60	−0.950
6	0.89	−0.992	0.50	−0.920	0.44	−0.946
9	0.94	−0.962	0.48	−0.949	0.36	−0.974
			Cotyledons			
3	1.19	−0.987	0.51	−0.987	0.28	−0.939
6	0.85	−0.952	0.26	−0.932	0.11	−0.868
9	1.10	−0.947	0.28	−0.960	0.05	−0.800

Kumar et al. (1978), Mattson et al. (1950), and Smithies (1960) who found a good correlation between low phytic acid content and cookability of legumes.

The high correlation between firmness of cooked beans and phytic acid phosphorous of the uncooked soaked beans suggests that phytic acid content could be used as an index of the cookability of red kidney beans. In our experiments, all samples with a phytic acid phosphorous level less than about 400 mg per 100g dry bean showed reduced cookability.

Minerals

The changes in calcium, magnesium, and potassium contents of the seed coat marcs during cooking are shown in Table 8. Calcium decreased during cooking, and the decrease was greater in the control than in the time samples stored at 32°C. The magnesium levels were one-third to one-fourth of the calcium levels but they showed a trend similar to the calcium during cooking. High values of calcium and magnesium in the seed coats seem to be associated with high firmness of the cooked bean, high total pectic substances and high water soluble pectic substances. The seed coats of the beans stored at 2°C had a relatively low potassium content after soaking for 18 hr at 25°C (Table 8). Cooking for 15 min caused more than a twofold increase. The potassium level declined when the beans were cooked longer than 15 min. The seed coats of beans stored at 32°C had a high potassium level which was almost unchanged after 15 min cooking and then declined slowly with longer cooking time. This pattern is in sharp contrast to the sawtooth pattern of change found in beans stored at 2°C The potassium content of the seed coats was not well correlated with the bean firmness, the total pectic substances or the water insoluble pectic substances content.

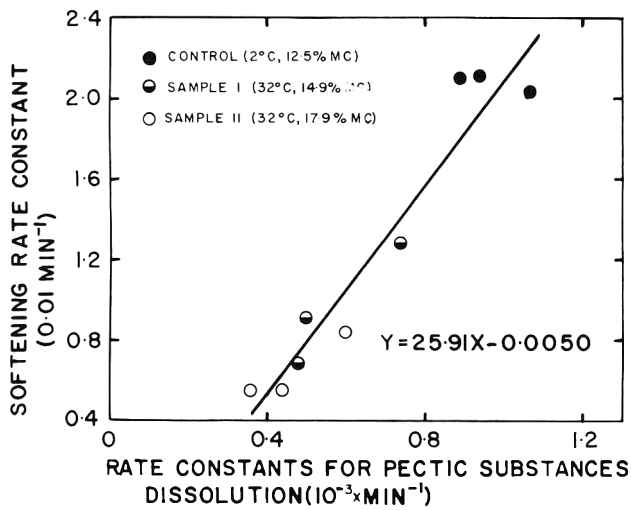

Fig. 4—Relationship between the apparent softening rate constant and the apparent pectic substances dissolution rate constant in bean seed coat. r = 0.96

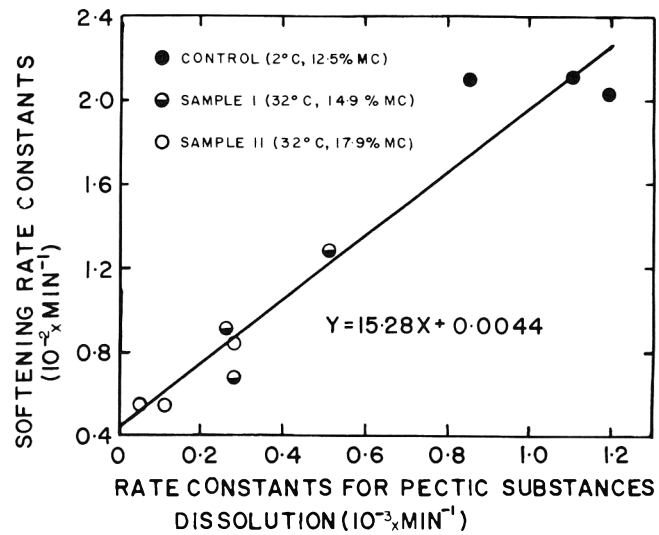

Fig. 5—Relationship between the apparent softening rate and the apparent pectic substances dissolution rate constant in bean cotyledon. r = 0.97

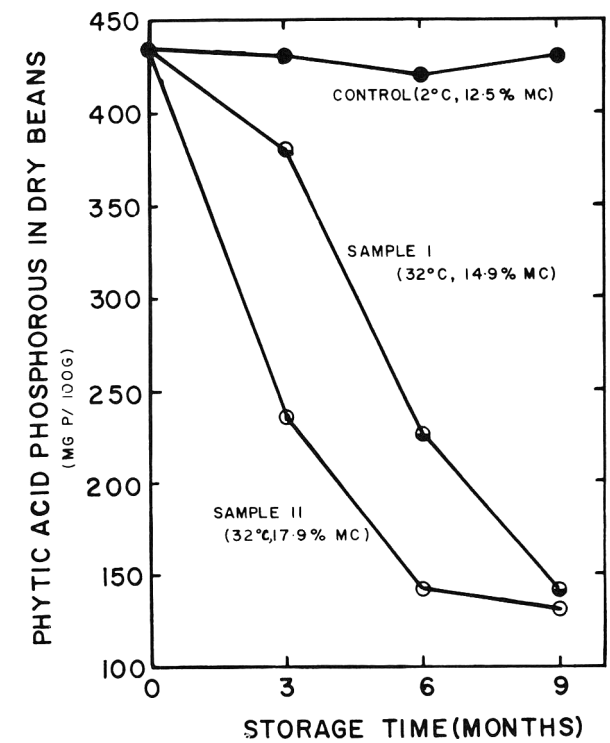

Fig. 6—Phytic acid phosphorous of soaked red kidney beans, after storing for 3, 6 and 9 months.

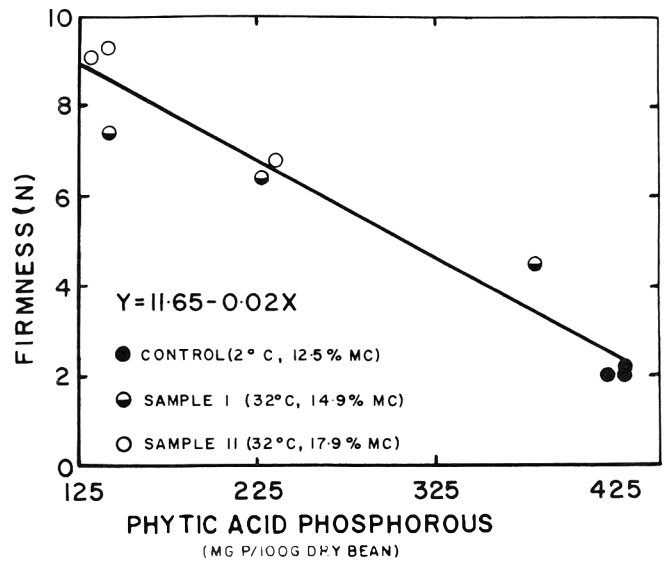

Fig. 7—Relationship between the phytic acid phosphorous of soaked beans and firmness after cooking for 45 min. r = −0.97. Pooled data for beans stored for 3, 6 and 9 months.

The changes in calcium, magnesium, and potassium contents of the cotyledon marcs during cooking are shown in Table 8. The magnesium contents of the cotyledons are about twice as high as the calcium contents which is the reverse of what was found in the seed coats (see Table 8). The uncooked soaked cotyledons lost both magnesium and potassium in an amount inversely proportional to the moisture content of the beans during storage. Additional magnesium and potassium were lost during cooking. There was little change in the calcium content of the control during cooking, and a small decrease in the samples stored at 32°C during cooking. This data supports the finding of Harman and Granett (1972) who reported an increase in magnesium and potassium leakage from aged peas during soaking. They attributed this to plasmalemma membrane breakage in the cotyledons.

Mattson (1946) suggested a direct relationship between cookability of dry peas and the ratio of monovalent to divalent cations. Table 9 lists the ratios found in this study. The correlation coefficient between the monovalent/divalent cations ratio and apparent softening rate constants was r = 0.93, and for the apparent pectin dissolution rate constants was r = 0.90 which supports Mattson's suggestion.

The relationship between phytic acid phosphorous content and the ratio of monovalent to divalent cations was negative in the seed coats (r = −0.83) and positive in the cotyledons of soaked beans (r = 0.85). This is consistent with the observation that aged beans with low phytic acid content, due to hydrolytic cleavage by phytase (Mattson et al., 1950), show a considerable loss of potassium ions from the cotyledon during soaking. Some of the potassium ions lost from the cotyledons are retained in the seed coats resulting in an increase in the ratio of monovalent to divalent cations.

The loss of cookability of dry bean seeds in storage is associated with a decrease in the phytic acid phosphorous content and alterations in the ratio of monovalent to divalent cations in the soaked beans. The seeds are left with less phytic acid and less monovalent cations which can solubilize

Table 7—Calcium, magnesium and potassium in dry seed coat marcs prepared from stored beans (mg/g dry marc)

Storage time (months)	Cook time (min)	Control (2°C, 12.5% H_2O)			Sample I (32°C, 14.9% H_2O)			Sample II (32°C, 17.9% H_2O)		
		Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
3	0	9.9	2.5	9.9	9.1	2.4	21.3	8.3	2.4	21.1
	15	6.1	2.4	20.7	7.6	2.5	21.1	7.8	2.4	22.1
	30	5.9	2.0	16.6	7.5	2.3	20.0	7.8	2.2	19.7
	45	5.2	1.7	13.1	7.6	2.1	17.3	7.7	2.2	18.6
6	0	9.5	2.5	8.3	7.9	2.2	21.2	8.3	2.1	18.3
	15	6.6	2.4	21.3	8.3	2.4	21.2	8.6	2.3	18.4
	30	6.7	2.0	17.4	8.1	2.2	19.0	8.3	2.2	17.3
	45	5.9	1.8	15.1	8.3	2.2	19.2	8.2	2.1	17.6
9	0	8.8	2.4	8.1	7.5	2.3	21.2	7.3	2.3	19.1
	15	6.0	2.3	21.3	7.1	2.4	20.6	7.4	2.4	19.6
	30	5.8	2.0	17.2	6.8	2.3	19.7	7.0	2.3	18.8
	45	4.9	1.7	14.7	7.0	2.2	19.1	6.9	2.2	18.2

Table 8—Calcium, magnesium and potassium in dry cotyledon marcs prepared from stored beans (mg/g dry marc)

Storage time (months)	Cook time (min)	Control (2°C, 12.5% H_2O)			Sample I (32°C, 14.9% H_2O)			Sample II (32°C, 17.9% H_2O)		
		Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
3	0	0.50	1.20	10.75	0.55	1.07	8.94	0.55	0.71	5.93
	15	0.48	0.95	7.13	0.45	0.86	5.24	0.37	0.69	6.19
	30	0.45	0.84	6.01	0.42	0.78	5.10	0.34	0.59	5.32
	45	0.45	0.74	5.03	0.34	0.66	4.51	0.34	0.63	5.57
6	0	0.46	1.27	11.20	0.43	0.77	6.73	0.37	0.61	4.73
	15	0.55	0.97	7.97	0.51	0.73	5.70	0.36	0.62	4.70
	30	0.48	0.70	5.51	0.39	0.59	4.90	0.31	0.64	4.61
	45	0.47	0.74	5.79	0.39	0.60	4.74	0.30	0.60	4.68
9	0	0.47	1.25	11.02	0.51	0.83	7.28	0.49	0.63	5.20
	15	0.53	0.97	7.61	0.50	0.76	5.56	0.42	0.63	5.19
	30	0.50	0.81	6.22	0.47	0.70	5.05	0.39	0.58	5.03
	45	0.49	0.75	5.84	0.45	0.67	4.66	0.39	0.58	5.04

Table 9—Ratio of monovalent to divalent cations in soaked beans

	Storage time (months)	Control (2°C, 12.5% H_2O)	Sample I (32°C, 14.9% H_2O)	Sample II (32°C, 17.9% H_2O)
Seed coat	3	0.80	1.85	1.99
	6	0.70	2.09	1.75
	9	0.72	2.16	1.99
Cotyledon	3	6.32	5.52	4.71
	6	6.47	5.61	4.83
	9	6.41	5.43	4.64

the pectic substances through chelation and ion exchange during the cooking process.

Future studies on this problem could investigate the factors responsible for making the aged bean membranes more permeable to small molecular weight solutes. Harman and Mattick (1976) found a good correlation between free radical formation as the result of lipid oxidation and damage to the plasmalemma membrane of aged peas. The finding by Mejia (1979) in regard to the oxidation of polyphenolic substances during aging of dry beans also opens new possibilities in connection with membrane permeabilities in aged beans and peas.

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constants. The results showed that for all cultivars moisture and temperature affected the rate constants (k) of deactivation. There was an increase in rate of deactivation with an increase in moisture and temperature levels. The combination of 25% moisture and 45°C gave the highest rate constants.

Boiling treatment of high tannin sorghum was accompanied by a logarithmic reduction of extractable tannin. The plot of ln (a − x) against time for the three sorghum cultivars yielded straight lines (Fig. 2) again suggesting first order kinetics. The regression lines for the three cultivars were drawn and the regression and correlation coefficients calculated. Both regression and correlation coefficients (Table 4) were highly significant. The rate constants were similar and ranged from 0.031–0.035 min^{-1} for the three cultivars.

The result demonstrating that anaerobic storage treatment responds to moisture and temperature changes is of importance for the practical application of this process. Moisture and temperature conditions can be selected to achieve the desired rate of tannin deactivation during anaerobic storage. The results of the boiling treatment showed that most of the tannin was deactivated by 50 min of boiling and the tannin content for the three cultivars was then <1% (Fig. 2).

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Chemical Composition and Functional Properties of Acylated Low Phytate Rapeseed Protein Isolate

LILIAN U. THOMPSON and YOUNG SOOK CHO

ABSTRACT

Protein isolates were prepared by isoelectric precipitation or dialysis of the first and second aqueous extracts of rapeseed flour which was unmodified or acylated by succinic or acetic anhydride. Acylation of the flour lowered the protein and phytic acid contents without significantly altering the amino acid composition of the isoelectrically precipitated protein isolates from the two extracts. The acylated protein isolates from the isoelectrically precipitated extracts had high nitrogen solubility, emulsifying and fat absorption properties. Isoelectrically precipitated and dialyzed acylated proteins did not differ in nitrogen solubility, but dialyzed unmodified protein isolates had nitrogen solubility which was considerably greater than that of isoelectrically precipitated proteins.

INTRODUCTION

HIGH PHYTIC ACID is one of the factors limiting the food applications of rapeseed protein (Jones, 1979). Phytic acid binds by electrostatic attraction to basic or acidic amino acids and/or minerals thus reducing their bioavailabilities (Cheryan, 1980). Acylation, a method of protein modification often used to improve functional properties, can increase the net negative charges of the protein, introduce bulky side groups and change the protein conformation (Kinsella, 1976). Hence it can affect the degree of protein-mineral-phytate interactions. Therefore, with an ultimate goal of preparing a low phytate rapeseed protein isolate of good functional properties, we determined in our earlier studies (Thompson and Cho, 1984; Cho and Thompson, 1984) the extractability and precipitation behaviors of nitrogen, phytic acid and minerals in dehulled, defatted rapeseed flour (RF) which was acylated by various levels of succinic or acetic anhydride. Results showed that high level of protein acylation (63% succinylation or 87% acetylation) provides preferential improvement in extractability of protein over phytic acid in the first aqueous extract of acylated RF and low recovery of phytic acid in the protein after isoelectric precipitation or dialysis. The extractability of phytic acid was greater in the second than in the first extract of acylated RF control. Thus separate processing of the first and second extracts was recommended in our previous studies if an isolated protein of negligible phytic acid content is required. Recovery of the protein in the second extract of acylated RF was also recommended if a further increase of 8-11% in protein yield from the RF is desired.

In order for new protein isolates to be acceptable, they should maintain or enhance the quality of the food in which they are used. They should not only have satisfactory nutritional and toxicological properties but also good functional properties (Kinsella, 1976).

The objective of this study was to evaluate the chemical composition and some functional properties of protein isolates prepared from the first and second aqueous extracts of rapeseed flour which had been acylated with high levels of succinic or acetic anhydride.

Authors Thompson and Cho are affiliated with the Dept. of Nutritional Sciences, Univ. of Toronto, Toronto, Canada M5S 1A8.

MATERIALS & METHODS

DEHULLED, solvent extracted rapeseed flour was obtained from the Food Research Institute, Canada Department of Agriculture, Ottawa. Promine-D, a commercial soy protein isolate, was obtained from Central Soya Corporation (Chicago, IL) and fresh eggs from a local supermarket. Dialysis membrane tubing (Spectrapor®, M.W. cut off: 6,000-8,000, cylinder diam 20.4 mm) was obtained from Spectrum Medical Industries, Inc. (Los Angeles, CA). All chemicals were analytical grade unless otherwise specified. Deionized water was used for protein isolates preparation.

Sample preparation

Defatted RF was dispersed in deionized water (1:20, w/v) and acylated with succinic or acetic anhydride (0.186g or mL, respectively, per g protein) at pH 8.5 for 1 hr at room temperature and centrifuged to yield extract I as described previously (Thompson and Cho, 1984). The unmodified control was treated in the same manner except that no acylating agents were added. The residual RF was reextracted with deionized water (1:20, w/v) at pH 8.5 for 1 hr and recentrifuged to provide extract II. All extracts were adjusted to their isoelectric pHs (pH 4, 3.5 and 3.0 for extract I of unmodified RF, extract I of acylated RF and all extracts II, respectively) (Cho and Thompson, 1984) with 1N HCl, and centrifuged at 1000 x *g* for 25 min. To determine the effect of alcohol treatment, a portion of the precipitate from unmodified or acetylated extract I was washed twice with 50% ethyl alcohol (5 alcohol:1 precipitate, v/v) followed by centrifugation. Both alcohol treated and untreated precipitates, were washed twice with deionized water (5 water:1 precipitate, v/v), redispersed in the same amount of water, adjusted to pH 7 with 1N HCl and freeze-dried. All samples were ground to pass through a 100 mesh and analyzed for chemical composition and functional properties.

Extracts I and II with or without 0.1M sodium ethylene diaminetetraacetic acid (EDTA) were dialyzed in Spectrapor membrane tubings against distilled water at 4°C for five days. Distilled water was changed twice a day. The nitrogen and phytic acid recoveries in the retentates have been reported (Cho and Thompson, 1984). The retentates were freeze-dried and tested for nitrogen solubility in this study.

Chemical analysis

Crude protein, ash and moisture were determined by standard AOAC (1980) procedures, phytic acid by the method of Latta and Eskin (1980) and glucosinolate by the technique of Wetter and Youngs (1976). Calcium and zinc were measured by atomic absorption spectroscopy (Prasad et al., 1966). Amino acids were measured by using a Beckman Model 120 amino acid analyzer after acid hydrolysis of the sample with 6N HCl for 24 hr at 110°C under vacuum. The ninhydrin procedure (Rosen, 1957; Grant, 1963) was employed to estimate the extent to which the free amino groups of the proteins had reacted with acylating agents, with the unmodified protein isolate as control.

Evaluation of functional properties

Nitrogen solubility was determined at pH 7 according to AACC (1969) methods as described by Thompson et al. (1982a). Whipping capacity was calculated as % volume increase of a 50 mL 3% (w/v) sample dispersion or as % volume increase per g protein after whipping with a high speed Sunbeam electric mixer for 6 min (Lawhon and Cater, 1971).

The centrifugation technique (Lin et al., 1974) was followed in the measurement of fat absorption capacities. The freeze-dried protein isolate (0.5g) was mixed with 3.0 mL corn oil with a glass rod in a 15 mL graduated centrifuge tube for 1 min. After a holding period of 30 min, the tube was centrifuged (1000 x *g*, 25 min) and

the volume of free oil was read. Fat absorption was expressed as the amount (mL) of corn oil bound per g sample or protein.

Emulsifying activity index (EAI) was determined according to the turbidimetric technique of Pearce and Kinsella (1978). To prepare emulsions, 1 mL corn oil and 3 mL 0.5% (w/v) aqueous protein isolate dispersion were homogenized in an Omni-mixer with micro attachment (Ivan Sorvall, Newtown, CT) at the lowest speed setting for 1 min at room temperature. Aliquots (1 mL) of the emulsion were diluted with 0.1% (w/v) sodium dodecyl sulfate and the absorbance was determined at 500 nm. EAI denotes the area of interface stabilized per g sample or protein.

Color of the uniformly ground (100 mesh) freeze-dried samples was determined with a spectrophotometer equipped with reflectance accessory. The lightness value (Y_{CIE}) was calculated from the tristimulus values (X,Y,Z) as described by Clydesdale and Francis (1969). Bulk density was determined by weighing the samples (5g) in a cylinder (25 mL) and after tapping the cylinder 10 times, noting the sample volume. It was expressed as g per mL of sample.

All functional properties of RF and Promine D and whipping properties of fresh egg white were determined for comparison with those of the unmodified and acylated protein isolates.

Two batch preparations were made per sample and each preparation was analyzed for chemical composition and functional properties at least in duplicate.

RESULTS & DISCUSSION

THE PROTEIN CONTENT of rapeseed protein isolates (RPI) prepared from extract I of unmodified RF was 85.2% (Table 1). The protein content of the succinylated (S-RPI) and acetylated rapeseed protein isolates (A-RPI) from extract I were lower at 75.3 and 76.5%, respectively, probably due to the succinic or acetic acid residues bound to the proteins and the NaCl produced during neutralization. S-RPI had lower protein content than A-RPI despite its lower degree of modification (Table 1) since the succinic acid residue which was bound to the protein is a larger molecule than the acetic acid residue in A-RPI. In addition, because of the large number of negative charges present in S-RPI, there was a greater NaOH requirement for neutralization and hence greater NaCl production in S-RPI.

Lower phytic acid contents in S-RPI and A-RPI than RPI from extract I were expected since the extractability of phytic acid from RF was decreased by acylation (Thompson and Cho, 1984). A-RPI showed a slightly lower phytic acid content than S-RPI, probably due to a lower phytic acid extractability at the high level of RF acetylation.

The RPI, S-RPI and A-RPI from extract II showed protein contents about 17% less than the respective protein isolates from extract I. However, their ash contents were 1 to 2 times and the phytic acid contents 3 to 14 times greater than those from respective protein isolates from extract I (Table 1). These compositional trends are not surprising based on the extractability and precipitation patterns of minerals and phytic acid in acylated rapeseed protein (Thompson and Cho, 1984). However, for all protein isolates from extract II, the ratio of protein to phytic acid concentration (10.9, 20.5 and 21.5 for RPI, S-RPI and A-RPI, respectively) was greater than what might be expected from the extractability precipitation data (10.6, 10.1 and 12.9 for RPI, S-RPI and A-RPI, respectively) (Cho and Thompson, 1984) suggesting further losses of phytic acid during the washing steps prior to freeze drying of the precipitate. Greater losses of phytic acid occurred in the acylated than the unmodified samples.

No protein isolates have detectable glucosinolates. Glucosinolates are undesirable in rapeseed products since in the presence of myrosinase, they are hydrolyzed to compounds such as isothiocyanates, oxazolidinethione and nitriles which can cause enlarged thyroid and reduced weight gain (Fenwick, 1982). Except A-RPI from extract II, all the other protein isolates showed no detectable calcium. On the contrary, all the protein isolates contained zinc which was the highest in A-RPI and S-RPI from extract II.

Except for a small, insignificant reduction in lysine and isoleucine content by succinylation, there was no change in the essential amino acid composition of RPI upon acylation (Table 1). Similar observations have been reported on succinylated soy protein (Franzen and Kinsella, 1976a), sunflower (Kabirullah and Wills, 1982), and whey protein concentrates (Thompson and Reyes, 1980), although others reported no losses in amino acids (Franzen and Kinsella, 1976b). However, the protein quality of acylated protein isolates can not be predicted by the essential amino acid pattern. Some of the amino acids, especially lysine, are partly bound by acylating agents and may be partially unavailable (Groninger and Miller, 1979; Bjarnason and Carpenter, 1969; Siu and Thompson, 1982a, b). Therefore, S-RPI and A-RPI may have slightly lower nutritional quality than RPI, despite the similar amino acid patterns shown in Table 1. Contrary to this is the work of Johnson and Brekke (1983) which showed no impairment in in vitro digestibility of pea protein isolates upon acylation of lysine up to 95%. S-RPI and A-RPI from extract I had 63 and 87%, respectively, of the amino groups acylated; extract II had 20 and 55%, respectively (Table 1).

Some functional properties of RPI, S-RPI and A-RPI

Table 1—Chemical composition[a] of unmodified (RPI), succinylated (S-RPI,, and acetylated (A-RPI) rapeseed protein isolates prepared from respsective extracts I or II by isoelectric precipitation

	RPI		S-RPI		A-RPI	
	I	II	I	II	I	II
Protein (Nx6.25), %	85.2	68.9	75.3	57.5	76.5	60.7
Ash, %	4.2	7.2	4.4	6.1	4.0	5.5
Phytic acid, %	2.2	6.3	0.3	2.8	0.2	2.8
Glucosinolates, mg/g sample	b	b	b	b	b	b
Calcium, ppm	b	b	b	b	b	20
Zinc, ppm	7.7	4.2	8.0	14.0	6.8	14.6
Essential amino acids[c] (g/100g protein)						
Lys	5.1	6.0	4.8	6.0	5.1	5.9
Thr	5.3	5.5	5.8	6.6	5.8	5.5
Cys + Met	2.8	3.4	3.0	3.1	3.2	2.6
Phe + Tyr	8.7	8.7	9.0	9.1	8.2	9.4
Val	4.7	4.9	5.5	5.3	5.9	5.8
Ile	4.4	4.6	3.7	3.5	4.8	4.6
Leu	8.7	8.5	8.7	8.6	8.7	8.6
Chemical modification, %	0	0	63	20	87	55

[a] Dry basis
[b] Not detectable
[c] Tryptophan not analyzed

prepared from respective extracts I and II of RF are presented in Table 2. Acylation of the RF greatly increased the nitrogen solubility of protein isolates. S-RPI from extract I hydrated instantaneously and remained dispersed in solution. A-RPI from extract I was also highly water soluble but a little less than S-RPI. Increased solubility is often observed in acylated proteins (Franzen and Kinsela, 1976b; Eisele and Brekke, 1981). Nitrogen solubility of RPI (9.9%) from extract I was poor which might be due to extensive aggregation of protein during isoelectric precipitation or denaturation by acid. Nakai et al. (1980) also reported low dispersibility of isoelectrically precipitated rapeseed protein due to inadequate resuspension. Phytic acid might also play a role in lowering the nitrogen solubility (Smith and Rackis, 1957). Phytic acid content in RPI from extract I (2.2%) was 8 to 10 times greater than the acylated counterpart (Table 1). Gillberg and Tornell (1976) clearly demonstrated the ability of phytic acid to decrease the protein solubility and even added sodium phytate to increase protein precipitation yield.

RPI from extract II had better (27.4%) nitrogen solubility than RPI from extract I even though it had more phytic acid (9.9%), probably due to differences in their protein composition. Rapeseed has a complicated protein composition and contains proteins with widely different isoelectric pH and molecular weights (Lonnerdal and Jansen, 1972, 1973; Lonnerdal et al., 1977). The proteins in extract I appear to differ from that in extract II since they exhibit different isoelectric pH's (Cho and Thompson, 1984). Greater nitrogen solubility of A-RPI from extract II than S-RPI might be due to higher protein modification in the case of the former (Table 1). All the acylated protein isolates exhibited better nitrogen solubility than RF or Promine D. Greater nitrogen solubility of acylated proteins was due to increased protein-water interaction as the net negative charges increased upon acylation (Kinsella, 1976).

Emulsifying activity index of protein isolates from extract I was improved with acylation to levels better than that of Promine-D (Table 2). The increase however was not linearly related to the increase in nitrogen solubility. This could be explained by the fact that emulsifying property is dependent not only on solubility but also on hydrophile-lipophile balance (HLB) of the particular protein (Nakai, 1983). If the HLB of the protein is close to the optimal HLB for the oil, then emulsion capacity and stability of the protein will be high. Acylation of the proteins which increases net negative charges (Kinsella, 1976) might be expected to unfold the protein structure and expose more hydrophobic groups which then could change the HLB towards a more favorable level. The EAI of RPI and A-RPI from extract II were greater than those from extract I probably due to the previously mentioned differences in protein composition and HLB between the extract I and II protein isolates.

S-RPI and A-RPI from extracts I and II had poorer whipping properties than the RPI, RF and egg white (Table 2). A good foam-forming protein should reduce the surface and interfacial tension of the liquid and form structural continuous, cohesive films around air vacuoles (Kinsella, 1976). This property is dependent on the viscosity, hydrophobicity, solubility and net charge density of the proteins (Nakai, 1983). While increased viscosity, hydrophobicity and solubility favor foaming, increased net charge density tends to decrease foam stability since it prevents protein-protein interactions required in a continuous film around air bubbles. There is, therefore, a balance that should be maintained among these factors to obtain a good stable foam. The S-RPI and A-RPI had lower whipping properties than RPI probably because of the excessive net negative charges associated with their high degree of acylation. Thompson and Reyes (1980) also observed poor whippability in succinylated cheese whey protein concentrate. High whipping capacity of RPI from extract II was comparable to that of egg white but their foam characteristics were different. RPI from extract II had a soft, flowing, cream colored foam with medium-size air bubbles while egg white had the more desirable hard, brittle white foam with small-size air bubbles.

Acylation of the RF resulted in a slight but insignificant decrease in the bulk density of RPI from extract I. Difference in bulk density cannot be attributed to particle size and shape as well as protein concentration during drying as all were ground to uniform size and protein isolate concentration was standardized during freeze drying. Any decrease in the bulk density arising from acylation could probably be due to unfolding of the interfolded polypeptides as acylation replaces the short range attractive forces in the native protein with short range repulsive or neutral ones of two to four carbon fragments (Gounaris and Perlman, 1967; Habeeb, 1967). Such unfolding could result in loosening of the texture (Kinsella, 1976). All the bulk densities, however, were greater than those of RF. All protein isolates from extracts II, in general, had lower bulk densities than those from extract I. Since protein isolates from extract II have a lower degree of acylation, they differed in bulk densities from those from extract I probably due to large differences in their protein content and composition (Table 1).

The fat absorption capacity of RPI from extract I expressed as ml/g sample did not change with succinylation

Table 2—Functional properties of unmodified (RPI), succinylated (S-RPI) and acetylated (A-RPI) rapeseed protein isolates prepared from respective extracts I or II by isoelectric precipitation

		Nitrogen solubility	Emulsifying activity index[a]		Whipping capacity		Bulk density	Fat absorption		Color
		%	m^2/g sample	m^2/g protein	A[b]	B[c]	g/ml	mL/g sample	mL/g protein	$Y_{C\ E}$
RPI	I	9.9	110	130	378	299	0.29	3.0	3.6	41.9
	II	27.4	137	230	600	593	0.19	3.6	5.3	36.0
S-RPI	I	99.5	132	177	145	130	0.24	3.0	4.0	30.4
	II	70.7	127	227	115	137	0.23	3.0	5.4	29.6
A-RPI	I	91.4	136	179	132	116	0.28	3.4	4.5	33.3
	II	89.1	143	246	155	178	0.21	3.4	5.9	38.2
RF		54.6	117	257	356	521	0.17	3.8	8.4	65.4
Promine-D		64.4	119	131	138	101	0.36	2.1	2.3	69.1
Egg white		–	–	–	661[d]	–	–	–	–	–

[a] Denotes area of interface stabilized per unit weight sample or protein (Pearce and Kinsella, 1978).
[b] % volume increase of 50 ml 3% sample dispersion
[c] % volume increase per g protein
[d] % volume increase of 50 ml fresh egg white

but slightly increased with acetylation. This may be related to their differences in degree of acylation. Choi et al. (1983) observed a big change in fat absorption of cottonseed only at acylation levels greater than 60%. Expressed as ml/g protein, however, the fat absorption increased with both succinylation and acetylation. The increase in fat absorption may be attributed to greater exposure of hydrophobic groups as the proteins unfold during acylation (Kinsella, 1976). Also, it can partly be due to differences in bulk density since fat absorption (second column values in Table 2) and bulk density correlated significantly ($r = 0.79$, $p < 0.05$ or $r = -0.83$, $p < 0.01$ when the value for RF is included) as observed by Wang and Kinsella (1976). Fat absorptions of all protein isolates were better than that of Promine D but less than that of RF.

All the protein isolates had greenish brown color and were darker than the cream colored RF or Promine-D (Table 2). Unlike other studies (Franzen and Kinsella, 1976a, b), the acylated protein isolates were darker than the unmodified RPI. This might be attributed to high extraction of polyphenolic components along with the protein upon acylation of RF. Thompson et al. (1982c) also reported that the higher the amount of nitrogen recovered, the higher was the amount of pigments which coprecipitated with the proteins and contributed to the darkness of the product. For both extracts, the S-RPI was slightly darker than the A-RPI.

In the previous study (Cho and Thompson, 1984), dialysis technique of preparing protein isolates was tried to improve the nitrogen retention and remove the residual acylating agent and nonproteinacious constituents of the extracts. Extract II with EDTA was dialyzed against distilled water to further reduce its high phytic acid content. In this study, the nitrogen solubility of S-RPI from extract I and A-RPI from extracts I and II with or without EDTA prepared by dialysis method was determined and was found to be high (84~97%) and not significantly different from the corresponding protein isolates prepared by isoelectric precipitation (Table 2). The solubility of S-RPI from extract II was improved from 71 to 89% by dialysis method. The dialysis technique also improved the nitrogen solubility of RPI from extract I from 10 to 64% and that from extract II from 27 to 45%. These results implied that isoelectric precipitation was the main factor hindering solubilization of RPI and that poor nitrogen solubility by isoelectric precipitation can be overcome by acylation.

Phenolic constituents in rapeseed which are responsible for the green and brown colors of alkali-treated rapeseed products (Kozlowska et al., 1975) can bind with protein, reversibly by hydrogen bonding and irreversibly by oxidation followed by covalent condensation (Loomis and Battaile, 1966). Therefore, washing with ethanol, a hydrogen bond breaking agent, was tried to disrupt the interactions between proteins and polyphenols. Alcohol treatment did not significantly change the chemical composition and functional properties of RPI and A-RPI. However, it resulted in a considerably lighter color in the A-RPI, ($Y_{CIE} = 43.9$) and RPI ($Y_{CIE} = 45.6$) although still darker than the color of RF and Promine-D (Table 2). Alcohol treatment in conjunction with sulfite treatment has been suggested for better color lightening of the rapeseed products (Keshavarz et al., 1977; Thompson et al., 1982c).

In conclusion, acylation of the RF followed by aqueous extraction and isoelectric precipitation provided protein isolates with negligible phytic acid content and unaltered essential amino acid composition. The protein has high solubility, emulsifying and fat absorption properties and may be useful in products such as beverages, wieners, meat patties and dairy analogues. A second aqueous extract of the residual acylated RF provided protein isolates with phytic acid which was greater than that of the first extract but still lower than that prepared from the second extract of unmodified RF. It is worthwhile recovering also this protein as it may find food applications similar to that of the first extract protein isolate; it has functional properties which are either comparable to or better than that of the first extract. The color of all acylated products are dark, but could be improved by alcohol or preferably combined alcohol and sulfite treatment.

The application of acylated proteins in food systems has been successfully tried (Creamer et al., 1971; Melnychyn and Stapley, 1973; Thompson and Reniers, 1982; Thompson et al., 1982b, 1983). However, acylated proteins have not been commercialized due to limited data on their safety and toxicity. The only toxicity study done (Creamer et al., 1971) showed that mice fed acetyl casein were lighter in weight and had smaller litters than mice fed casein. However, no histological changes in the organ were observed in mice fed acetyl casein for three generations. Succinyl amino acids appear to be absorbed (Siu and Thompson, 1982b) but their metabolic fates are not clear. Obviously, more biological testing is needed before acylated proteins such as those produced in this study can be widely used.

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Inhibition of *Clostridium botulinum* Growth from Spore Inocula in Media Containing Sodium Acid Pyrophosphate and Potassium Sorbate with or without added Sodium Chloride

M. K. WAGNER and F. F. BUSTA

ABSTRACT

The effects of sodium acid pyrophosphate (SAPP), sodium chloride (NaCl) and/or potassium sorbate (PS) on the growth from heat-activated spores of three individual strains or a mixture of ten strains of *Clostridium botulinum* in peptone-yeast extract-glucose broth at pH 5.55 or 5.85 were measured spectrophotometrically at A_{630nm}. Growth ratios (GR = treatment/control) based on time to reach A_{630} = 0.35 or 0.04 were calculated and used to compare effects of additives on strains. SAPP, NaCl, PS, and pH exhibited independent significant main effects ($p \leq 0.01$) on delaying growth in most *C. botulinum* strains tested. Combinations of additives without NaCl consistently caused an increase in the GR and an increase in organism sensitivity to additives in the medium. Treatments containing SAPP (0.2 or 0.4%) and PS (0.13 or 0.26%) were more effective for delaying growth than other formulations tested.

INTRODUCTION

NITRITE has been used for decades in cured meat products for preservation by prevention of toxin formation by *Clostridium botulinum* (Pivnick et al., 1970). In recent years, however, nitrite has been implicated as a precursor of carcinogenic nitrosamines (Gray and Randall, 1979). Consequently, work continues toward a means of decreasing or eliminating the use of nitrite by finding a chemical alternative or by altering the process. The need for such investigations is detailed in a recent publication by the National Academy of Sciences (NAS, 1982).

Examples of chemical antibotulinal agents which are promising as nitrite replacements include potassium sorbate (PS) and sodium acid pyrophosphate (SAPP). Potassium sorbate was effective in combination with low levels of nitrite in delaying botulinal toxin production in temperature-abused bacon (Sofos et al., 1980), whereas SAPP was effective in delaying botulinal toxin production in chicken (Nelson et al., 1983) and beef/pork (Wagner and Busta, 1983) frankfurter emulsions.

Another antibotulinal alternative approach to nitrite involves addition of greater amounts of sodium chloride (NaCl) to the product. However, reductions of NaCl have become the trend in cured meat products (Terrell, 1983), thus complicating the nitrite dilemma. Recent work on reduction of NaCl in cured meat products has been directed primarily to water binding capacity (Puolanne and Terrell, 1983b), as well as the physical, chemical and sensory properties in reduced NaCl products such as frankfurters (Puolanne and Terrell, 1983a). Information is needed on the interactions of various nitrite alternatives such as PS and SAPP in the presence of reduced NaCl levels and the effects of such combinations on *C. botulinum* growth and toxin production.

This research was conducted at the Dept. of Food Science & Nutrition, Univ. of Minnesota, 1334 Eckles Ave., St. Paul, MN, 55108. Author Wagner is now affiliated with the Dept. of Food Science, Univ. of Wisconsin, 1605 Linden Drive, Madison, WI, 53706. Author Busta is now affiliated with the Dept of Food Science & Human Nutrition, Univ. of Florida, Gainesville, FL, 32611.

The objective of this research was to observe the effects of PS and SAPP at various concentrations alone and in combination with NaCl on the growth from spores of different strains of *C. botulinum* in media systems.

MATERIALS & METHODS

Spore inoculum

The *C. botulinum* inoculum used was either one of three strains (Lamanna B, 52A, 62A) or a mixture of equal proportions of ten strains [five type A (36A, 52A, 62A, 77A 12885A) and five type B (ATCC 7949, 41B, 53B, 213B, Lamanna B)]. The original spore suspensions were provided by Swift and Co. Research Center (Oak Brook, IL). Sporulation of the strains and preparation of spore crops were performed according to the methods described by Chistiansen et al. (1973). Harvested spores were stored in distilled water at 4°C until used. Individual strains were enumerated using Lee tubes (Ogg et al., 1979), and inocula were adjusted to yield ca. 10^3-10^4 spores/mL of inoculated medium. The inoculum volume of spore suspensions was 0.1 mL/5 ml of medium.

Medium preparation

Peptone-yeast extract broth was prepared according to Holdeman et al. (1977) and modified following the procedure by Blocher et al. (1982). The modified medium, designated peptone-yeast extract-glucose (PYEG) broth, was used in all experiments. The medium for Lee tubes was PYEG + 15g agar/L. Various combinations of SAPP (0, 0.2, 0.4%), NaCl (0, 1.25, 2.50%) (Spectrum Chem. Mfg. Corp., Redondo Beach, CA) and/or PS (0, 0.13, 0.26%) were added to appropriate liquid media treatments prior to pH adjustment and before filter sterilization. The SAPP and PS were provided by Monsanto Co. (St. Louis, MO).

The pH of all treatments was adjusted to either 5.55 or 5.85 by the addition of 1N or 6N/HCl or NaOH and measured with a glass combination electrode and an Orion 620 meter. Each pH adjusted treatment was filter sterilized using 0.45 μm cellulose nitrate membrane filters (Micro Filtration Systems, Dublin, CA) and dispensed into 12.7 mm glass colorimeter tubes (5 mL/tube). The pH of each treatment after filter sterilization was within ca. ± 0.1 pH units of the initial pH adjustment.

Test procedure

Sterile PYEG in treatment tubes was inoculated with the appropriate spore suspension and heat activated at 80 ± 2°C for 15 min, overlaid with sterile molten vaspar and incubated at 37°C.

Absorbance at 630 nm (A_{630}) was measured with a Bausch and Lomb Spectronic 20 spectrophotometer at approximately 4-hr intervals. The arithmetic mean A_{630} for each treatment (10 tubes/treatment) was calculated for each measurement using the respective uninoculated treatment tube to blank the spectrophotometer.

Data analysis

Time to reach an A_{630} of 0.35 or 0.04 was estimated by linear interpolation between the last average $A_{630} < 0.35$ or 0.04 and the first average $A_{630} > 0.35$ or 0.04 for each treatment. An A_{630} of 0.35 was chosen for most spore suspensions because it required at least four of the ten replicate tubes per treatment to reach simultaneous substantial growth. An A_{630} of 0.04 was chosen using similar criteria mentioned above for other spore suspensions which consistently reached maximum $A_{630} < 0.35$, yet displayed a readable average A_{630} of ca. 0.04. The $A_{630} = 0.35$ and the $A_{630} = 0.04$ were calculated for the ten-strain mixture using the same original data.

Growth ratios (GR) were calculated for each treatment (GR = Hr to A_{630} of 0.35 or 0.04 for treatment/Hr to A_{630} of 0.35 or 0.04 for control). The larger the GR, the more inhibition had occurred. Controls were represented by additive-free treatments at the same pH as the test treatments.

Statistical analysis

The study was designed as two replicates of a split plot experiment where two levels of pH were randomized to laboratory days (the whole plots) and a 3x3x3 factorial was randomized in three sets of nine combinations for day by confounding two degrees of freedom for a three-way interaction with days. All 27 combinations of each additive, SAPP, NaCl, and PS at three different levels were tested under each pH (5.55 and 5.85). On any given day a constant pH was used with one-third the replicate (9 combinations) of the additives. Data were analyzed using IVAN at the University of Minnesota (Weisberg and Koehler, 1979). Statistical differences among means were tested using f-values at the 5 and 1% levels (Snedecor and Cochran, 1980).

RESULTS & DISCUSSION

Effect of pH on C. botulinum growth

Significance of pH was observed in *C. botulinum* strains Lamanna B ($p \leq 0.05$), 52A ($p \leq 0.01$) and 62A ($p \leq 0.01$) (Table 1). Results from this statistical analysis indicate variation in strain sensitivity to hydrogen ion concentration (as measured by pH). Fig. 1a displays the differences among *C. botulinum* strains in pH sensitivities based on GR using average A_{630} = 0.35, whereas Fig. 1b displays the same pH comparisons based on GR from an average A_{630} = 0.04. In both Fig. 1a and b, the individual strain responses to pH 5.55 and to 5.85 can be seen for 52A, Lamanna B and 62A. Blocher et al. (1982) previously showed that spores of all ten strains used in the composite inoculum exhibited delayed growth at pH 5.65 based on predicted GR data. An increase in GR, at a lower pH of 5.55, also is indicated by most results presented in Fig. 1. The data on the composite inoculum indicated that not all ten strains exhibit delayed growth when used together. This is shown by the nonsignificant effect of pH on the ten-strain mixtures at both GR = 0.35 and GR = 0.04 (Table 1). Blocher et al. (1982) also showed various responses depending on strain or spore suspension. Other factors such as medium, growth temperature and inoculum size have also been shown to influence the variability of growth of *C. botulinum* between the pH range 4.0 - 6.0 (Ito and Chen, 1978).

Effect of SAPP, NaCl and PS on C. botulinum growth

All three major additives, SAPP, NaCl and PS, exhibited independent significant main effects ($p \leq 0.01$) on the overall design of the experiment for most strains tested (Table 1). Only PS displayed a significant influence on growth by strain 52A ($p \leq 0.01$). Fig. 2a and b show the variations in GR among levels of additives employed for ten-strain mixture (A_{630} = 0.35), Lamanna B and 52A; as well as for the ten strain mixture (A_{630} = 0.04) and 62A, respectively.

Effect of SAPP. Increasing levels of SAPP (0, 0.2, and 0.4%) caused subsequent increases in GR values for all strains tested (Fig. 2a and b). For example for strain 52A, in treatments containing 0, 0.2 and 0.4% SAPP the GR's were 1.3, 2.2 and 4.0, respectively. These results are consistent with earlier work showing SAPP to be effective in delaying *C. botulinum* spoilage and toxin production in pork slurries (Jarvis et al., 1979), chicken emulsions (Nelson et al., 1983), and beef/pork emulsions (Wagner and Busta, 1983). The effect of phosphate buffer on inhibition of *C. botulinum* by antioxidants and related phenolic compounds was similar to that of SAPP (Reddy and Pierson, 1982). The increase in GR indicating increased sensitivity to additives in media containing SAPP may have been due to the polyphosphates causing cell deformation during outgrowth as reported by Seward et al. (1982) using *C. botulinum* type E in microculture; however, inhibition of growth in the present study was directly related to additive concentration. Seward et al. (1982) speculated that the similarities in

Table 1—Analysis of variance for growth of Clostridium botulinum *spores in Peptone-Yeast Extract-Glucose broth containing various levels of sodium acid pyrophosphate, NaCl and/or potassium sorbate*

		A_{630} = 0.35						A_{630} = 0.04			
		Ten-strain Mixture		Lamanna B		52A		Ten-strain Mixture		62A	
Source	df	MS	F	MS	F	MS	F	MS	F	MS	F
Replicates (R)	1	15.270	3.24NS	0.952	1.14NS	5.118	2.37NS	0.152	0.09NS	16.357	1.19NS
pH (H)	1	19.978	4.24NS	5.787	6.95*	78.319	36.28**	1.510	0.84NS	173.150	12.60**
R x H	1	17.448	3.70NS	1.799	2.16NS	7.369	3.41NS	11.702	6.52*	4.364	0.32NS
+Error—1	8	4.7143		0.8332		2.1588		1.7956		13.7436	
Sodium acid pyrophosphate (S)	2	14.733	6.34**	4.795	5.52**	6.361	1.24NS	8.919	49.38**	25.329	7.31**
NaCl (N)	2	12.278	5.28**	5.932	6.83**	11.790	2.29NS	2.654	14.70**	25.875	8.05**
Potassium sorbate (P)	2	13.862	5.97**	18.171	20.93**	78.728	15.31**	9.326	51.64**	93.760	27.07**
HS	2	3.943	1.70NS	1.156	1.33NS	1.983	0.39NS	0.020	0.11NS	8.100	2.34NS
HN	2	14.362	6.18**	2.397	2.76NS	5.356	1.04NS	1.913	10.59**	14.791	4.27*
HP	2	8.032	3.46*	5.522	6.36**	48.865	9.50**	1.053	5.83**	41.588	12.01**
SN	4	2.723	1.17NS	0.847	0.98NS	7.496	1.46NS	0.359	1.99NS	2.976	0.86NS
SP	4	1.562	0.67NS	1.135	1.31NS	2.755	0.54NS	0.232	1.28NS	1.183	0.34NS
NP	4	4.723	2.03NS	2.620	3.02*	3.266	0.64NS	1.281	7.09**	3.661	1.06NS
HSN	4	3.229	1.39NS	0.843	0.97NS	4.727	0.92NS	0.175	0.97NS	1.525	0.44NS
HSP	4	1.814	0.78NS	1.124	1.30NS	3.245	0.63NS	0.302	1.67NS	1.176	0.34NS
HNP	4	3.918	1.69NS	1.234	1.42NS	2.193	0.43NS	0.302	1.67NS	3.841	1.11NS
Error—2	60	2.324		0.868		5.142		0.181		3.464	

NS = Not significant
* = Significant at $p \leq 0.05$
** = Significant at $p \leq 0.01$

+Error—1 is for days within pH levels and is confounded with 2 df in the SxNxP interaction.

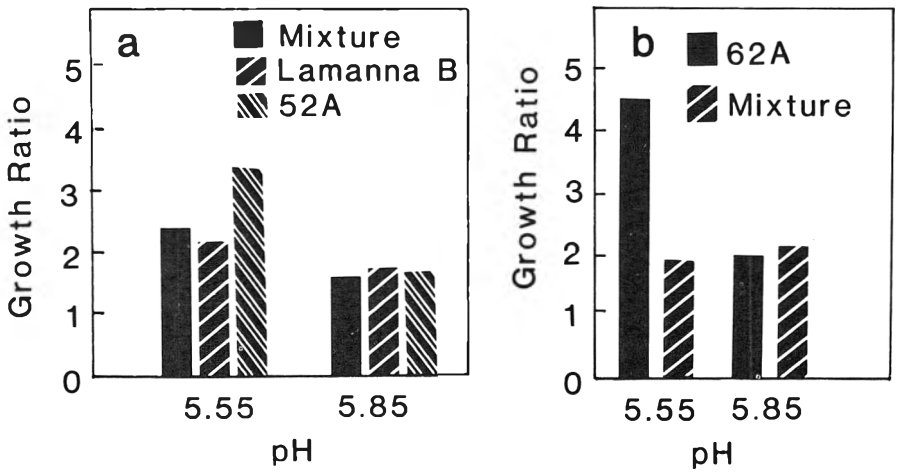

Fig. 1—Effect of pH 5.55 and 5.85 on growth of Clostridium botulinum: (a) ten-strain mixture, Lamanna B and 52A (A_{630} = 0.35) and (b) ten-strain mixture and 62A (A_{630} = 0.04).

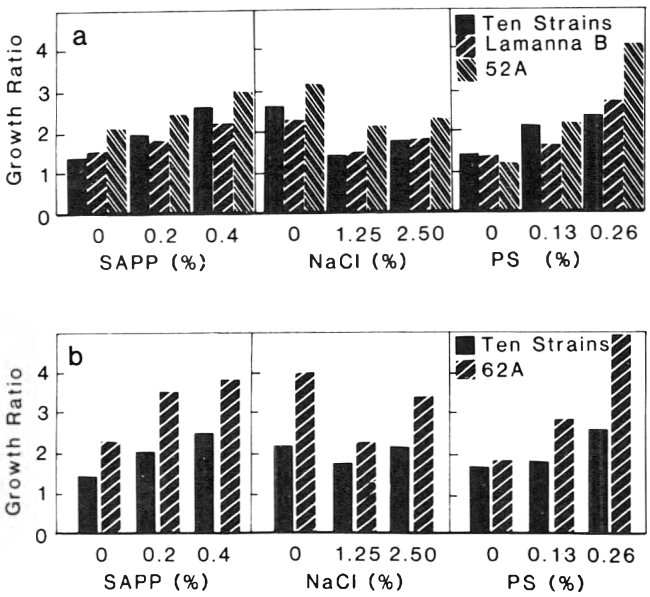

Fig. 2—Effect of SAPP, NaCl or PS on growth of Clostridium botulinum: (a) ten-strain mixture, Lamanna B and 52A (A_{630} = 0.35) and (b) ten-strain mixture and 62A (A_{630} = 0.04).

morphological cell deformations which occurred in the presence of either PS or hypophosphite might suggest similar mechanisms of action for polyphosphates and PS on growing vegetative cells.

Phosphates perform three basic chemical functions in cured meat systems: control pH by buffering; sequester metal ions; and act as polyanions to increase ionic strengths of solutions (Steinhauer, 1983). The latter two functions were of concern in this study. Short chain polyphosphates (especially pyrophosphates such as SAPP) are best for sequestering heavy metal ions such as iron and copper. The increase in GR observed with increase of SAPP may indicate ions essential for cell development were made unavailable. The influence of SAPP on ionic strength was considered inconsequential because the amount of SAPP utilized in the experiment contributed only minimally to overall ionic strength (Table 2). Experiments utilizing the addition of essential cations to solutions containing SAPP in the model systems used in these experiments have yet to be conducted to validate these claims.

Effect of NaCl. Increasing the levels of NaCl (0, 1.25 and 2.50%) did not appear to cause responses in GR values similar to those with SAPP (Fig. 2a and b). Inhibition of *C. botulinum* growth was more prevalent with treatments

Table 2—Ionic strengths and percentages of additives in PYEG broth

Additive	Ionic strength[a]	Percentage
SAPP	0.08	0.2
	0.16	0.4
NaCl	0.21	1.25
	0.43	2.50
PS	0.01	0.13
	0.02	0.26

[a] Based on 100% moisture content, $Y = \Sigma \frac{1}{2} M z^2$, where Y = ionic strength, M = molarity, and z = charge of ion.

containing 0% NaCl compared to higher levels (1.25 and 2.50%). Pivnick and Thacker (1970) also observed that *C. botulinum* spores grew to a greater extent in media containing NaCl than in media without NaCl.

Treatments containing 1.25% NaCl enhanced *C. botulinum* growth more than either 0% or 2.50% NaCl. These results suggest that *C. botulinum* spore germination may be restricted at higher NaCl concentrations, as was previously shown by Ando (1974). Media without added NaCl may not have sufficient ions for successful spore germination. NaCl was the largest contributor to ionic strength in the treatments employed in this experiment (Table 2). The decrease in GR values in treatments containing 1.25% NaCl may be due to the presence of sufficient ions for spore germination and outgrowth. Further explanations for NaCl tolerance by certain strains of *C. botulinum* has been suggested by Kiss et al. (1978) who speculated a relationship between salt tolerance and DNA reapir capacity, and by Tesone et al. (1981) who observed that NaCl in the growth medium raised the upper limit of temperature for growth of *C. perfringens.*

Effect of PS. Increased levels of PS (0, 0.13 and 0.26%) resulted in increased GR values for all strains tested (Fig. 2a and b). The responses were similar to those observed for SAPP.

PS has been reported to inhibit germination (Smoot and Pierson, 1981). Sorbate inhibition of growth was dependent on strain of *C. botulinum* spores and different spore suspensions of the same strain (Blocher et al., 1982). This difference in strains or spore suspensions was observed in Fig. 2a and b.

Interactions of pH and NaCl effects on C. botulinum growth

The interactions of pH and NaCl were significant for the ten-strain mixture (A_{630} = 0.04) ($p \leq 0.01$) and for strain 62A ($p \leq 0.05$) (Table 1). Comparison of data showing the influence of pH 5.55 and 5.85 on *C. botulinum* growth for

the ten-strain mixture (A_{630} = 0.35) is made in Fig. 3a. Results for the ten-strain mixture (A_{630} = 0.04) and 62A are shown in Fig. 3b.

In general, the ten-strain mixture (A_{630} = 0.35) and strain 62A (A_{630} = 0.04) show a larger GR and an increased sensitivity to additives in the media at pH 5.55 compared to pH 5.85. Pivnick and Thacker (1970) reported that certain salt (NaCl) levels (1-4%) were more inhibitory toward *C. botulinum* in medium at pH 6.0 rather than at pH 7.0. Emodi and Lechowich (1969) also showed that the tolerance of *C. botulinum* to salt is lower at pH 6.5 than 7.2.

Results from the ten-strain mixture (A_{630} = 0.35) (Fig. 3a) show a difference in GR for spores in 0% NaCl treatments at pH 5.55 (GR = 3.7) and at pH 5.85 (GR = 1.4), yet no difference at other NaCl levels (Fig. 3a), suggesting inhibition due to pH and not due to a NaCl/pH interaction. Similar results were obtained for strain 62A at pH 5.55 (Fig. 3b). The only NaCl/pH interaction resulting in inhibition occurred for 62A at pH 5.55 in the presence of 2.50% NaCl. The 1.25% NaCl-containing treatments enhanced growth at pH 5.55. This enhancement of growth may be due to the effect of ions on spore germination and outgrowth.

Lynt et al. (1982) reported that proteolytic strains of *C. botulinum* were more tolerant to high salt concentration than nonproteolytic strains; however, results from this study showed variation even among different proteolytic strains (Fig. 3a and b).

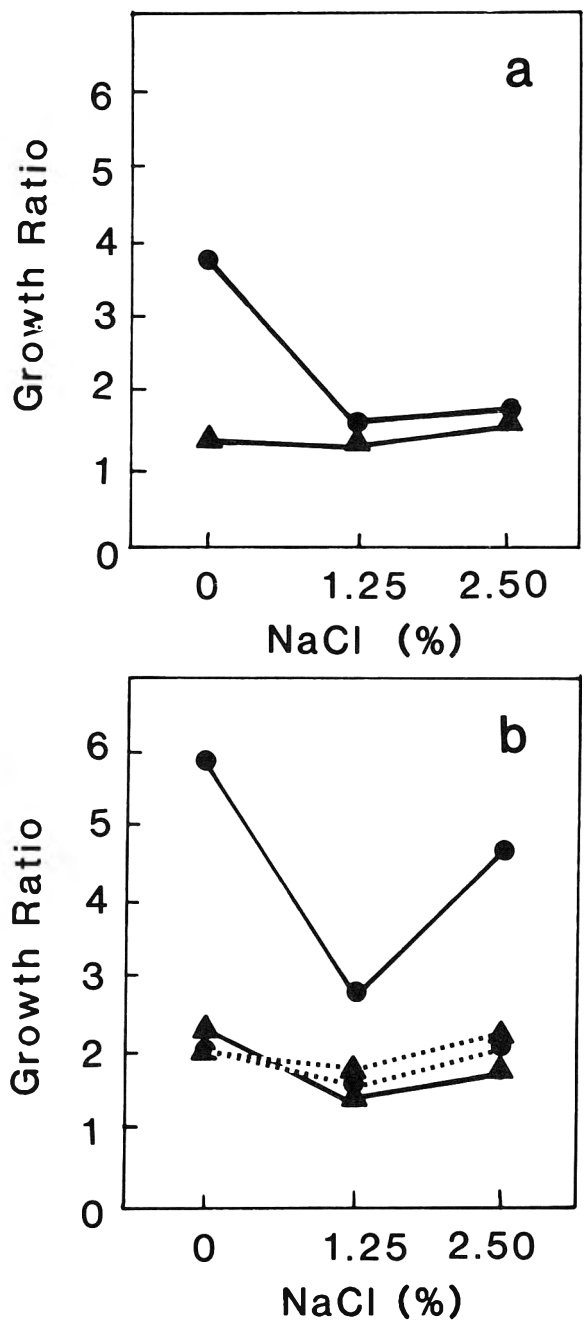

Fig. 3—Influence of pH and/or NaCl on growth of Clostridium botulinum: (a) ten-strain mixture (A_{630} = 0.35) at pH 5.55 (●) and 5.85 (▲) and (b) ten-strain mixture (▲) and 62A (●) (A_{630} = 0.04) at pH 5.55 (—) and pH 5.85 (- - -).

Interactions of pH and PS effects on C. botulinum growth

Interactions of pH and PS were significant ($p \leq 0.01$) for most strains tested (Table 1). The exception was the ten-strain mixture (A_{630} = 0.35) which showed significance at the $p \leq 0.05$ level. The influence of pH and PS effects on growth can be seen for the *C. botulinum* ten-strain mixture (A_{630} = 0.35), Lamanna B and 52A at pH 5.55 (Fig. 4a) and at pH 5.85 (Fig. 4b). The same effects of pH and PS interaction for *C. botulinum* ten-strain mixture (A_{630} = 0.04) and 62A are shown in Fig. 5.

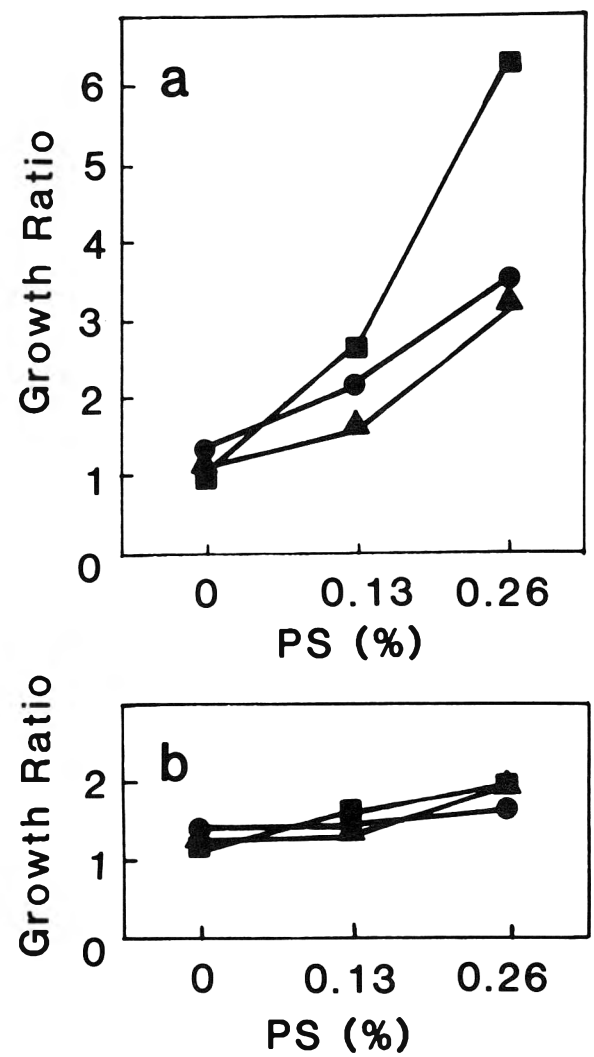

Fig. 4—Influence of pH and/or PS on growth of Clostridium botulinum: ten-strain mixture (●), Lamanna B (▲) and single strain 52A (■) (A_{630} = 0.35) at a pH of: (a) 5.55 and (b) 5.85.

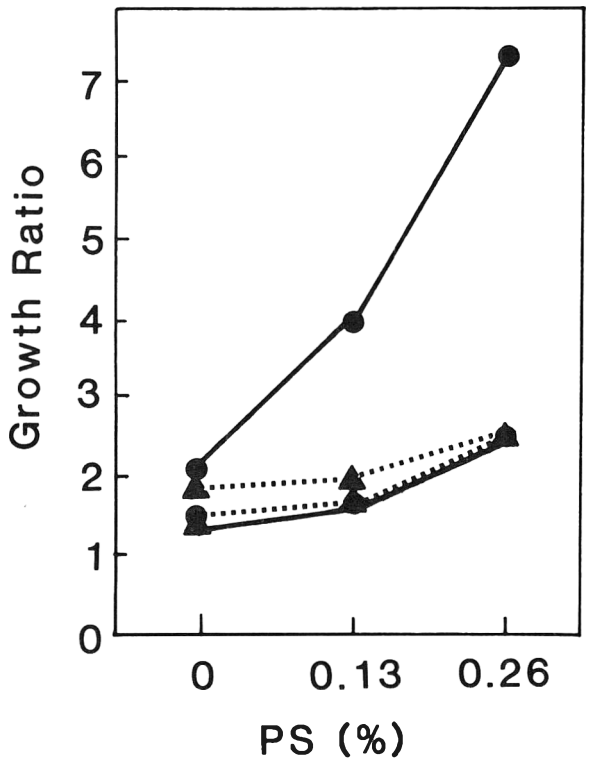

Fig. 5—Influence of pH and/or PS on growth of Clostridium botulinum: ten-strain mixture (▲) and single strain 62A (●) (A_{630} = 0.04) at pH 5.55 (—) and pH 5.85 (- - -).

Smoot and Pierson (1981) previously reported that PS was a strong inhibitor of germination at pH 5.7 with reduced effectiveness as an inhibitor at a higher pH of 6.2 or 6.7. Blocher et al. (1982) also reported *C. botulinum* sensitivities to reduced pH in combination with PS. Results from Fig. 4a, b, and Fig. 5 are consistent with both observations.

Again, variation in sensitivity to reduced pH and PS content was noted among strains tested, with 52A (Fig. 4a) and 62A (Fig. 5) displaying a greater degree of increased sensitivity to PS levels at the low pH tested. This was more than either the ten-strain mixture or Lamanna B. The combination of 0.26% PS and pH 5.55 was the most favorable for increased strain sensitivity to additives in the media for all strains tested.

Interactions of NaCl and PS effects on C. botulinum growth

Significant NaCl and PS interactions ($p \leq 0.05$) were observed for only Lamanna B in this study (Table 1). The influence of various levels of NaCl (0, 1.25, and 2.50%) and PS (0, 0.13, and 0.26%) on GR of Lamanna B is shown in Fig. 6. A slight increase in strain sensitivity to additives in the media was observed with increasing NaCl levels from 1.25% to 2.50%. However, the largest GR was observed for combinations containing 0% NaCl and 0.26% PS. These results agree with earlier observations of inhibitory activity of PS on *C. botulinum* (Blocher et al., 1982). Although this two-factor interaction was statistically significant ($p \leq 0.05$), it was small compared with the main effects observed separately for NaCl and PS ($p \leq 0.01$) (Table 1).

Observations from nonsignficant two-factor interactions

Several two- and three-factor interactions were not significant (Table 1). Some of these interactions have been reported by other workers, however, and require mention.

Effect of SAPP and NaCl. The effects of sodium phosphates with and without NaCl, on the ability of *Moraxella-*

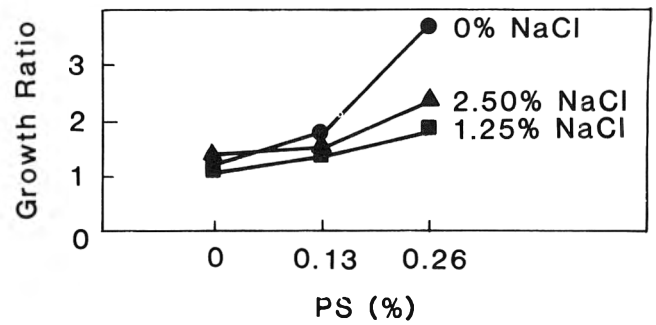

Fig. 6—Influence of NaCl and/or PS on growth of Clostridium botulinum strain Lamanna B.

Acinetobacter cells to grow were determined by Firstenberg-Eden et al. (1981). Combinations of SAPP and NaCl exhibited a synergistic inhibitory effect. Roberts et al. (1981) showed a reduction in spoilage by *C. botulinum* spore outgrowth in a pork slurry system when polyphosphate was used in combination with 4.5% salt. Results obtained from the present study showed no significant SAPP/NaCl interaction (Table 1) for any *C. botulinum* strain tested.

Effect of SAPP and PS. Synergistic effects of SAPP and PS on *C. botulinum* inhibition are well known; however, no significant interaction between the two was observed in results obtained from this study (Table 1). Work by Ivey and Robach (1978) using *C. botulinum*-inoculated perishable, canned, comminuted, cured pork showed that either SAPP or sodium hexametaphosphate displayed statistically significant synergistic interactions with sorbic acid. Interactions also were observed by Seward et al. (1982) when normal growth of *C. botulinum* was prevented by the addition of 0.5% tripolyphosphate to media containing 1.5% PS at pH 7.1, and the inhibition was more than in media containing PS alone.

Interactions of SAPP, NaCl and/or PS effects on C. botulinum growth

Observations involving two (nonzero) levels of SAPP are plotted for the nine combinations of NaCl and PS and are compared to the zero level of SAPP response in Fig. 7: (a) (A_{630} = 0.35), ten-strain mixture; (b) (A_{630} = 0.35), 52A; (c) (A_{630} = 0.35) Lamanna B; (d) (A_{630} = 0.04), ten-strain mixture; (e) (A_{630} = 0.04), 62A. All strains showed an increase in GR and an increased sensitivity to additives in the media for treatment combinations containing SAPP (0.2 and 0.4%), NaCl (0%) and PS (0.13 and 0.26%).

The most favorable combination for inhibiting growth of the ten-strain mixture of *C. botulinum* was 0.2% SAPP in combination with 0.26% PS (Fig. 7a and d). A combination of 0.4% SAPP and 0.26% PS delayed growth most extensively in 52A (Fig. 7b) and Lamanna B (Fig. 7c). In Fig. 7e, a 0.2% SAPP, 2.50% NaCl and 0.26% PS combination was the most inhibitory to 62A with 0.4% SAPP, 0% NaCl and 0.26% PS producing about the same GR value for 62A as it had for 52A and Lamanna B.

CONCLUSIONS

SAPP, NaCl, PS and pH each independently exhibited a main effect ($p \leq 0.01$) on the growth of most strains of *C. botulinum* used in this study. In addition, combinations of SAPP (0.2 and 0.4%) and PS (0.13 and 0.26%) were the most effective treatments for delaying growth of *C. botulinum*. Finally, combinations of additives excluding NaCl consistently increased GR values and sensitivity to additives in the media at both pH 5.55 and 5.85.

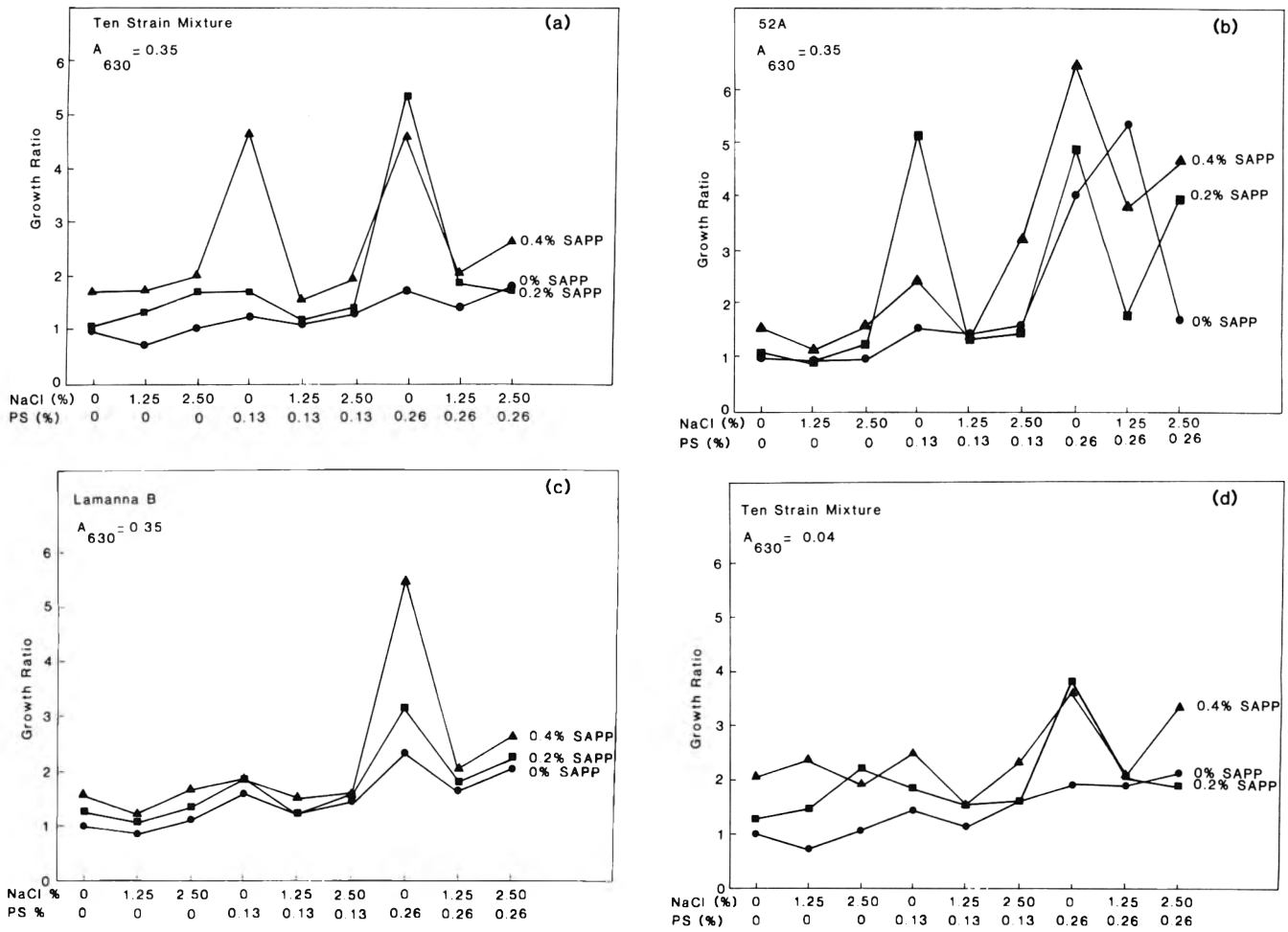

Fig. 7—Effect of SAPP, NaCl and/or PS on growth of Clostridium botulinum: (a) ten-strain mixture, (b) 52A, and (c) Lamanna B (A_{630} = 0.35); and (d) ten-strain mixture and (e) 62A (A_{630} = 0.04).

Findings suggest that the reduction of NaCl from 2.50% to 1.25% in this system may decrease antibotulinal effects observed for combinations of SAPP and PS used in this study. However, the elimination of NaCl can increase that antibotulinal effect. These conclusions are drawn from media model systems and may be somewhat altered in meat environments.

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Sensory Analysis of Carbonated Apple Juice Using Response Surface Methodology

M. R. McLELLAN, J. BARNARD, and D. T. QUEALE

ABSTRACT

Carbonated apple juice, produced at various levels of soluble solids (Brix) and carbonation, was studied using response surface methodology with treatment levels based on a rotatable design. Sensory attributes of aroma, sweeteners, sourness, body, carbonation level, and acceptability were evaluated and response models derived where appropriate. Aroma intensity was not significantly affected by level of soluble solids or carbonation level. A model including linear and quadratic effects was derived for sweetness response and perceived carbonation level. Sourness and body (mouthfeel) were modeled with significant linear effects. The model derived for the acceptability of the juice had a ridge of high acceptability running through the range of typical soluble solids and carbonation levels.

INTRODUCTION

APPLE JUICE is a popular beverage in the U.S. and the world markets. It is perceived as a wholesome and nutritious, all-day beverage and as such is gaining in use and popularity with large increases in sales over recent years (Anon., 1982). With the increasing use of apple juice, a carbonated apple-based beverage may become more popular and capture a part of this expanding market. There exists a need to better understand the effect of carbonation on the perception of quality parameters of apple juice. There have been a number of reports suggesting and evaluating the use of carbonation as both a preservative and flavor enhancer in apple juice; however, few have investigated the effects on specific sensory attributes.

Carpenter (1933) first reported on the public acceptability of carbonated verses noncarbonated apple juice. Irish (1933) suggested various levels of pure juice for carbonated fruit beverages. Included was apple juice, for which he recommended a minimum of 15% in the carbonated beverage. Production of carbonated apple juice in cans was investigated by Celmer and Cruess (1937). This study compared quality of carbonated fruit juices packed in tin verses glass containers. Arengo-Jones (1939) reported on the use of dry ice for production of carbonated apple juice, a technique which is out of date compared to current commercial practices. Brown et al. (1939) studied the preservation and quality of carbonated versus noncarbonated apple juice. Their work indicated the significance of the preservative effects of carbonation on apple juice. Bright and Potter (1970) reported on overall acceptability of carbonated versus noncarbonated apple juice. Their findings indicated an overall slight preference for carbonated apple juice.

Response surface Methodology (RSM) is a unique tool used to identify the combination of quantitative levels of two or more factors that will produce an optimum response, usually a maximum or minimum. An excellent introductory paper on the use of RSM in the food sciences has been published by Giovanni (1983) and should be referenced for further general information on the technique.

Authors McLellan and Queale are affiliated with the Dept. of Food Science & Technology and Author Barnard is affiliated with the Dept. of Computer Services, Cornell Univ., New York State Agricultural Experiment Station, Geneva, NY 14456.

MATERIALS & METHODS

Materials

A blend of apples consisting of approximately 80% R.I. Greening, 10% McIntosh and 10% Cortland were pressed for juice. The juice was clarified for 2 hr at 49°C using pectinase (Klerzyme 200, GB Fermentation Industries Inc., Charlotte, NC), cooled through a tube and shell heat exchanger and then filtered. A precoat of 6.8 kg per 9.29 m^2 of filter area (Filter Cel, Johns-Manville, Denver, CO) was applied to the plate and frame filter. Body feed was maintained between 0.5% and 1% during filtration. The filtered juice was piped into 50 gal. drums where 500 ppm sodium benzoate was added as an anti-microbial agent. The drums of juice were stored at 0°C until carbonated. The filtered juice was of 12.8°Brix and had a titratable acidity of 0.39% as malic acid; the pH was 3.72.

Soluble solids of some sample lots were adjusted to range between 8° and 14°Brix based on the experimental design. The range was selected to be analogous but somewhat below typical juice samples (Mattick and Moyer, 1983). Samples requiring an increased Brix were adjusted with sucrose. Those requiring a decrease in Brix were adjusted with a malic acid buffer solution in order to maintain a standard titratable acidity.

Carbonation

Juice samples were adjusted for soluble solids and carbonated according to the experimental design. The range of carbonation, 0 - 4 volumes of CO_2, was typical of that found in the carbonated beverage industry (Phillips and Woodroof, 1981). The carbonation unit (Zahm Carbonating Pilot Plant Unit, Zahm and Nagel Co., Inc., Buffalo, NY) allowed CO_2 to enter the juice through a submerged aeration stone. Pressure was maintained for a predetermined period to allow equalization of dissolved and gaseous CO_2. A complete sweep and pressurization of each bottle was made with CO_2 just prior to filling. Juice was held at 0°C during carbonation and filling. Bottles were dried and chilled to −40°C before filling. The bottles were closed with crown caps.

Sensory analysis

Samples were submitted to an experienced panel consisting of eight persons for a Quantitative Descriptive Analysis (QDA) (Stone et al., 1974). Panelists were selected on their ability to discriminate and reproduce results from difference tests. All panelists had experience with QDA type panels and with various processed apple products. Each attribute on the ballot was interpreted using a typical QDA non-numeric linear scale with a low-medium-high or similar notation (Table 1). Numerical interpretation was handled by digitizing the ballot scales to produce an answer re-

Table 1—Sensory attributes included on the quantitative descriptive analysis ballot with appropriate scale notations

Attribute	QDA scale notation
Fruity Apple Aroma	low – medium – high
Sweetness	low – medium – high
Sourness (Tartness)	low – medium – high
Body	thin – thick
Carbonation Level	low – medium – high
Acceptability	low – medium – high

flecting a 0.0 - 10.0 scale in tenths. The technique and basis for digitizing is described elsewhere (McLellan and Cash, 1983). Testing was carried out in a sensory evaluation laboratory, in partitioned booths under standardized daylight conditions. Samples, 80 mL, at room temperature, were poured into wine glasses and submitted in groups of three to the panelists. Each was coded using a three digit random number. All samples including replicates were given to panelists in a random order. Water was present for rinsing. Panelists were instructed to evaluate each sample presented, individually, for attributes in the following categories: aroma, taste, mouthfeel and acceptability.

Experimental design and statistical analysis

A rotatable design was used in this project to minimize the number of experimental units involved (Mullen and Ennis, 1979). Nine replicates of each treatment combination were submitted to the panelists. Appropriate treatment combinations used in the design are shown as coded levels and actual levels in Table 2.

Statistical analysis was accomplished using a GENSTAT (Alvey et al., 1977) program written by one of the authors (Barnard, 1984). Actual methodology for calculations can be derived from elementary text books (e.g. Gill, 1978).

RESULTS & DISCUSSION

A VARIANCE RATIO was calculated for a model with linear effects only and also for a model which included both linear and quadratic effects, for each of the sensory attributes. Additionally, a variance ratio for lack of fit of the respective model was calculated. A summary of pertinent models is given in Table 3.

Analysis of variance indicated no significant effect of carbonation or soluble solids on the aroma intensity of the samples. Carbon dioxide does not have an aroma of itself and based on these results the effervescence in the poured beverage did not significantly impinge on the effect of the apple juice aroma volatiles present in the headspace over the range of the study.

The sensory response of sweetness was modeled with significant linear effects. With the inclusion of quadratic effects, the model was significant at P = 0.10. Although not statistically significant at P = 0.05, the quadratic certainly seems worth considering as a plausible model (Fig. 1). Lack of fit however, was marginally significant indicating the possibility that additional parameters are needed in the model.

The analysis of sourness response indicated only significant linear components in the model. Lack of fit was not significant. The coefficients for the linear terms in the model for sourness, unlike the linear terms in the model for sweetness, were not equal and opposite. The linear term coefficient for carbonation was 0.98; however, the coefficient for the effect of Brix was only 0.15. Sourness was affected to a much lesser degree by a change in Brix than by a respective change in carbonation.

A significant linear model was generated for sensory response to body, a descriptor of the mouthfeel of the beverage. The model proposes a positive effect of soluble solids on body and a negative effect of carbonation on body. Lack of fit was not significant indicating adequacy of the model.

Perceived carbonation was modeled with significant linear and quadratic effects. The response surface illustrates the sensitivity of the carbonation response to soluble solids at the two extremes of volumes of CO_2 used (Fig. 2). Though lack of fit was sharply reduced in going to a model with both linear and quadratic terms, it was still marginally significant indicating missing components to the model.

The acceptability of the carbonated beverage was modeled with both linear and quadratic terms. The re-

Table 2—Level combinations for the two variables, carbonation and soluble solids used in the rotatable design for response surface analysis

Level of carbonation		Level of soluble solids	
(coded)	Vol. CO_2 (actual)	(coded)	Brix (actual)
−1.0	0.6	−1.0	9.0
−1.0	0.6	1.0	13.0
1.0	3.5	−1.0	9.0
1.0	3.5	1.0	13.0
1.414	4.0	0.0	11.0
−1.414	0.0	0.0	11.0
0.0	2.0	1.414	14.0
0.0	2.0	−1.414	8.0
0.0	2.0	0.0	11.0

Table 3—Summary of response surface models. The independent variable 'Y' is the sensory response. Variables for actual levels of soluble solids and carbonation are 'B' (Brix) and 'C' (Vol. of CO_2), respectively.

Attribute	Model	Significance (P)
Sweetness	— Linear and Quadratic Model $Y = 4.81 + 0.25(B) - 1.66(C) + 0.04(B^2) + 0.07(BC) + 0.09(C^2)$	0.10
Sourness	— Linear Model $Y = 4.86 - 0.07(B) + 0.69(C)$	0.01
Body	— Linear Model $Y = 2.54 + 0.23(B) - 0.20(C)$	0.01
Carbonation	— Linear and Quadratic Model $Y = 2.82 - 0.24(B) + 2.28(C) + 0.026(B^2) - 0.14(BC) + 0.22(C^2)$	0.01
Acceptability	— Linear and Quadratic Model $Y = -6.87 + 1.7(B) + 2.6(C) - 0.05(B^2) - 0.12(BC) - 0.28(C^2)$	0.01

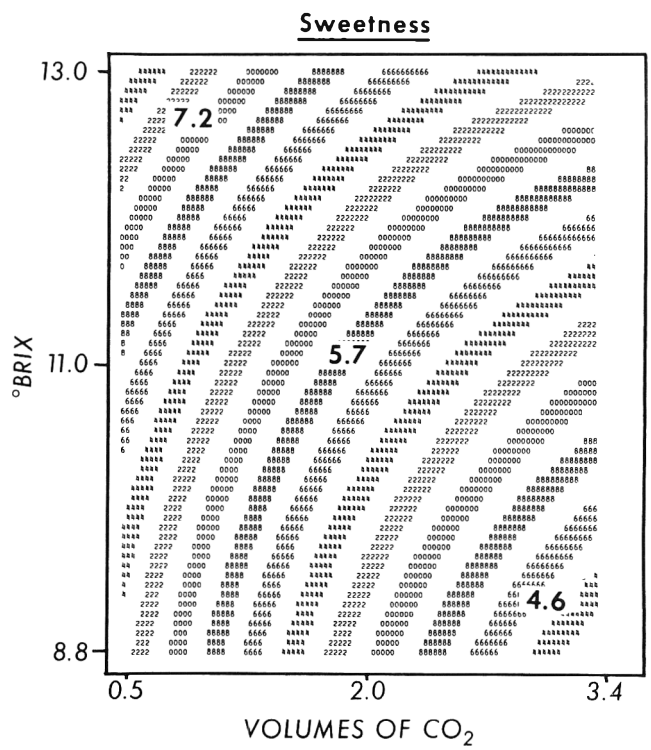

Fig. 1—Response surface relating level of carbonation and soluble solids to sweetness response.

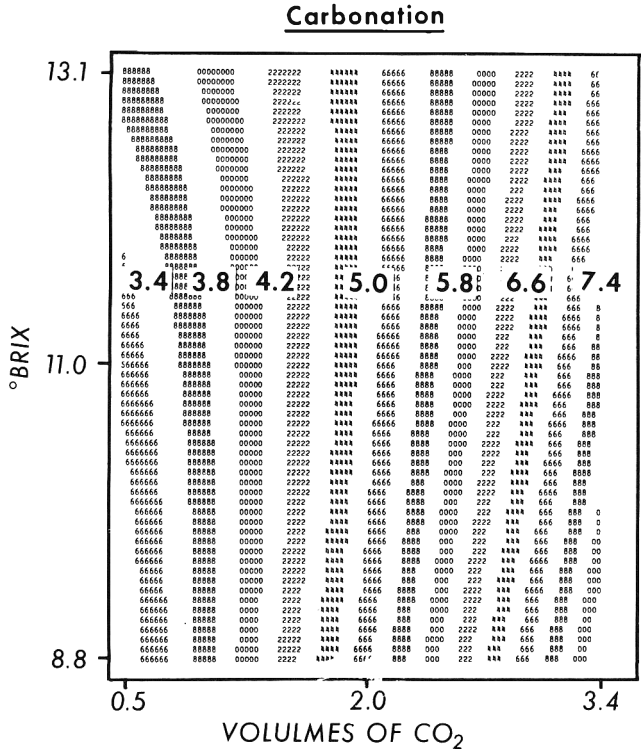

Fig. 2—Response surface relating level of carbonation and soluble solids to perceived carbonation.

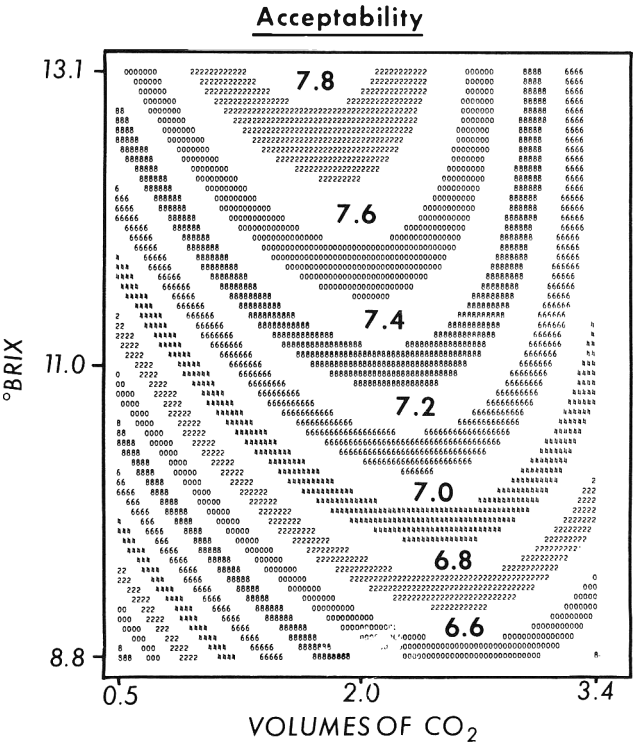

Fig. 3—Response surface relating level of carbonation and soluble solids to level of acceptability.

sponse surface indicated a ridge of higher values reaching a theoretical optimum just outside of the range of the experiment (Fig. 3). An interpretation of the response surface leads to the proposition that a specific optimum level of carbonation existed for each level of soluble solids in the carbonated apple juice. A maximum was calculated for the surface at 15.06° Brix and 1.30 volumes of CO_2. Knowing the equation for the ridge line, optimum levels of carbonation based on soluble solids can be derived.

$$\text{Vol. } CO_2 = 5.79 - 0.29\,(\text{Brix}) \qquad [1]$$

Eq. [1] was generated for the rising ridge in the response surface plot of acceptability level. The ridge was associated with the least sensitive canonical axis as determined by the latent roots.

CONCLUSIONS

THE LINEAR MODELS have been proposed for the sensory response to sourness and body (mouthfeel) in carbonated apple juice. Both were reasonable in their estimates and could be applied with confidence.

Three models including linear and quadratic effects were suggested for sweetness, perceived carbonation and acceptability. The models for sweetness and carbonation, though significant, may involve more than this preliminary experiment suggests; however, both offer a reasonable and significant starting point in the effort to model sensory response of these attributes in carbonated apple juice. Modeling the sensory response of acceptability, this third model posed a significant finding, that being a ridge of high acceptability running through the range of the experiment. The ridge indicated an optimum level of carbonation depending upon the level of soluble solids.

All the models proposed here will need additional work to verify and further extend their applicability. This is especially true about the models proposed for perceived carbonation and sweetness response.

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Relative Importance of Color, Fruity Flavor and Sweetness in the Overall Liking of Soft Drinks

HELY TUORILA-OLLIKAINEN, SEIJA MAHLAMÄKI-KULTANEN, and RAKEL KURKELA

ABSTRACT

Sixteen soft drink samples, in which appearance (colorless or colored), fruity flavor (with or without a flavorant) and sweetness (5 or 9% sucrose) were systematically varied, were rated for their pleasantness of appearance, flavor (odor, taste and sweetness separately) and overall liking by 448 young adults and children. In terms of sample parameters sucrose concentration and the presence of a flavorant significantly influenced overall liking, whereas color had little significance. In terms of sensory attributes pleasantness of taste and sweetness mainly determined overall liking. The responses of young adults and of children were in the main rather similar, except that the children seemed to put more emphasis on sweetness.

INTRODUCTION

SENSORY ATTRIBUTES of foods and beverages are commonly categorized as appearance, texture, and flavor, i.e. odor and taste. These attributes naturally overlap (see e.g. Kramer, 1972; Hutchings, 1977), and in some foods they can be distinguished more easily than in others. Their relative importance in the overall liking or acceptance of a product has been studied in questionnaires (Schutz and Wahl, 1981; Szczesniak, 1972; Szczesniak and Kleyn, 1963) and in the case of specific food products in sensory tests (e.g. DuBose et al., 1980; Ennis et al., 1979; Pangborn et al., 1960; Pangborn and Leonard, 1958; Schutz, 1954). The authors generally agree that overall flavor or its separate dimensions critically affect product liking. However, appearance and texture may also play a considerable role.

Authors Tuorila-Ollikainen and Mahlamäki-Kultanen were formerly affiliated with the Technical Research Centre of Finland, Food Research Laboratory, Biologinkuja 1 A, SF-02150 Espoo 15, Finland. Author Tuorila-Ollikainen is now with the Dept. of Food Chemistry & Technology, Univ. of Helsinki, SF-00710 Helsinki 71, Finland. Author Mahlamäki-Kultanen current affiliation is The Finnish Recreational Sports Association, Topeliuksenkatu 41a, SF-00250 Helsinki 25, Finland. Author Kurkela is affiliated with the Dept. of Food Chemistry & Technology, Univ. of Helsinki, SF-00710 Helsinki 71, Finland.

Color and sweetness of soft drinks have, besides their role in hedonic quality, nutritional and safety aspects (Anon., 1980; Bierman, 1979). The present study was, therefore, aimed at determining systematically their relative importance, as well as the importance of flavor, to the overall liking of soft drinks.

MATERIALS & METHODS

Sample preparation and presentation

Two series of carbonated soft drinks, in which appearance, fruity flavor and sweetness level varied, were prepared according to a normal industrial recipe; the series are called here RASPBERRY and PEAR series (see Table 1). Within both series, those having a colorant, a flavorant and 9% sucrose are similar to typical commercial Finnish soft drinks. Samples containing 5% sucrose were clearly less sweet, but they still retained characteristic qualities of sweetened soft drinks, which was the desired effect of this sweetening level.

The samples were prepared by the method commonly used in product development laboratories of the Finnish soft drink industry. Minor ingredients were dissolved in liquid sucrose (65% w/w), added to bottles of carbonated water and evaluated between 2 and 14 days after preparation. Carbonation is relatively insensitive towards the ingredients used in this study (Hoffström, 1982).

Both series were presented to subjects in sets of four samples in an incomplete block design (plan 11.10, Cochran and Cox, 1957). The coded samples (60 mL) were presented at 10–13°C in randomized orders in 125 mL transparent polyethylene cups, placed on white-covered trays. The subjects rated the pleasantness of appearance and flavor (odor, taste and sweetness separately) as well as liking, on a 10 cm line anchored verbally at both ends (Stone et al., 1974). The scale was modified by indicating the possible response sites with nine equidistant bars. Prior to testing, the subjects were instructed in the use of the scale, but not in sniffing or tasting techniques. The subjects evaluated one attribute of all the samples at a time – first appearance, then odor, sweetness and taste. Taste was defined as an attribute covering all oral perceptions except sweetness. Finally, subjects evaluated overall liking. No rinsing water was provided. After the performance of the sensory test the subjects filled out a questionnaire, in which, among other things, their opinions concerning the importance of sensory attributes were queried.

Table 1—Sample ingredients per liter of beverage

Ingredient	Source	Sample no.							
A. RASPBERRY drinks		1	2	3	4	5	6	7	8
Sucrose (%)	Finnish Sugar Co. Ltd.	5	5	5	5	9	9	9	9
Sodium benzoate (mg)	Merck	90	90	90	90	90	90	90	90
Amaranth (E123) (mg)[a]	Williams Ltd., England	–	40	–	40	–	40	–	40
Raspberry flavor	Fructus Fabriker, Sweden	–	–	+	+	–	–	+	+
B. PEAR drinks		9	10	11	12	13	14	15	16
Sucrose (%)	Finnish Sugar Co. Ltd.	5	5	5	5	9	9	9	9
Sodium benzoate (mg)	Merck	90	90	90	90	90	90	90	90
Tartrazine (E102) (mg)[b]	Williams Ltd., England	–	4	–	4	–	4	–	4
Patent blue V (E131) (mg)[b]	Nederlandse Kleustofindustri, Holland	–	0,4	–	0,4	–	0,4	–	0,4
Citric acid-1-hydrate (g citric acid)	Merck	–	–	1,35	1,35	–	–	1,35	1,35
Pear flavor	Fructus Fabriker, Sweden	–	–	+	+	–	–	+	+

[a] Red color
[b] Green color

Table 2—Mean overall liking of soft drink samples in different subgroups

Sample no.		Sample characteristics			Overall liking[a]			
					RASPBERRY		PEAR	
RASP-BERRY	PEAR	Color	Flavor	Sweetness (% sucrose)	Adults	Children	Adults	Children
1	9	–	–	5	3.3 ab	2.7 a	3.4 ab	3.4 a
2	10	+	–	5	2.6 a	2.6 a	3.1 a	3.7 ab
3	11	–	+	5	3.5 ab	3.0 ab	3.5 ab	3.8 ab
4	12	+	+	5	3.1 ab	4.0 b	3.4 ab	4.1 ab
5	13	–	–	9	3.2 ab	4.1 b	3.9 ab	4.9 bd
6	14	+	–	9	3.9 b	4.1 b	4.2 b	5.4 d
7	15	–	+	9	5.4 c	7.0 c	5.8 c	7.2 c
8	16	+	+	9	5.6 c	7.0 c	5.9 c	7.0 c

[a] Each mean consists of 56 scores. Means within columns followed by the same letter are not significantly different at the 95% level using Tukey's alternate procedure (TUKEYB) test.

Subjects

A total of 448 subjects, of whom one half (N = 224) were young adult college students (mean age 20.2, s.d. 2.5 yr) and the other half (N = 224) were school children (mean age 11.0 s.d. 1.0 yr), participated in the study. In both age groups, half of the subjects (N = 112) evaluated samples of the RASPBERRY and the other half (N = 112) samples of the PEAR series. In each group half (N = 56) were females.

Testing was conducted during school hours, before lunch. Sessions took place in a canteen (children) or in an auditorium (young adults). Testing was carried out during a mild period in autumn. Normal fluorescent lighting was provided in the testing rooms.

Analysis of results

The data were analyzed by computer programs from the Statistical Package for the Social Sciences (Nie et al., 1975). The scores given on the graphical scale were converted to integers from 1 to 9 (1 = extremely unpleasant and 9 = extremely pleasant). All the variables were treated as interval scale level variables. Correlations were calculated as Pearson's product moment correlation coefficients.

RESULTS

THE RATINGS of overall liking showed an identical grouping for the two most preferred samples in all the subgroups (Table 2). For both young adult subgroups the remainder of the grouping was also similar. An interesting feature in the young adult subgroups was that colored, unflavored samples with 5% sucrose (nos. 2 and 10) received poorer ratings than the other "defective" samples 1, 3, 4, 5, 9, 11, 12, and 13. The ratings of children in the PEAR series were grouped according to sucrose concentration, but this was not the case in their RASPBERRY series. The range of ratings was wider in the children than in the young adult subgroups.

Analysis of variance of the overall liking ratings indicated that sucrose concentration was crucial for overall liking [RASPBERRY, adults $F(1,440) = 58.83$ and children $F(1,440) = 132.77$; PEAR, adults $f(1,440) = 70.77$ and children $F(1,440) = 123.47$; $p < 0.001$ in all cases). The flavorant (plus an acid addition in PEAR drinks) was also important [RASPBERRY, adults $F(1,440) = 42.02$ and children $F(1,440) = 85.44$; PEAR, adults $F(1,440) = 28.52$ and children $F(1,440) = 29.28$; $p < 0.001$ in all cases]. Moreover the interaction flavor x sucrose concentration was significant [RASPBERRY, adults $F(1,440) = 20.73$ and children $F(1,440) = 24.02$; PEAR, adults $F(1,440) = 15.02$ and children $F(1,440) = 13.52$; $p < 0.001$ in all cases]. The presence of color was significant for only one group of subjects [RASPBERRY, adults $F(1,440) = 7.68$, $p < 0.01$], being considered negative when combined with the lower level of sucrose (5%) but positive when combined with the normal sucrose concentration.

Among the sensory ratings, sweetness, taste and overall liking were the most closely correlated (Table 3). The correlation of odor with other attributes was weaker, and appearance correlated only slightly with other sensory attributes. This trend was similar in all the subgroups. Multiple regression analysis was carried out to predict the significance of sensory attributes for overall liking. Because of multicolinearity caused by the taste x sweetness interaction, the combination of these two parameters was taken as one independent variable. In all models, computed separately for the four subgroups, taste and sweetness were significant, but their interaction was not (Table 4). Odor was of less significance, and appearance was of almost negligible importance. In the different subgroups, up to 60% of the overall liking responses were explained by the pleasantness of the sensory attributes. In general, the regression equations of the young adults and children corresponded to each other, although in the equations of the data from the younger group, sweetness seemed to become more pronounced.

Verbally expressed significances of different sensory attributes for the acceptance of soft drinks displayed rather

Table 3—Correlation coefficients between pleasantness ratings of sensory attributes

	Appearance	Odor	Sweetness	Taste	Overall liking
A. RASPBERRY drinks					
Adults					
Appearance	1.00	0.17	0.03	0.09	0.08
Odor		1.00	0.22	0.24	0.26*
Sweetness			1.00	0.66***	0.58***
Taste				1.00	0.76***
Overall liking					1.00
Children					
Appearance	1.00	0.33*	0.11	0.13	0.14
Odor		1.00	0.31*	0.39**	0.39***
Sweetness			1.00	0.78***	0.67***
Taste				1.00	0.74***
Overall liking					1.00
B. PEAR drinks					
Adults					
Appearance	1.00	0.28*	0.06	0.10	0.22
Odor		1.00	0.29*	0.30*	0.26
Sweetness			1.00	0.73***	0.64***
Taste				1.00	0.75***
Overall liking					1.00
Children					
Appearance	1.00	0.28*	0.23	0.18	0.21
Odor		1.00	0.29*	0.27*	0.32*
Sweetness			1.00	0.65***	0.62***
Taste				1.00	0.69***
Overall liking					1.00

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 4—Pleasantness of taste, sweetness, odor and appearance as factors explaining overall liking of soft drinks: stepwise multiple regression analysis, regression coefficients

Subject group	Taste	Sweetness	Taste x Sweetness	Odor	Appearance	Constant	R^2 (%)
A. RASPBERRY drinks							
Adults	0.66***	0.13**	–	0.10*	–	0.27	58.85
Children	0.52***	0.22***	–	0.13***	–	0.49	58.24
B. PEAR drinks							
Adults	0.59***	0.21***	–	–	0.19***	−0.30	60.96
Children	0.48***	0.29***	–	0.13**	–	0.46	53.78

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 5—Reports by subjects on the importance of sensory attributes in the acceptance of soft drinks

Attribute	Importance[a] Adults (N = 224)	Children (N = 224)	Significance t value
Taste	3.9 ± 0.4	3.8 ± 0.6	1.79
Sweetness	3.0 ± 0.8	3.3 ± 0.8	3.34***
Odor	2.6 ± 0.7	2.5 ± 0.8	2.19*
Appearance	2.4 ± 0.8	2.1 ± 0.8	3.42***

[a] 1 = indifferent, 4 = extremely important; means and standard deviations are given.
* $p < 0.05$
*** $p < 0.001$

similar trends for both groups (Table 5). Taste was the most important criterion of acceptance. Children put more emphasis on sweetness, whereas the young adults considered appearance and odor to be more important than did the children.

DISCUSSION

THE ANALYSIS OF FACTORS influencing overall liking, whether performed on the level of sample parameters, sensory attributes or verbally expressed opinions, revealed the significance of taste and sweetness for hedonic responses to soft drink samples. This is in agreement with Schutz and Wahl (1981), who, in their questionnaire study, found that flavor was particularly important for liquid food products, and with Szczesniak (1972), who reported flavor as the main criterion of beverage preferences among teenagers. The comments by the subjects of Pangborn et al. (1960), and of Pangborn and Leonard (1958), as reasons for sensory responses to flavored drinks also emphasize the significance of sweetness and flavor.

Sweetness appears to occupy an important position among flavor attributes. Members of societies otherwise diverging considerably in their taste habits share the preference for sweetness (Moskowitz et al., 1975). Sweetness has also been suggested to constitute one of the basic dimensions regulating preference behavior (Pilgrim and Kamen, 1959). In addition to the present work, other sensory studies have also shown that sweetness forms an important criterion for overall liking (Ennis et al., 1979; Pangborn et al., 1960; Pangborn and Leonard, 1958). A sucrose concentration in soft drinks of around 10% was preferred by a majority of American subjects (Pangborn, 1980) and the same was true in the present study.

In her analysis, Schiffman (1979) found that beverages were evaluated according to two criteria: sweetness and familiarity. In the present study the samples containing a flavorant and a familiar sucrose concentration (9%) obviously fulfilled these criteria and were therefore rated highly in the overall liking. Positive association between flavor and sweetness was also noted by Pangborn and Leonard (1958).

Color had only a slight, if any, influence on overall liking. However, it is well known that color functions as a necessary cue for flavor identification (e.g. DuBose et al., 1980; Hall, 1958), and that even a slight deviation from the accustomed hue may considerably change a preference rating (Martens et al., 1983). This explains the need for artificial coloring in many products which themselves are colorless. In the present study the samples were not identified as "raspberry" or "pear" drinks, but only as soft drinks. The lack of flavor specification most probably explains the insignificance of color; in an earlier study flavor specification was found to influence color ratings (Tuorila-Ollikainen, 1982). The fact that adult subjects reacted negatively to colored, but otherwise defective samples also suggests that the principal role of color is as an arouser of expectations and associations. However, the result also calls for a critical examination of the "need" for artificial colorants; these needs may fluctuate. It is worthy of note that on the Finnish soft drink market colorless soft drinks constitute about one-fifth of total sales (Martinoff, 1982). Thus, the subjects were most probably familiar with both colorless and colored soft drink products.

Pleasantness ratings of different sensory attributes only partially explained overall liking responses. This reflects the complex, multidimensional nature of a hedonic response (Hirschman and Holbrook, 1982). The sensory attributes measured in this study may have covered the sensory dimensions of the product, but they could not cover the possible mental images and fantasies which, together with the actual sample properties, create the sample image.

In general the reactions of young adults and children were similar, but children seemed to put more emphasis on sweetness at the expense of other sensory attributes. The importance of sweetness to younger age groups has previously been indicated by Pangborn and Leonard (1958) and by Desor et al. (1975). The result may be an indication of truly different sensory preferences, but it may also imply an inability of children to comprehend or appreciate the complex spectrum of properties which make up a certain product.

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Determination of Individual Simple Sugars in Aqueous Solution by Near Infrared Spectrophotometry

GERALD G. DULL and ROBERTO GIANGIACOMO

ABSTRACT

An approach is presented for using near infrared region (NIR) spectrophotometry to determine the individual simple sugars (glucose, fructose, and sucrose) in aqueous solutions ranging from 3 - 52% (w/w). The 95% confidence limits of the method for glucose, fructose, and sucrose were ± 1.3, 1.0, 0.9%, respectively.

INTRODUCTION

MEASUREMENT of soluble solids is a generally accepted practice used in following quality changes in a number of fruits and vegetables. Recognizing that soluble solids is a measure of sugars primarily, but not exclusively, the capability of following the concentrations of specific simple sugars rapidly and nondestructively during development and postharvest storage of fruits and vegetables is a critical need in evaluating the quality of these products. Near infrared region (NIR) spectrophotometry is the most promising of several nondestructive methods which have the potential of meeting this need. This technique is presently in wide use in determining chemical constituents and quality parameters in agricultural products, as discussed in a recent review (Polesello and Giangiacomo, 1983). However, most of these determinations are applied to dry or low moisture products (less than 15% moisture) and are not practicable for fresh fruits and vegetables.

In the course of developing a nondestructive method for measuring specific simple sugars in fruit tissues by NIR spectrophotometry, we have encountered a number of obstacles. Preliminary indications (Dull et al., 1978) have encouraged us to continue our efforts in this area. In that work, it was pointed out that sugar spectra have absorption bands in the wavelength region from 900 - 1300 nm. Water also has absorption bands in this region. We attributed our difficulties in that work to the absorption of radiation by water which was strong in comparison to the absorption by sugars. We reasoned that it would be necessary to establish first that it was possible to obtain satisfactory NIR measurements on individual dry sugars. Successful results were obtained with dry sugars in the range 950 - 1850 nm (Giangiacomo et al., 1981). With the knowledge that the NIR spectra of simple sugars contain sufficient information to enable the spectrophotometric determination of dry sugars, we applied the same technique to sugar solutions, taking advantage of new computer programs which were available for processing the spectral data.

This paper includes the results of our studies on the determination of glucose, fructose, and sucrose in aqueous solutions in the concentration range 3 - 52% (w/w) using NIR spectrophotometry.

Author Dull is affiliated with the USDA-ARS, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30613. Author Giangiacomo is affilaited with the Istituto Sperimental Per La Valorizzazione, Tecnologica Dei Prodotti Agricoli, Milano, 20133, Italy.

MATERIALS & METHODS

Sample preparation

Samples of glucose, fructose, and sucrose used in this work were established as pure single sugars by high performance liquid chromatography. Samples of each sugar were prepared in distilled water at concentrations of 1.5, 8, 15, 24, 35, 48, and 60% on a w/w basis. Each solution was replicated four times. These samples were designated as the calibration set. A second set of sugar samples was prepared using the concentrations of 3, 12, 20, 36, and 52% for each sugar, replicated four times. This set of samples was designated as the prediction set.

Spectral data acquisition and processing

The instrument used in this work was a modified SpectroComputer produced by the Neotec Company. It is made up of a Cary monochromator, a lead sulfide detector, and a Data General computer. The system is described in detail elsewhere (Rosenthal et al., 1976). We used a transmittance (T) geometry in which the incident radiation passed through the sample. Since the instrument is a single beam type, it was necessary to use the empty cell as a reference standard. The cell path length was 2.74 mm. The sample cells used in this work were constructed with an aluminum body and quartz end plates. The path length was varied by using appropriate spacers between the top and bottom end plates. Since the path length (and therefore the sample volume) was fixed, the predicted concentrations were on a % weight/volume basis. The results were converted to our standard concentration units (% weight/weight) by dividing the spectral data by the density of each specific sample. The specific wavelength range used in this study was 1550 - 1850 nm. The monochromator slit was set at 2 mm, which gave an effective bandpass of 7 nm. We collected 1000 data points in each spectrum with a wavelength increment of 0.3 nm per data point. The spectral data were recorded as log 1/T. The instrument noise level was found to be 0.0015 optical density (OD) units, peak to peak, over the spectral range being studied.

The methodology used in this work depended upon the computation of derivatives of spectra in relation to changes in component concentrations. The best results were obtained using second derivative data processing. The data were processed in a second derivative form using the procedure and computer programs described by Norris and Massie (1981). The second derivative for the log 1/T at a nominal wavelength B was obtained from the calculation A + C − 2B, where A and C represented values for log 1/T at wavelengths below and above B. The separations between A, B, and C are termed gaps. The exact number of data points in these gaps was optimized by stepwise linear regression analysis with a computer program which gave the highest correlation coefficient and lowest possible standard error of estimate. The computer program made it possible to use a second derivative at a specific wavelength or the ratio of two second derivatives at two different wavelengths. Norris (1983) stated that the use of the derivative ratio was necessary to compensate for path length variations due to different particle sizes and constituents. In the ratio method, the regression analysis component of the program compared all the ratios of the second derivative at a wavelength to the second derivative at each wavelength in the region scanned and identified the wavelengths of the second derivative ratio giving the highest correlation with the chemical data. The wavelengths in this ratio are referred to as the numerator and denominator wavelengths. Both single derivatives and derivative ratios were used for this study.

Each calibration and prediction sample was scanned three times and the scans were averaged to give a single spectrum for each replicate of each concentration of each sugar. The spectral data for the calibration set were processed to develop a regression equation

which could be used for prediction purposes. Spectral data were collected for prediction samples and the data were used to predict the sugar concentrations in that set.

Statistical analysis

The performance of the NIR procedure was followed by calculating the standard error which is the square root of the sum of the squares of the differences between the actual and predicted values divided by n-2. The standard error for the calibration set is called the standard error of calibration (SEC). The standard error for the prediction set is called the standard error of prediction (SEP). The standard errors and confidence limits were calculated according to Little and Hill (1978).

RESULTS & DISCUSSION

IT SHOULD BE NOTED that the general approach of classic spectrophotometry was the basis for this work; however, the acquisition and mathematical treatment of spectral data which were correlated with component concentrations represented a major departure from the standard procedure. The absorption at a single wavelength was not adequate for the determination of sugars in aqueous solutions. For this reason, the derivative spectrophotometry approach was used.

In preliminary work, the region from 1000 - 2200 nm was scanned using a cell path length of 0.254 mm, and a problem of nonreproducibility of selection of wavelengths for each sugar was encountered. However, the work did produce information which substantiated the contention that the wavelength region of 1550 - 1850 nm contained spectral information which can be used in the determination of specific sugars. We reasoned that the nonreproducibility was due to inadequate absorption by the sugars in comparison to the absorption by water in the wavelength region scanned. To minimize this problem, a wavelength region was selected which represents a spectral window between two major water absorption bands at 1450 and 1940 nm. This choice permitted use of a longer cell path (2.74 mm) which resulted in a more favorable detection of absorption by the sugars.

Absorption spectra of 35% solutions of glucose, fructose, and sucrose in the wavelength range 1550 - 1850 nm are presented in Fig. 1. To the eye, and based upon classic spectrophotometry, there did not appear to be sufficient spectral character to enable the use of these data for prediction purposes. With the use of second derivative processing, subtle changes in these spectra were enhanced to such a degree that clear differences between the three sugars appear, as shown in Fig. 2. To illustrate the changes in spectral character with concentration of the sample, the second derivative spectra of glucose solutions of three different concentrations are shown in Fig. 3 and are compared with pure water. The wavelengths selected with the computer program for the highest correlation between spectral and chemical data are listed in Table 1. The correlation coefficients for the relationship between spectral data and sugar concentrations equaled or exceeded 0.9995. In the case of glucose, use of the second derivative ratio, as opposed to a single second derivative value, consistently resulted in a higher correlation and a lower standard error of calibration (SEC). For fructose and sucrose, the single derivative gave the best results. The regression equation constants and standard error of calibrations are presented in Table 2. The regression equations were used to compute the concentrations of sugars in the prediction samples. The prediction results are presented in Table 3.

The 95% confidence limits for predictions of glucose, fructose, and sucrose were ±1.3, 1.0, and 0.9%, respectively. This level of analytical precision is in agreement with the limitations imposed by the inherent instrument noise level of 0.0015 OD units. This noise level equates to a sugar concentration difference of ±0.8%. Therefore, the combination of instrument noise and sampling error result in a minimum detectable difference in sugar concentration of 2%.

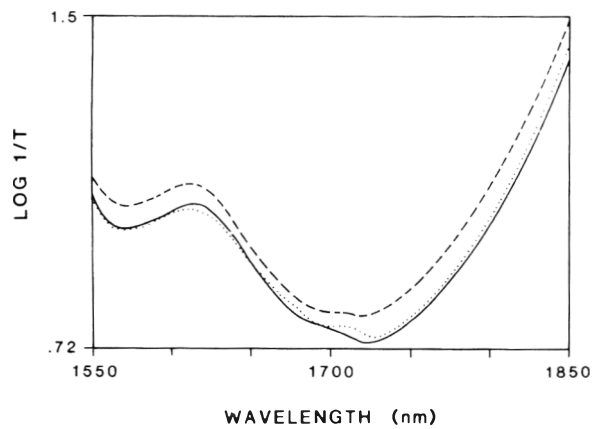

Fig. 1—Absorption spectra of glucose (- - -), fructose (· · ·), and sucrose (—)in 35% (w/w) aqueous solutions.

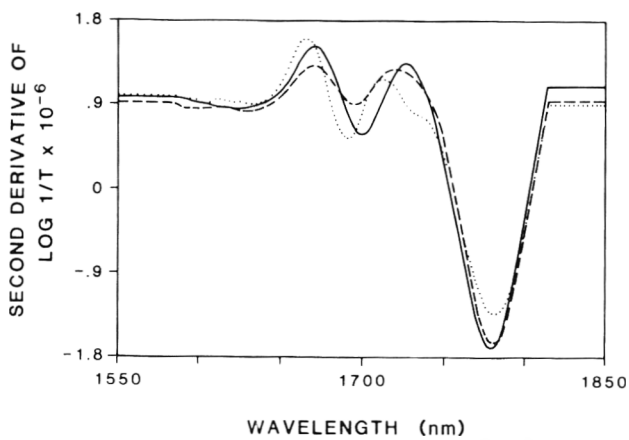

Fig. 2—Second derivative spectra of glucose (- - -), fructose (· · ·), and sucrose (—) in 35% (w/w) aqueous solution.

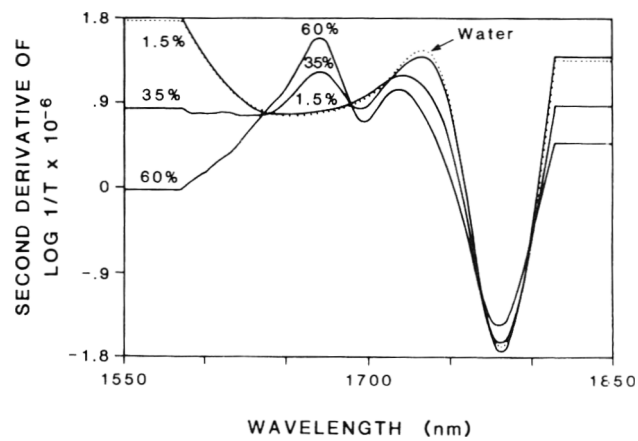

Fig. 3—Second derivative spectra of glucose solutions at three different concentrations compared with pure water.

Table 1—Numerator and denominator wavelengths at which the highest correlations occurred between second derivative data and concentrations of glucose, fructose, and sucrose in aqueous solutions

	Numerator wavelength nm	Denominator wavelength nm	Correlation coefficient r
Glucose	1600	1687	0.9996
Fructose	1727	–	0.9999
Sucrose	1732	–	0.9995

Table 2—Regression equation constants and standard errors of calibration (SEC) for correlations of glucose, fructose, and sucrose concentrations with second derivative data (Y = A + BX)

Sugar	A	B	SEC
Glucose	58.4	−31.8	0.61
Fructose	69.5	491.4	0.29
Sucrose	91.8	672.6	0.66

CONCLUSIONS

ALLOWING for instrumentation modifications to reduce the instrument noise level to 0.0001 OD units, we believe the accuracy of prediction can be markedly improved over the entire concentration range 1.5 - 60% for each sugar. Preliminary tests indicate that the method will enable one to determine all three sugars in a mixture with a single scan, but additional work is needed to develop the calibration procedure.

These results substantiate the contention that NIR spectrophotometry can be used successfully to determine the concentration of glucose, fructose, and sucrose in aqueous solutions.

Table 3—Concentrations of glucose, fructose, and sucrose predicted with NIR spectrophotometry and compared with actual concentrations

	Actual Conc. % w/w	Predicted conc[a] % w/w		
		Glucose	Fructose	Sucrose
1	12.00	11.34	11.97	12.29
2	36.00	35.16	36.18	36.52
3	3.00	3.04	2.69	2.96
4	52.00	51.96	51.65	53.09
5	20.00	19.60	20.55	20.28
6	3.00	4.27	2.59	2.87
7	12.00	11.43	13.48	12.21
8	36.00	35.48	36.03	36.50
9	20.00	19.46	19.28	19.77
10	52.00	51.39	51.71	52.05
11	36.00	36.05	35.92	36.14
12	20.00	19.44	19.38	20.73
13	52.00	51.61	52.34	52.06
14	3.00	3.31	3.50	2.58
15	12.00	11.43	12.40	12.49
16	3.00	3.65	3.23	2.51
17	20.00	20.57	19.39	20.43
18	36.00	36.50	36.25	36.35
19	52.00	51.98	51.98	52.68
20	12.00	10.62	11.84	12.30

[a] SEP = 0.67, 0.53, 0.47 for glucose, fructose, and sucrose respectively.

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Interaction of Sucrose with Starch During Dehydration as Shown by Water Sorption

P. CHINACHOTI and M. P. STEINBERG

ABSTRACT

Component interactions in the sucrose-starch-water system were investigated. Starch, sucrose, a mechanical starch:sucrose mixture and four powders obtained by freeze drying dilute aqueous mixtures were equilibrated to a_w ranging from 0.33–0.93. Both raw and gelatinized starch were included. Sucrose-starch interaction was determined by measuring the reduction in water sorption as compared to theoretical. Only the freeze-dried mixtures showed interaction. Interacted sucrose, calculated as the sucrose that did not bind water, (1) decreased from a maximum at 0.86 a_w to zero at 0.936 a_w, and (2) rose sharply with increasing sucrose-starch ratio to a maximum at a ratio of 0.4 and decreased to zero with further increase in ratio to 1.5. More sucrose interacted with gelatinized than raw starch.

INTRODUCTION

INTERACTIONS between constituents of a food can be of profound importance. Most foods contain much water at some stage in their biological development and/or processing so water can be very important to such interactions. The hydration of macromolecules in salt solutions is extremely complex (Neale and Williamson, 1956; Schumaker and Cox, 1961). Bull and Breese (1970) found ion-water-protein interactions at relatively high salt concentrations and estimated the rates of water and solute bound on egg albumin by an isopiestic method.

In case of the NaCl-water-casein system (Gal, 1975; Gal and Hunziker, 1977; Gal and Singer, 1965), an enhanced ionic-protein interaction was reported. Their explanation was based on the hypothesis that NaCl existed in an equilibrium among crystalline, amorphous, bound and dissolved phases. This was supported by similar experiments in other systems, such as NaCl and soy protein (McCune, 1981), NaCl and paracasein (Hardy and Steinberg, 1984) and others (Leeder and Watt, 1974; McLaren and Rowen, 1951; Bull, 1944). It was concluded that the amount of salt bound by a protein increased with increasing salt concentration to a maximum and remained constant upon further addition of salt; this salt binding capacity depended on moisture content.

Preferential interaction of proteins with salts and sugars in concentrated solutions was found by Arakawa and Timasheff (1982). It has been well recognized that there is a strong chemical reaction between proteins and reducing sugars in many food systems such as cornmeal proteins and lactose and sucrose (Racicot et al., 1981) and between casein and sugars (Minson et al., 1981; Lonergan et al., 1981).

Solute-solute interaction has been studied in a carbohydrate system. Janado and Nishida (1981) reported increased solubilities of hydrophobic solutes such as octanol and heptanol as the concentration of sugars increased. This was explained on the basis that as sucrose concentration increased, a_w decreased, causing a reduction in number of hydrogen bonds and an enhnacement of nonpolar interactions.

Authors Chinachoti and Steinberg are affiliated with the Dept. of Food Science, Univ. of Illinois, Urbana, IL 61801.

Various charged carbohydrate macromolecules, e.g. gums, have been reported to show strong interactions with sugars as well as salts, even in gels at the high a_w of 0.99 (Rey and Labuza, 1981). The effect of sugars on physical and chemical properties of a food system has been shown to be related to their a_w suppression properties (Spies and Hoseney, 1982; Hester et al., 1956; Baxter and Hester, 1958; Salem and Johnson, 1965; Ghiasi et al., 1982).

The objective was to study the sucrose-starch-water system in order to test for interaction between sucrose and starch as determined from a reduction in water sorption. Most foods containing starch have been heated so the starch has been gelatinized. Solutes such as oligosaccharides have been found to enter the gel phase of starch and interact there (Brown and French, 1977). Therefore, it was important to study interaction in gelatinized as well as raw starch.

MATERIALS & METHODS

Materials

Corn starch (Argo, Best Foods, Inc., Englewood Cliffs, NJ), 11.1% moisture w.b. was used in both the crystalline and gelatinized forms. Gelatinized starch was prepared by adding 200g distilled, deionized water to 10g starch and heating at 100°C for 30 min. Microscopic observation showed that gelatinization was more than 95% complete. Sucrose was analytical grade (Mallinckrodt, Inc., St. Louis, MO).

Corn starch and sucrose were used to prepare eight samples as follows: (1) starch was mixed with deionized, distilled water (2g water to 1g starch), frozen at −40°C and freeze-dried (room temperature, about 3 microns Hg pressure, 24 hr); (2) crystalline sucrose; (3) freeze-dried sucrose prepared by dissolving crystalline sucrose in sufficient deionized, distilled water to make a 20% w.b. solution, frozen and freeze dried as above; (4) starch and sucrose (dry) were mechanically mixed in the proportion of 9:1; (5) starch was mixed with water as above and a sufficient amount of the 20% sucrose solution described above was added to give sucrose: starch ratio d.b. of 1:9. The two liquids were mixed by stirring at room temperature to minimize the possibility of heat effects, equilibrated for 2 hr, frozen and freeze-dried as above; (6) same as 5 but in 2:8 ratio; (7) same as 5 but in 1:1 ratio; (8) same as 5 but in 9:1 ratio.

All freeze-dried samples showed a vacuum oven moisture between 3 and 5%.

Sorption isotherms

Each of the 8 dry samples was equilibrated against 9 saturated salt solutions at 25°C (Stokes and Robinson, 1949; Greenspan, 1977) to obtain a sorption isotherm over the range 0.33–0.93 a_w. The cell used was a modification of the PEC (Lang et al., 1981) in that the sample was placed in a 10 mL weighing bottle held in a well at the bottom while the saturated salt solution was outside the well. Moisture content was determined from weight gain. Each time the bottle was weighed, the sample was stirred with a tiny rod that had been tared with the bottle. Duplicate determinations were made at each a_w; the deviation from the mean ranged from 1.2 ± 0.5% at low moisture content to 12.4 ± 1.0% at high moisture content. Therefore, only averages are presented here.

RESULTS & DISCUSSION

Raw starch, sucrose, and a mechanical mixture

The starch (sample 1) showed a linear isotherm (Smith, 1947); the line parameters are in Table 1.

Crystalline sucrose (sample 2) sorbed a negligible amount of water at each a_w below 0.86 while at higher a_w, the data points fell on a smooth curve (Fig. 1) which intersected the 0.86 a_w line at 0.5g water/g sucrose, comparing favorably with the reference (Dittmar, 1935) concentration for a saturated sucrose solution at this temperature.

Sorption data for sample 4 (Fig. 2) gave two straight lines of different slopes, one below 0.86 a_w and another above. The connection was made by a vertical line at 0.86, the saturation a_w for sucrose, based on Fig. 1, where sucrose alone showed a vertical line at this a_w. Thus, the lower line shows sorption by starch alone, and the upper line shows sorption by sucrose as well as by starch.

Lang and Steinberg (1980) developed a mass balance equation based on the premise that the moisture content of a mixture was equal to the sum of the moisture contents sorbed by the components. They tested the equation at three a_w levels with each of three mechanical sucrose-starch mixtures (1:9, 2:8, and 4:6) and found that the calculated moisture contents agreed with the experimental values within about 2%. They concluded that the components sorbed independently of each other. Using the data for sucrose and starch in Table 1, the data in Fig. 1 were subjected to the same test. The discrepancy between calculated and experimental moisture contents for the eight a_w ranging from −2.4 to +3.4%, led to the same conclusion.

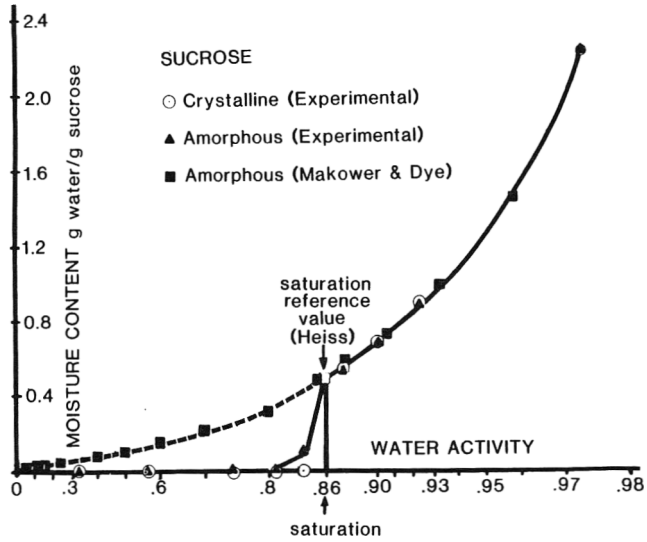

Fig. 1—Sorption isotherm data at 25°C for crystalline and amorphous sucrose.

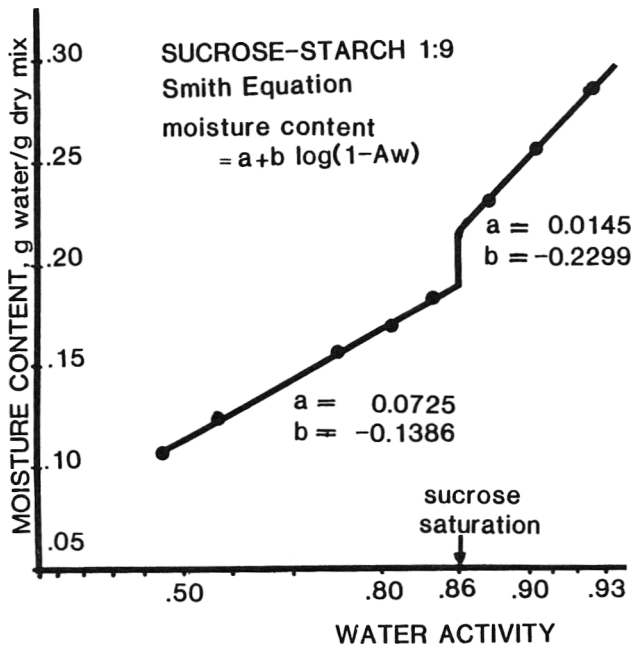

Fig. 2—Sorption isotherm data at 25°C for sucrose-starch 1:9 prepared by mixing the dry components.

Freeze-dried mixtures

Fig. 3 shows the data for these freeze-dried mixtures as well as for freeze-dried starch (sample 1) and freeze-dried sucrose (sample 3). These data were subjected to the same mass balance test as the mechanical mixtures. The 5 points of experimental and calculated values for the 2:8 mixture (sample 6) at a_w *below* 0.86 fell on the same line (Fig. 4). The calculated values were obtained assuming that sucrose did not bind water. Thus, none of the sucrose sorbed water as predicted by the mass balance equation which assigns zero sorption to sucrose at a_w below 0.86. Also, the starch sorbed its expected, or normal, amount of water.

Table 1—Comparison of calculated and experimental values for the linear regression line parameters of the Smith isotherms (25°C). Moisture content = a + b log (1 − a_w)

Sucrose:starch ratio	Intercept a		Slope b		Exp. Corr. Coef. r
	Calc.	Exp.	Calc.	Exp.	
Water activity range 0.329-0.860					
1:9	0.073	0.069	−0.144	−0.155	0.997
2:8	0.065	0.062	−0.128	−0.126	0.965
1:1	0.040	0.054	−0.080	−0.055	0.983
9:1	0.008	0.011	−0.016	−0.011	0.997
0.36-0.925					
starch	–	+0.081	–	−0.160	0.998
1:9	−0.016	−0.010	−0.303	−0.247	0.999
2:8	−0.116	−0.272	−0.449	−0.563	0.999
1:1	−0.411	−0.299	−0.881	−0.737	0.996
9:1	−0.805	−0.560	−1.459	−1.247	0.999
sucrose	–	−0.903	–	−1.603	0.998

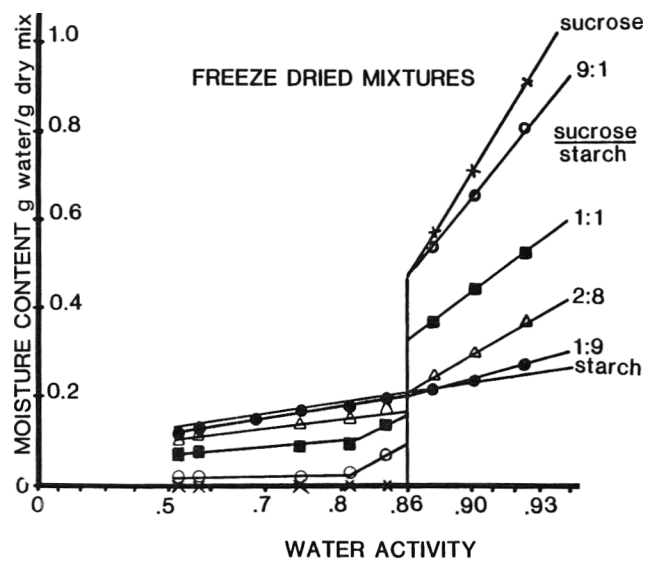

Fig. 3—Sorption isotherm data at 25°C for solids obtained by freeze drying solutions of sucrose, starch and four sucrose-starch mixtures.

However, there was a marked discrepancy between experimental and calculated moisture content at a_w *above* 0.86 (Fig. 4); the experimental value was below that predicted by the mass balance. The comparison was repeated for the other mixtures and the data are summarized in the form of Smith plot parameters in Table 1. The experimental moisture content was always below the calculated. Evidently, one component was not sorbing its full measure of water. Since starch sorbed its full complement of water at a_w below 0.86, as discussed just above, it was concluded that starch continued to sorb its full complement of water at a_w above 0.86. Therefore, sucrose was the component not sorbing its full complement of water, i.e., some of the sucrose had interacted with the starch and thus lost its water binding ability.

Water sorption by sucrose component

The water sorbed by the sucrose was taken to be the total water less the water sorbed by the starch. The Smith plot parameters for the a_w range 0.86–0.925 (Table 1) were used to calculate the experimental moisture content of the mixture for a number of a_w values at 0.02 increments. From Smith plot line parameter data for starch alone (Table 1), the moisture bound by the starch in 1g mixture at each a_w was similarly calculated. The difference between these two values gave the water sorbed by the sucrose in 1g mixture. This was divided by the weight ratio of sucrose to mixture to obtain g water bound by sucrose per g total sucrose. The ratio of total sucrose to mixture was divided by ratio of starch to mixture to obtain the ratio of total sucrose to total starch.

Sample calculation of the amount of interacted sucrose for a sucrose-starch 2:8 mixture at a_w 0.92:

(1) Water sorbed by the mixture (M_m):

$$M_m \text{ g water/g mixture} = a_m + b_m \log (1 - 0.92) \text{ (Smith, 1947)}$$

From Table 1, a_m = intercept for the mixture = −0.272, b_m = slope for the mixture = −0.563; M_m = 0.3456 g water/g mixture.

(2) Water sorbed by starch in the mixture (M_{st}):

$$M_{st} \text{ g water/g starch} = [a_{st} + b_{st} \log (1 - 0.92)]$$

From Table 1, a_{st} = intercept for pure starch = +0.081; b_{st} = slope for pure starch = −0.160; M_{st} = 0.2565 g water/g starch

$$0.2565 \frac{\text{g water}}{\text{g starch}} \times 0.80 \frac{\text{g starch}}{\text{g mix}} = 0.2052 \frac{\text{g water}}{\text{g mix}}$$

Therefore, water sorbed by the sucrose in the mixture is $M_m - M_{st} = 0.3456 - 0.2052 = 0.1404$g water/g mixture;

$$= \frac{0.1404 \text{ g water}}{\text{g mix}} \times \frac{1 \text{ g mix}}{0.2 \text{ g sucrose}} = 0.702 \text{ g water/g sucrose}$$

This was plotted in Fig. 5 on the Y axis above its mixture ratio of 2/8 = 0.25 on the X axis for a_w = 0.92. All other points on Fig. 5 were similarly obtained.

Fig. 5 shows that the water sorbed by the sucrose component was greatly affected by a_w, as expected. However, it was also affected by the sucrose-starch ratio at below 1.0. Each curve started at the origin and the water sorbed by each gram of sucrose at each a_w increased with increasing ratio. Above a ratio of 1.0, the curves remained horizontal at the same level as that shown by sucrose alone at the infinity mark on the ratio axis. If the sucrose in the mixture sorbed independently, we should expect each curve to be horizontal over the entire ratio range. Thus, at the low ratios, a unit weight of sucrose was not sorbing its normal complement of water because the sucrose had interacted with starch in some manner that reduced its water-binding capacity. This was extended to the idea that in a given mixture at a given a_w, a portion of the sucrose had interacted with starch and bound no water while the remaining sucrose was free to bind its normal complement of water. On this basis, the curves of Fig. 5 could be used to calculate the amount of interacted sucrose.

Interacted sucrose

Interacted sucrose was calculated as the total sucrose minus free sucrose. Free sucrose was calculated on the basis of total water bound by the sucrose component, M_s g water/g sucrose. Thus, free sucrose may be obtained from the ratio of *experimental* total water bound by sucrose in the mixture to amount of water *expected* if all the sucrose showed normal water-binding. The latter was obtained from the Smith plot parameters for sucrose in Table 1. Thus:

$$\text{free sucrose} = \frac{M_s \times S}{a_s + b_s \log (1 - a_w)}$$

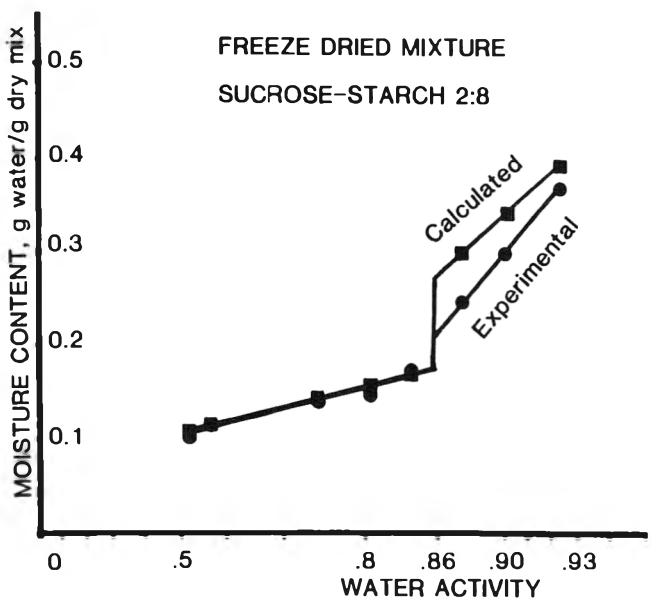

Fig. 4—Comparison of experimental and calculated (mass balance) isotherm data 25°C for one freeze-dried sucrose-starch mixture.

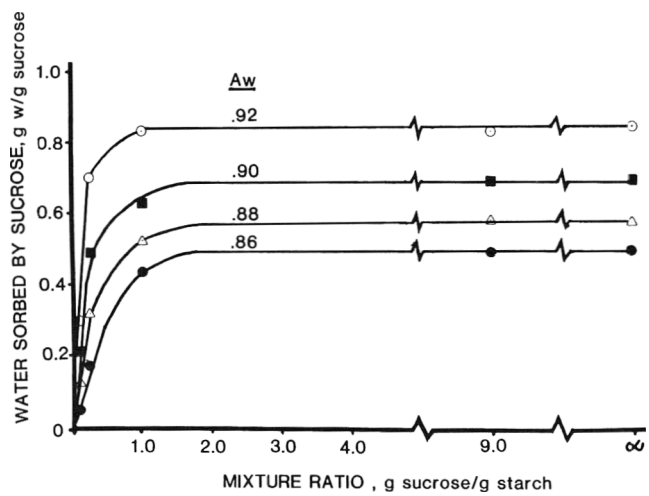

Fig. 5—Water sorbed by sucrose calculated as the difference between total water sorbed and water sorbed by starch as affected by mixture ratio and water activity.

where M_s = g *total water* sorbed by mixture/g sucrose; S = total sucrose in the mixture, g.

For example, Fig. 5 was entered at a mixture ratio of 0.4 and the water sorbed at a_w of 0.86 was found to be 0.26g water/g sucrose = M_s and S = 0.4g sucrose/g starch. Thus, free sucrose at this point

$$= \frac{0.26 \times 0.4}{-0.903 - 1.603 \times \log(1 - 0.86)} = 0.223g$$

and interacted sucrose = 0.4g − 0.223g = 0.177g. This value was plotted in Fig. 6 as 0.177g interacted sucrose/g starch against S = 0.4g sucrose/g starch on the 0.86 a_w curve. All other points were similarly obtained.

At each a_w, with increasing sucrose-starch ratio, interacted sucrose started at the origin, rose to a peak and fell back to zero (Fig. 6). Ascending and descending regression lines were calculated at each a_w. The intersection of these lines gave a "regression peak." The slopes and peak coordinates are given in Table 2. The peak occurred at the same total sucrose, about 0.35g, and its amplitude decreased by a factor of 5 with increasing a_w from 0.86 to 0.92. Both slopes also decreased with increasing a_w.

The interpretation of Table 2 is that interaction with starch was greatest (about 1/4g sucrose/g starch) at the lowest a_w where total sucrose concentration was the highest, i.e., moisture content was lowest. As the a_w increased, the moisture content increased, and this caused the equilibrium to shift away from interacted to free sucrose so that at 0.92 a_w only 1/20g sucrose remained interacted with 1g starch. Further analysis of the data showed a linear relationship between interacted sucrose and a_w with a slope of −3.07, Y intercept of 2.8738 and r value of 0.994. Extrapolation of this line indicated that interacted sucrose fell to zero at about 0.94 a_w.

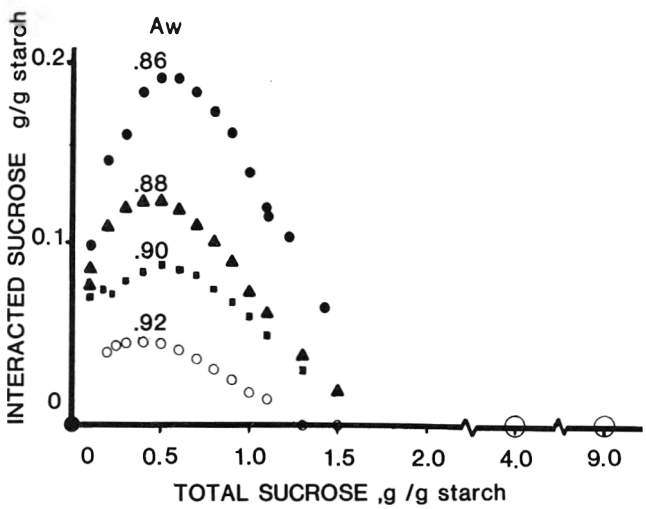

Fig. 6—Interacted sucrose in freeze-dried sucrose-starch mixtures as affected by mixture ratio and water activity.

At total sucrose concentrations below 0.4, interacted sucrose increased sharply with increasing total sucrose. There was an equilibrium between free and interacted sucrose such that the added sucrose induced a shift of the equilibrium from free to interacted sucrose. This resembles a typical interaction phenomenon, for instance, a strong ionic interaction such as that between protein and salt. In general, such strong interactions show a leveling off of the interaction vs concentration curve as a maximum interaction is obtained and the degree of interaction remains at the maximum level with further increase in reactant concentration (Bull and Breese, 1970; Gal, 1975; Gal and Singer, 1965; McCune and Steinberg, 1983; Hardy and Steinberg, 1983). Therefore, there are a limited number of binding sites on an ionic macromolecule to bind an ionic solute and the binding is irreversible. In contrast, at sucrose concentrations above 0.4, the present nonionic (sucrose-starch) system showed a rapid decrease in interaction; i.e., at higher sucrose concentration, the equilibrium moisture content was higher so that sucrose leaves the starch and enters into hydrogen bonding with the water. From this we can conclude that the sucrose-starch bond is weaker than hydrogen bonding. This is supported by Janado and Nishida (1981) who found that sugars and alcohols show some hydrophobic interactions.

Freeze-dried sucrose is amorphous and amorphous sucrose is highly hygroscopic (White and Cakebread, 1966; Mackower and Dye, 1956). It releases water upon crystallization (Iglesias and Chirife, 1978; Karel, 1973). Therefore, our explanation of the sucrose-starch interaction found here is that some of the amorphous sucrose formed during freeze drying interacted with the starch. The mechanical mixture did not show interaction because it contained no amorphous sucrose.

Gelatinized starch

The isotherm for gelatinized starch was linear between a_w 0.58 and 0.88. It showed the same moisture content as

Table 2—Effect of water activity on maximum amount of interacted sucrose and slopes of ascending and descending lines in Fig. 6

Water activity	Regression peak: g interacted sucrose/g starch	Regression peak: g total sucrose/g starch	Slopes g int. sucrose/g total sucrose: Ascending	Slopes g int. sucrose/g total sucrose: Descending
0.86	0.241	0.36	0.639	−0.188
0.38	0.160	0.33	0.424	−0.124
0.90	0.113	0.41	0.275	−0.090
0.92	0.052	0.28	0.181	−0.044

Table 3—Line parameters for experimental and calculated Smith plots, where moisture content (dry basis) = A + B log (1 − a_w), for sorption by mixtures of sucrose and either gelatinized or raw starch at a_w 0.86 − 0.93 and 25°C. Values at 0.90 a_w given to facilitate comparison

Sucrose/Starch ratio	Experimental: A intercept	Experimental: B slope	Experimental: Moisture content at 0.90 a_w (g water/g mixture)	Calculated: A intercept	Calculated: B slope	Calculated: Moisture content at 0.90 a_w (g water/g mixture)	Discrepancy at 0.9 a_w (g water/g mixture)
Gelatinized starch							
1:9	−0.1017	0.3458	0.244	−0.0093	−0.2892	0.280	0.036
2:8	−0.3008	−0.5755	0.275	−0.1085	−0.4350	0.327	0.052
1:1	−0.3262	−0.7652	0.439	−0.4064	−0.8729	0.467	0.028
8:2	−0.4329	−1.0262	0.593	−0.7043	−1.3108	0.607	0.014
9:1	−0.7517	−0.4033	0.652	−0.8039	−1.4571	0.653	0.001

Table 4—Effect of water activity on maximum amount of sucrose interacted with starch and on sucrose-starch ratio at this maximum: comparison of gelatinized with raw starch

	Ordinate g interacted sucrose/g starch		Abscissa g total sucrose/g starch		Ratio ordinate:abscissa g interacted sucrose/g total sucrose		
Water activity	Gelatinized	Raw	Gelatinized	Raw	Gelatinized	Raw	Diff
0.86	0.30	0.24	0.38	0.36	0.80	0.67	0.13
0.88	0.19	0.16	0.34	0.33	0.57	0.49	0.08
0.90	0.15	0.11	0.43	0.41	0.34	0.28	0.06
0.92	0.11	0.05	0.47	0.28	0.23	0.19	0.04

raw starch at a_w 0.58, a slightly lower moisture at a_w 0.88 and the same at a_w 0.902.

The experimental and calculated data for mixtures are in Table 3. Plots of experimental data were similar among mixtures and between gelatinized and raw starch for each mixture. The interacted sucrose calculations are in Table 4.

The effect of starch gelatinization on sucrose interaction was determined by comparing points of maximum interaction between mixtures with gelatinized and raw starch. The amount of interacted sucrose (ordinate) was higher in case of gelatinized starch at each a_w and this decreased sharply with increased a_w in both cases. The quotient of the peak ordinate and abscissa values in Table 4 gave the ratio of interacted to total sucrose at each a_w, also in Table 4. These ratios were higher for gelatinized starch at each a_w but the difference decreased with increasing a_w. This difference between gelatinized and raw starch was explained on the basis that gelatinization of starch converts some of its crystalline amylopectin fraction to the amorphous form (van den Berg, 1981).

SUMMARY

INTERACTION between sucrose and starch was calculated from a decrease in water sorption as compared to that obtained when each component sorbs independently. A mechanical mixture showed no interaction, but interaction was observed in a wetted and freeze-dried mixture. This was due to presence of amorphous sucrose in the freeze-dried samples but not in the mechanical mixtures. This interaction was strongly a_w dependent; at a given sucrose-starch ratio, it increased with decreasing a_w; at constant a_w, it increased with sucrose-starch ratio to a maximum at 0.5 and then decreased. The increased moisture content obtained upon equilibration of the high sucrose samples caused the sucrose to interact less with starch and more with water. Gelatinized starch showed more interaction with sucrose than did raw starch.

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A Research Note

A Simplified Method for Determination of Browning in Dairy Powders

RAHAMIM PALOMBO, ARIEH GERTLER, and ISRAEL SAGUY

ABSTRACT

An improved method for the determination of nonenzymatic browning in dehydrated dairy powders is described. The method involves liberation of the brown pigments from the protein molecules by means of a proteolytic enzyme, pronase. For complete proteolysis, the pronase mixture (2.53 mg enzyme/0.1g dry powder) was incubated at 45°C for 2 hr. After clarification, the browning index (defined as the optical density difference measured at 420 nm and 550 nm) was determined spectrophotometrically. The suggested method proved to be straightforward, easy to employ, and showed high accuracy and reproducibility. The procedure is suitable for routine laboratory analyses and its repeatability, as expressed by the coefficient of variation, was below 3%.

INTRODUCTION

FOODS are very sensitive and susceptible to quality losses due to chemical instability which depends on compositional and environmental factors. One reaction causing major food quality losses is nonenzymatic browning, known as the Maillard reaction. It may lead to undesirable deterioration due to the formation of chemically stable and nutritionally unavailable derivatives known as melanoidins (Karel et al., 1975; Mauron, 1981).

Dairy powders are most sensitive to nonenzymatic browning as they contain relatively high concentrations of lactose and proteins with high lysine level. In addition relatively high temperatures and water content during processing and prolonged storage are the major factors involved in the high susceptibility of dehydrated dairy products, as they are favorable conditions for the Maillard reaction (Bender, 1972; Henry et al., 1948; Labuza, 1972).

A method to determine nonenzymatic browning in dry milk products, which involves proteolysis of the protein molecules by means of a proteolytic enzyme, was reported by Choi et al. (1949). Labuza and Saltmarch (1981) improved the method by using a mixture of three enzymes which increased the proteolysis. These methods were designed to overcome the main factor limiting the extraction of the brown pigments from the dairy products which is related to the formation of unextractable complexes of protein pigments (Goldblith and Tannenbaum, 1966).

In the course of kinetic studies of browning deterioration of dairy products, presently undertaken by the authors, it was observed that the recommended methods published in the literature suffer from several drawbacks which hamper the receiving of reproducible nonenzymatic values in dairy powders.

This investigation was undertaken with the aim of developing a modified method which would provide better accuracy and reproducibility, and be suitable for routine laboratory work on nonenzymatic browning determination of dairy powders.

Authors Palombo and Gertler are affiliated with the Dept. of Agricultural Biochemistry, The Hebrew Univ. of Jerusalem, Faculty of Agriculture, Rehovot, 76-100 Israel. Author Saguy is affiliated with the Dept. of Food Technology, Agricultural Research Organization, the Volcani Center, Bet Dagan 50-250, Israel.

MATERIALS & METHODS

Dairy powders

Four commercial dairy powders were tested: (a) Whole milk powder – (Tnuva, Israel; 26.4% protein, 27.5% fat and 38.2% sugars); (b) Coffee whitener (DMV De Melkindustrie Veghel, BV-15 Veghel-Holland; 8% protein, 35% emulsified vegetable fat, and 51% sugars); (c) Whey powder – (DMV De Melkindustrie Veghel, BV-15 Veghel-Holland; 14.2% protein, 0.8% fat and 76.35 sugars); and (d) "Materna" (Maabarot Products Ltd., Maabarot, Israel; a spray-dried infant's first months milk powder; 11.1% protein, 25.9% fat and 55.5% lactose).

Pronase method

The proposed method was based on pronase proteolysis. The procedure was as follows: 1g of the dairy powder was dispersed in 5 mL deionized distilled water at 45°C. The mixture was mixed thoroughly and 1.5 mL was transferred to a 2.5 mL test tube which contained 0.4 mL of Pronase (Calbiochem-Behring P-53702; 45,000 P.U.K/mg) solution (10 mg enzyme/mL buffer tris, pH 7.00, with 50 mM $CaCl_2$), yielding ultimately 2.53 mg enzyme/0.1g dry powder. The test tubes were placed on a rack and incubated for 120 min at 45°C in a water bath. The rack was cooled in ice water and 150 μL trichloroacetic acid (100% TCA) were added to each tube. Centrifugation (20 min at 7000 rpm) and filtration (Whatman No. 1 filter paper) were used for clarification prior to the spectrophotometric determination.

Browning index

The optical density of the clear filtrates was determined on a Gilford (Model 250; Oberlin, OH) spectrophotometer. Samples were read in a 1 mL cuvette with 1 cm pass length. Water was used as blank. Browning index, OD, was calculated as:

$$OD = A_{420nm} - A_{550nm}$$

For practical purposes the browning index was expressed as OD/g dry solids.

RESULTS & DISCUSSION

THE OPTICAL DENSITY (OD) after an incubation period of 120 min and up to 240 min was similar at each time interval (30 min). Hence, for practical reasons 120 min reaction time at 45°C was adopted as a standard for the present study.

The relatively high incubation temperature (45°C) was utilized to comply with previous reports (Choi et al., 1949; Labuza and Saltmarch, 1981). Furthermore, only an insignificant decrease in browning was observed when incubation was done at lower temperatures. On the other hand, at 45°C the reaction time was reduced to a minimum, and thus determination efficiency was improved.

To assess the reproducibility of the suggested browning determination method, eight separate determinations were carried out on a typical dairy powder. The product chosen was the "Materna" powder (Table 1). For all the other dairy powders, four replicate determinations were carried out (Table 2). Also, in order to increase the browning index significantly, and to cause high protein denaturation, all the powders were severly heat treated. These severe conditions were used to test the ability of the pronase to proteolyze highly denatured proteins, thus simulating conditions

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A Research Note

Incidence of Enterococcal Thermonuclease in Milk and Milk Products

V. K. BATISH, HARISH CHANDER, and B. RANGANATHAN

ABSTRACT

Out of 728 enterococcal isolates recovered from 208 samples of milk and milk products, 216 isolates (29.7%) produced deoxyribonuclease (DNase), but among them only 31 (4.3%) were capable of exhibiting thermonuclease (TNase) activity. The incidence of DNase positive enterococci was maximum (63.4%) in Kulfi mix, but the number of thermonuclease positive enterococci was maximum (9.6%) in nonfat dry cow's milk. DNase positive enterococci were encountered in 78 (37.5%) samples, whereas only 22 (10.6%) of the samples showed the presence of thermonuclease producing enterococci. Although 30 samples (14.4%) contained staphylococcal TNase, thermonuclease of nonstaphyloccal origin was actually detected in 7 such samples. The latter, however, did not contain either *Staphylococcus aurcus* or their enterotoxins.

INTRODUCTION

THE PRODUCTION of deoxyribonuclease (DNase) by Streptococci groups A, B, C, F, G and L was first reported by Tillett et al, (1948), Brown (1950), and Gudding (1979). Later, group D Streptococci (Enterococcus) were also included in the list (Thomas and Nambudripad, 1974) Batish et al, 1982). However, in the latter case, many of the enterococcal strains recovered from dairy products produced DNase that resisted boiling for 15 min, indentical to thermonuclease (TNase) of *Staphylococcus aureus* (Bissonnetti et al., 1980; Park et al., 1982; Batish et al., 1982) and hence, they may interfere with the TNase test frequently used for the screening of foods for the presence of staphylococcal enterotoxins (Lachica et al., 1969; Tatini et al., 1975; Batish et al., 1978). In order to find the possibility of false reaction in the conventional TNase test due to enterococcal DNases, the incidence of DNase and TNase positive enterococci in milk and milk products sampled from various sources has been determined in the present investigation. Correlation of the occurrence of TNase positive enterococci with presence of TNase in these samples is also carried out.

MATERIALS & METHODS

Collection and analysis of samples

Samples of milk and milk products, including raw and pasteurized cow's milk, sweet cheese, cheddar cheese, butter, Kulfi (a frozen dairy product similar to ice cream but made from milk concentrated in open container without whipping), kulfi mix, ice cream, sweetened condensed milk and nonfat dry cow's milk and infant foods (spray dried), were collected aseptically from the local market as well as from the Institute's experimental dairy and examined for the presence of enterococci using citrate azide agar (Saraswat et al., 1965) after adjusting the concentration of sodium chloride at 6.5% and final pH at 9.6. All isolates were subjected to group D serology (Fuller, 1938) as well as Sherman's criteria (Sherman, 1937) to determine if they were 'true' enterococci. For further characterization of enterococci, the procedure of Deibel and Seeley (1974) was followed.

Authors Batish, Chander, and Ranganathan are affiliated with the Dairy Microbiology Division, National Dairy Research Institute, Karnal (Haryana) 132 001, India.

DNase activity

Enterococcal isolates were grown in Todd-Hewitt broth for 18 hr at 37°C and tested for DNase using methyl green DNA agar of the following composition: Sodium salt of calf thymus DNA (Sigma), 10 mg; 0.5% methyl green (BDH), 0.5 mL; 0.01M calcium chloride (BDH), 0.1 mL; 0.01M magnesium chloride (BDH), 0.1 mL; sodium chloride (BDH); 10g; noble agar (Difco), 10g, and 0.2M veronal buffer (pH 7.0), 100 mL. For determination of both DNase and TNase activities of enterococci, the method of Batish et al. (1982) was followed.

Differentiation of thermonuclease source

For confirming the presence of TNase of staphylococcal as well as nonstaphylococcal origin in samples showing TNase positive enterococci, the extraction procedure of Tatini et al. (1975) as modified by Batish et al. (1982) was followed. TNase extracts were prepared from the samples at pH 4.5 as well as at pH 3.8. TNase activity in these preparations was recorded both on toluidine blue DNA agar (Lachica et al., 1971) as well as on methyl green DNA agar media after heating the extracts at 100°C fro 15 min and 1 hr, respectively, using the procedure described above. The extraction of TNase of staphylococcal origin from the samples at pH 3.8 was very useful for their differentiation from TNases of nonstaphylococcal origin, since the latter could be extractable at the said pH. TNases of *S. aureus* were only slightly affected when heated at 100°C for 1 hr while TNases of enterococci were completely inactivated. TNases of enterococci, on the other hand were active when the pH of the DNase assay medium was adjusted to pH 6.7 as well as to pH 9.0. Staphylococcal TNase was not active at pH 6.7.

Detection of staphylococcal enterotoxins

From the TNase positive samples, staphylococcal enterotoxins were estracted by the method of Casman (1967). The microslide gel diffusion test (Casman et al., 1969) was used to detect extracted enterotoxin, with standard antisera supplied by Dr. M.S. Bergdoll.

Table 1—Distribution of deoxyribonuclease (DNase) positive enterococci isolated from milk and milk products

Type of sample	No. of samples	No. of entero-cocci tested[a]	No. of entero-cocci showing DNase activity	No. of entero-cocci showing TNase activity
Raw cow's milk	23	90	23(25.5)	3(3.3)
Pasteurized cow's milk	17	33	4(12.1)	0
Sweet cheese	10	36	9(25.0)	0
Cheddar cheese	25	105	53(50.5)	3(2.8)
Processed cheese	10	0	0	0
Butter	20	14	0	0
Kulfi	20	55	26(47.3)	1(1.8)
Ice cream	10	38	6(51.8)	0
Sweetened condensed milk	10	41	9(21.9)	12(4.8)
Nonfat dry cow's milk	27	125	41(32.8)	12(9.6)
Infant food	16	150	19(12.7)	9(6.0)
Total	208	728	216(29.7)	31(4.3)

[a] The number of enterococci tested equal the number isolated. Figures in parentheses indicate percentage.

Table 2—Incidence of samples of milk and milk products positive for thermonuclease (TNase)

Type of sample	No. of samples tested	No. of samples positive for staphylococcal TNase[a]	Type of staphylococcal enterotoxin detected	No. of samples positive for nonstaphylococcus (enterococcal) TNase
Raw cow's milk	23	6(26.7)	A, C, D	1(4.3)
Pasteurized cow's milk	17	1(5.9)	A	0
Sweet cheese	10	1(10.0)	A	1(10.0)
Cheddar cheese	25	4(16.0)	A, B	1(4.0)
Processed cheese	10	0	–[b]	0
Butter	20	2(10.0)	A	0
Kulfi	20	3(15.0)	A, B, C	2(10.0)
Kulfi mix	20	2(10.0)	A, B	0
Ice cream	10	1(10.0)	A, B	1(10.0)
Sweetened cond. milk	10	0	–[b]	1(10.0)
Nonfat dry cow's milk	27	7(25.9)	A, B, D	0
Infant food	16	3(18.7)	A, B	0
Total	208	30(14.4)		7(3.4)

[a] Data in parentheses indicate percentage
[b] Not detected

RESULTS AND DISCUSSION

IN THE PRESENT INVESTIGATION, 29.7% of the enterococcal isolates examined were positive for DNase, with the highest incidence of such organisms (63.4%) observed in kulfi mix (Table 1). However, only 4.3% of the DNase positive enterococci were capable of producing TNase. The incidence of TNase positive enterococci was maximum (9.6%) in nonfat dry cow's milk. Infant foods and sweetened condensed milk samples also contained appreciable numbers of TNase positive enterococci. Recovery of TNase positive enterococci from dairy products examined in this study were in agreement with Thomas and Nambudripad (1974) and Batish et al. (1982) who also reported thermostable DNase producing enterococci in milk and milk products, especially in dried milk. The presence of TNase positive enterococci in such foods may be viewed with concern, since many of them are capable of inducing food poisoning syndromes in laboratory animal models (Batish et al., 1982).

DNase production by enterococci also has been reported by other workers. While surveying DNase production by microorganisms other than staphylococci, Park et al. (1980) reported some strains of *S. faecalis* produced DNase that were heat resistant. Bissonnetti et al. (1980) also reported TNase production by *S. faecalis* strains isolated from naturally contaminated cheese. Conversely, several other workers (Brown, 1950; Lachica and Deibel, 1969) failed to demonstrate DNase production among enterococci. The probable reason for the failure of some of these workers to demonstrate DNase production may be due to different assay conditions used for determining the enzyme activity. Those workers used staphylococcal TNase assay procedures for enterococcal DNases also. Methyl green DNA agar medium developed in this laboratory (Batish, 1982 was found to be suitable for detection of enterococcal DNases. Many of the enterococcal isolates that exhibited poor TNase activity on conventional Toluidin blue DNA agar used for the assay of staphylococcal TNases were capable of showing a good Tnase activity on methyl green DNA agar medium.

The TNases of staphylococcal origin were detected in 30 samples (14.4%), whereas TNase of nonstaphylococcal origin were found in 7 samples (3.4%) (Table 2). Two samples of Kulfi and one each of raw cow's milk, sweet cheese, cheddar cheese, ice cream, and sweetened condensed milk were positive for enterococcal TNase. Samples containing enterococcal TNases did not contain either *S. aureus* or their enterotoxins. However, they did contain more than 10^5 cells of TNase positive enterococci per mL or g, thereby suggesting enterococcal nature of TNase in these samples. On the other hand, all of the 30 samples positive for staphylococcal TNase also contained staphylococcal enterotoxin, A, B, C or D. A high population of TNase positive *S. aureus* (10^6 cells per mL or g) were recorded in these samples, except in one pasteurized cow milk and three infant food samples which appeared to contain performed staphylococcal enterotoxins and TNase. In view of the presence of nonstaphylococcal TNase in foods, samples exhibiting positive TNase may lead to an incorrect interpretation with regard to contamination by *S. aureus*. Hence, due consideration must be given to nonstaphylococcal TNase potentially present in the food products, before finally interpreting the results on the basis of TNase test.

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A Research Note

Nondestructive Color Measurement of Fresh Broccoli

R. L. SHEWFELT, E. K. HEATON, and K. M. BATAL

ABSTRACT

A major limitation in the colorimetry of fresh broccoli has been the curvature of the head. Total chlorophyll concentration and visual appearance have thus been the methods of choice for color measurement. Samples from six cultivars selected for obvious visual color differences were presented to an older (Gardner C-4) and a newer (Gardner XL-845) generation colorimeter using both whole head and shaved floret sample presentations. No significant differences were observed in the total chlorophyll concentrations of the cultivars, but distinction was made among the cultivars using the shaved floret presentation for the older model and the whole head presentation for the newer model. Newer generation colorimeters thus provide a rapid nondestructive means of assessing fresh broccoli color.

INTRODUCTION

LOSS OF GREEN COLOR is a major limiting factor in shelf-life reduction in the storage of fresh broccoli. The most widely accepted means of evaluating broccoli color has been the subjective scale described by Lipton and Harris (1974) or Wang (1977). Chlorophyll analysis has been used by several investigators (Fuller et al., 1977; Wang, 1979; Batal et al., 1982) as an objective alternative, but it is a tedious, destructive technique. Colorimetry has been used in the past to evaluate broccoli color (Batal et al., 1982; Shewfelt et al., 1983) with limited success. The major limitation of past colorimetry studies has been the inability of the instruments to compensate for the curvature of the broccoli head. Samples were thus presented to the colorimeter as individual florets shaved from the head. Not only is this technique destructive, the underside of the florets start to yellow earlier than the surface facing the normal viewer giving an inaccurate representation of the whole head. The curvature leads to diffraction (or scattering) of the light which cannot be appropriately interpreted by the light collector (Francis and Clydesdale, 1975).

The field of colorimetry has advanced rapidly in the past few years and several instruments have been developed using fiber optics (Hammel, 1981) that are supposed to correct for the curvature of many fruits and vegetables. The purpose of this study is to determine if one of the newer generation instruments can provide an objective, nondestructive method for the color evaluation of fresh broccoli.

Authors Shewfelt and Heaton are with the Dept. of Food Science, Univ. of Georgia Experiment Station, Experiment, GA 30212. Author Batal is with the Dept. of Horticulture, Univ. of Georgia Coastal Plains Station, Tifton, GA 31793.

MATERIALS & METHODS

SIX CULTIVARS of broccoli (CVS 'Corsair', 'Emperor', 'Excalibur', 'Green Beret', 'Green Duke' and 'Southern Comet') grown in test plots at Tifton, GA were selected for marked differences in visual color at a similar level of maturity. From each cultivar six heads (four heads for the cultivar 'Emperor') were selected as representative, harvested within a 2-day period, cooled and top-iced. The heads were transported to Experiment, GA, a distance of approximately 150 miles, for the physical and chemical analyses. Samples were stored at 1°C with ice added when necessary until color measurements could be performed 1 wk after harvest.

Color measurements were performed on each whole head and then on florets shaved from each head using both the Gardner C-4 colorimeter, an older generation instrument, and the Gardner XL-845 colorimeter that employs fiber optics. Florets from each head were placed in appropriate sample cups and covered with a black nonglossy finished covering to protect from stray light during measurement. The instruments were adjusted using a green standard tile (L = 50.4, a = −25.2, b = +6.2). Derived functions for color difference (ΔE) and hue difference (ΔH) were calculated using the formulae:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

$$C = (a^2 + b^2)^{1/2}$$

$$\Delta H = [(\Delta E)^2 - (\Delta L)^2 - (\Delta C)^2]^{1/2}$$

where ΔL, Δa, Δb and ΔC are differences in the color values of each sample from the standard tile (Anon., 1979).

Total chlorophyll concentration was determined by the procedure of Lebermann et al. (1968) as modified by Batal et al. (1982). Statistical analysis was performed using the standard analysis of variance and Duncan's multiple range techniques (Helwig and Council, 1979).

RESULTS & DISCUSSION

THE SIX CULTIVARS used in the study provided a wide range of shades of green color with little variation of color between samples within a cultivar. A bluish tinge, common to certain cultivars in late autumn, was apparent in the 'Emperor' and 'Excalibur' samples.

Table 1—Total chlorophyll and instrumental color values of six cultivars of fresh broccoli using the Gardner C-4 colorimeter with shaved florets presentation and the Gardner XL-845 colorimeter with the whole head presentation (values represent a single measurement on each of four heads for 'Emperor' and six heads for each of the other cultivars)

Cultivar	Total chlorophyll (mg/100g)	Gardner C-4 (shaved florets)				Gardner XL-845 (whole head)			
		L	ΔE	ΔH	$\tan^{-1}$b/a	L	ΔE	ΔH	$\tan^{-1}$b/a
Corsair	27.0[a]	38.8[b]	22.2[a]	16.5[b]	119.8[a]	29.2[b]	28.5[ab]	9.4[ab]	133.2[c]
Emperor	25.4[a]	45.3[a]	20.6[bc]	18.8[a]	116.8[a]	41.9[a]	25.7[c]	14.0[a]	230.8[a]
Excalibur	25.5[a]	44.0[a]	20.2[bc]	17.2[b]	118.8[a]	34.1[b]	29.0[a]	10.9[ab]	191.4[b]
Green Beret	24.5[a]	42.9[a]	21.0[b]	17.6[b]	118.5[a]	32.7[b]	26.3[bc]	8.0[bc]	147.3[c]
Green Duke	24.0[a]	43.2[a]	20.7[bc]	17.4[b]	118.0[a]	33.5[b]	26.0[bc]	8.5[abc]	153.4[bc]
Southern Comet	28.6[a]	45.7[a]	19.6[c]	17.8[b]	119.8[a]	32.2[b]	28.0[abc]	3.7[c]	167.3[bc]

[a-c] Means within a column with the same letter are not significantly different from each other ($p \leq 0.05$).

Despite the obvious differences in visual appearance, no significant differences were detected in the total chlorophyll concentrations of samples from different cultivars (Table 1). It is apparent that factors other than chlorophyll concentration are contributing to the visual appearance of the broccoli.

No significant differences were observed in any of the instrumental color values when using the older colorimeter with the whole head presentation (data not shown). When the shaved florets were presented to this instrument, some distinction between cultivars was observed (Table 1). The function providing the greatest discrimination was ΔE. This instrument as well as other older instruments of similar design are apparently not able to compensate for differences in the curvature of the broccoli head.

The newer model detected significant differences between cultivars for L and ΔE values with the shaved floret presentation (data not shown). The whole head presentation using the newer colorimeter provided the greatest distinction between the cultivars observed in the study (Table 1). The widest range of differences was observed in the hue angle ($\tan^{-1} b/a$). The two cultivars evidencing a bluish tinge are separated from the rest by hue angles greater than 180° indicating a negative b value (greater blue than yellow character).

Although both colorimeter models were able to detect differences in the color of broccoli cultivars used in the study, the whole head presentation is clearly preferable to the shaved head presentation. The nondestructive nature of the whole head sample presentation provides multiple evaluation of the same heads during shelf-life studies. In addition, the surface evaluated objectively by the instrument and subjectively by the consumer are the same when the whole head presentation is employed. Thus the newer generation colorimeters provide a rapid, nondestructive alternative to chlorophyll analysis in the objective measurement of broccoli color.

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DETM OF BROWNING IN DAIRY POWDERS . . . From page 1609

Table 1—Browning index (OD/g dry solid) reproducibility (eight replicates) in "Materna" powder exposed to various heat treatments

Heat treatment (hr at 90°C)	Average	Standard deviation	Coefficient of variation (%)
–	0.075	0.002	2.9
50	0.183	0.003	1.6
100	0.296	0.003	1.1

Table 2—Browning index (OD/g dry solid; average + standard deviation; four replicates) in several dairy powders exposed to various heat treatments

Heat treatment (hr at 90°C)	Whole milk	Coffee whitener	Whey powder
–	0.200+0.006	0.067+0.002	0.745+0.006
50	0.508+0.007	0.548+0.003	1.192+0.017
100	0.856+0.002	1.346+0.010	1.577+0.051

which may be pertinent during "high temperature-long time" processes.

The nonenzymatic browning data derived (Table 1) showed that the coefficient of variation for all the browning indexes was below 3%. This low variability indicated that the method suggested was highly reproducible and accurate. Furthermore, the derived data (Tables 1 and 2) showed that the method was not affected by protein denaturation. Similar repeatability was reported for browning determination in citrus products (Meydav et al., 1977).

As the dairy powders analyzed contained a wide range of protein concentrations (8-26%) typical to other similar products, it may be assumed that the enzyme concentration used would be effective to liberate the brown pigments in other dairy products.

In conclusion, the suggested method for determination of nonenzymatic browning in dairy powders proved to be straightforward, easy to employ and to be highly reproducible. Furthermore, the procedure is suitable for routine laboratory analyses and its repeatability, as expressed by the coefficient of variation, was below 3%.

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A Research Note

Improved Sieve Method for Measurement of Lyophoresis in Applesauce

J. N. NOGUEIRA and M. R. McLELLAN

ABSTRACT

Measurement of lyophoresis in applesauce may yield erroneous results due to variability in a previously accepted sieve method. To overcome this problem an improved sieve method is proposed in which the techniques and equipment were selected to give more accurate results over a larger range of applesauce types. Besides accuracy and simplicity, this new technique provides differentiation of samples where measurement of lyophoresis with the earlier method is barely detectec. This new method with increased sensitivity brings a significant improvement to a technique familiar to the apple processing industry.

INTRODUCTION

CONSISTENCY is an important attribute of quality in applesauce. The U.S. standards for grade of canned applesauce (1982) allocates 20 points out of 100 to this factor of quality. However, evaluation of this attribute in applesauce is not as simple as it is for other food products.

Toldby and Wiley (1962) recommended that consistency in applesauce be determined objectively by measuring separately the lyophoresis of the sauce and the mounding of the mass of sauce. Mounding of applesauce can be easily evaluated with the Adams consistometer. Although a few methods are available for measurement of lyophoresis in applesauce (Toldby and Wiley, 1962; Powers et al., 1978; Drake et al., 1979) none of them seems to be completely adequate in terms of simplicity, accuracy and/or time involved.

The objective of this study was to develop a simple and accurate procedure for measurement of lyophoresis in applesauce. The direction of the work was to study modifications on several steps of the sieve method described by Toldby and Wiley (1962).

MATERIALS & METHODS

LYOPHORESIS of different applesauce samples was measured using the original sieve method (Toldby and Wiley, 1962) and an improved technique developed for comparison. Three applesauce samples were prepared for evaluation in this study. The preparation of these samples was such that the sauce produced would have different consistencies.

Sample 'A' was prepared from R.I. Greening apples immediately after harvest with a raw fruit firmness of approximately 20 lbf. These apples were processed under standard conditions by the method of LaBelle et al. (1960). The process was modified such that the finisher screen size was 0.158 cm (1/16in.) and the finisher impeller speed was 700 rpm. The average particle size in sample 'A' as determined by the method of Kimball and Kertesz (1952) was 0.4864 mm. Bostwick reading at room temperature was 3.9 cm.

Sample 'B' was prepared from Rome apples harvested and stored at 0°C until the fruit firmness dropped to approximately 15 lbf. These apples were then processed under the standard conditions with the following modification. The finisher screen was 0.238 cm (3/32in.) and the finisher impeller speed was adjusted to 900 rpm. The average particle size of sample 'B' was 0.7834mm and the corresponding Bostwick reading was 3.13 cm at room temperature.

Sample 'C' was prepared from R.I. Greening apples harvested and stored at 0°C until the fruit firmness dropped to approximately 10 lbf. These apples were then processed under the standard conditions with a 0.317 cm (1/8in.) finisher screen and a finisher impeller speed of 700 rpm. The average particle size of sample 'C' was 0.7626 mm and the corresponding Bostwick reading was 3.43 cm at room temperature.

Original sieve method (I)

The three different samples of applesauce (A, B and C), each in triplicate, were used for evaluation of this procedure. The following steps were taken: (1) 200g of applesauce were weighed into a No. 1 can; (2) A United States Standard Sieve No. 20, a glass funnel with a diameter of 11 cm, and a 25 mL measuring cylinder were placed on the top of the can; (3) The system was turned over in a laboratory stand and timing started; (4) Exactly 10 min later, the volume of liquid in the measuring cylinder was determined and recorded as a measure of lyophoresis.

Improved sieve method (II)

Another set of samples (A, B and C), in triplicate, were used to evaluate this procedure. The procedure developed for this method was as follows:

(1) The entire contents of a 303 can were well mixed. 200g were weighed into a 1000 mL glass beaker. [This beaker was cut to measure a height of 8 cm in order to allow the sauce to flow faster and give a larger contact area of the sieve to sauce.].

(2) A United States Standard Sieve No. 20, a glass funnel with a diameter of 12.5 cm, and a 25 mL measuring cylinder were placed on the top of the beaker.

(3) The complete system was turned upside down and placed in laboratory stand.

(4) After 5 min, the beaker was lifted leaving only the sauce on the screen. Care was taken to assure a uniform flow rate of the sauce to the sieve for each group of replicates.

(5) At exactly 10, 15, 20 and 25 min after the system was turned upside down, the volume of liquid was determined and recorded as a measure of lyophoresis. [This was done to select the most suitable time for measurement of lyophoresis.]

Coefficients of variability were calculated for the results obtained from both methods (I and II). Standard deviations were also determined for the data obtained for sample C with the improved method.

RESULTS & DISCUSSION

MEASUREMENTS of lyophoresis with the new sieve method presented a better degree of accuracy than the same measurements obtained with original sieve method (Toldby and Wiley, 1962), for all applesauce samples used in this experiment (Table 1). It is this increased accuracy which warrants this technique to be considered a significant improvement. This accuracy is based on methods and equipment which are better suited to allow lyophoresis to take place and to measure this lyophoresis over a broader range.

The coefficient of variability calculated for the results obtained from both methods indicated that the improved method, at any time of measurement, was better than the original method (CV = 27.6%). As also shown in Table 1 and Fig. 1, 20 min was the time for highest accuracy for lyophoresis measurement with the improved method (CV =

Author Nogueira, on sabbatic leave at the New York State Agricultural Experiment Station, is affiliated with the Dept. of Rural Technology of ESALQ-Univ. of Sao Paulo, 13400-Piracicaba, SP., Brazil.

Author McLellan is affiliated with the Dept. of Food Science & Technology, Inst. of Food Science, New York State Agricultural Experiment Station, Cornell Univ., Geneva, NY 14456.

Table 1—Measurement of lyophoresis with improved and original sieve methods

Sample	Rep	Improved method (mL) 10 min	15 min	20 min	25 min	Original method (mL) 10 min
A	1	5.4	8.6	10.9	12.0	0.0
	2	5.4	9.6	11.1	12.6	<1.0
	3	4.2	7.7	10.8	12.2	<1.0
B	1	17.0	19.6	21.8	22.4	5.1
	2	16.3	19.0	21.2	22.0	3.3
	3	16.4	18.9	21.0	22.1	3.1
C	1	17.0	20.0	24.0	25.6	3.7
	2	18.8	21.6	24.6	24.9	6.4
	3	19.2	22.0	24.9	26.2	5.4
CV (%)		6.1	5.2	1.9	2.2	27.6

1.9%). On the other hand, this method presents the flexibility to be used with 15 or even with 10 min with a coefficient of variability of 5.2% and 6.1%, respectively.

Another advantage of this new technique is the fact that it provides differentiation of samples where measurement of lyophoresis with the original method is barely detected. Sample A in Table 1 illustrates this observation. It should be stressed here that the accuracy of the improved method depends mainly on two factors: a well mixed applesauce sample and a very uniform flow rate of sauce from the beaker to the sieve, after the system is turned upside down. This is important to assure repeatable measurements for replicates.

In terms of simplicity and time involvement this new procedure is also highly advantageous over other methods such as the photographic method (Toldby and Wiley, 1962) and the drip method described by Powers et al. (1979) and Drake et al. (1979).

CONCLUSION

WE PROPOSE the following method for measurement of lyophoresis in applesauce: 200g of a well homogenized sample are weighed out in a 1000 mL glass beaker with 8 cm height. A United States Standard Sieve No. 20, a glass funnel with a diameter of 12.5 cm, and a 25 mL measuring cylinder are placed on the top of the beaker. The system is then turned upside down and held in place in a laboratory stand. After 5 min, the beaker is lifted up leaving the sauce on the screen. At 20 min after the system was turned upside down, the volume of liquid is determined and recorded as a measure of lyophoresis.

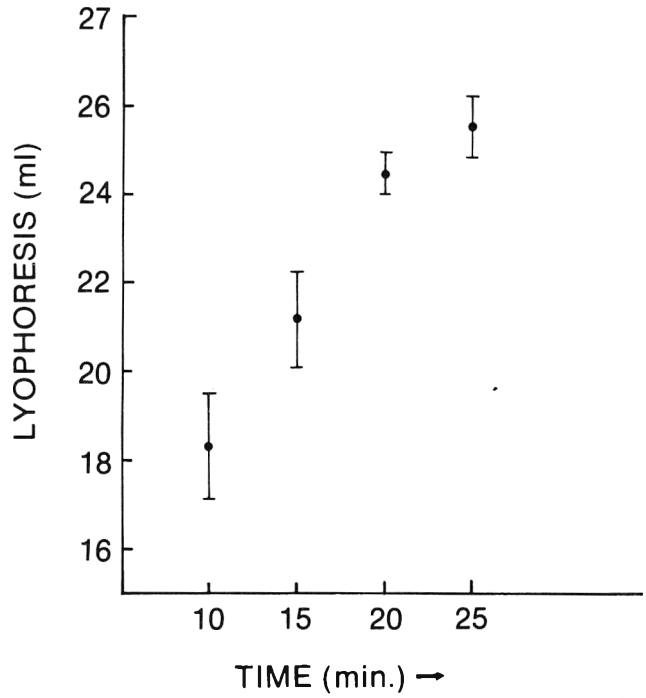

Fig. 1—Lyophoresis measurements for different times using the improved sieve method on sample 'C'.

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A Research Note

Isolation and Further Characterization of a Heat Resistant Peroxidase Isoenzyme from Cauliflower

C. Y. LEE and A. P. PENNESI

ABSTRACT

A heat resistant isoenzyme was isolated from the snowball cauliflower and was analyzed for its thermal inactivation characteristics. Although it exhibited less than 5% of the total peroxidase activity, it showed strong heat stability in that it took more than 30 min at 50°C to reduce activity by 50%. The rate of inactivation followed first order kinetics with an Ea value of 21.3 Kcal/mol. In order to prevent all peroxidase catalyzed reactions in cauliflower during storage, this heat resistant isoenzyme must be completely inactivated.

INTRODUCTION

PEROXIDASE (EC 1.11.1.7, donor:hydrogenperoxide oxidoreductase) is known to be one of the most heat stable enzymes in plants and under certain conditions of limited heat treatment of vegetables it may regain activity during storage (Schwimmer, 1944; Lu and Whitaker, 1974). It is considered to have a direct relationship to the adverse changes in flavor and color of raw and under-blanched vegetables. Rosoff and Cruess (1949) were the first to report the presence of peroxidase in cauliflower. Since then a very limited number of papers on this vegetable and enzyme have been published (Böttcher, 1975), but they were all based on peroxidase activity within cauliflower tissue. Recently Lee et al. (1984) isolated cauliflower peroxidase into three isoenzymes by using hydrophobic chromatography. They reported that the isoenzyme giving over 94% of the total peroxidase activity was very heat-labile in that it took only 15 min at 50°C to reduce its activity by 98%. This result was in contrast to many previous reports that had shown peroxidases from various sources to be very heat stable. Some reports suggested that several vegetables contained both heat sensitive and heat resistant peroxidase (Yamamoto et al., 1962; Ling and Lund, 1978). The objective of this research was to isolate the minor cauliflower isoenzyme and to study its heat inactivation.

MATERIALS & METHODS

THE PERSISTENT WHITE CURD and snowball cauliflower selections (Dickson and Lee, 1980) were grown during the 1982 and 1983 seasons at this experiment station. The peroxidases were extracted from mature cauliflower by homogenizing with cold acetone. The procedures for the separation of the isoenzymes on a hydrophobic chromatographic column, the purification of the enzyme, and the measurement of enzyme activity have been published previously (Flurkey and Jen, 1978; Lee et al., 1984; Wissermann and Lee, 1980).

Heat inactivation of the peroxidase isoenzyme was performed over a temperature range 40–90°C with the heating times varying from 0–60 min. After heating for a given period in a Haak water bath (Model FK), the solutions were rapidly cooled by immersing the tubes in an ice water bath; the samples were then assayed immediately. The percent peroxidase activity remaining after treatment was calculated from the initial activity.

Authors Lee and Pennesi are affiliated with the Institute of Food Science, New York State Agricultural Experiment Station, Cornell Univ., Geneva, NY 14456.

RESULTS & DISCUSSION

MUCH OF THE DIFFICULTY in understanding peroxidase is due to the presence of multiple isoenzymes in various products. Therefore, in order to study the specific characters of peroxidase from a given product, it is essential to isolate first the individual isoenzymes. We were able to separate the three isoenzymes using hydrophobic chromatography and step gradient elution from the presistent white cauliflower; however, due to the minute quantity and low peroxidase activity, we were unable to characterize the minor isoenzymes from this cultivar in the previous study (Lee et al., 1984). The snowball variety, which tends to turn brown on exposure to sunlight, contained a relatively large quantity of peroxidase (approximately 10 times that of the persistent white curd). As shown in Fig. 1, it also yielded three isoenzymes (A, B, and C) that had elution profiles identical to those of the persistent white cauliflower. Therefore, it was possible to isolate enough enzyme to study its heat inactivation properties. In our earlier study it had been observed that component C was the isoenzyme that gave about 94% of the peroxidase activity and that it was heat-labile (Lee et al., 1984). Since isoenzyme A had been found to be more heat stable, we used it for the heat inactivation study.

With guaiacol as the substrate, isoenzyme A showed a pH optimum of 6.5 at 22°C. This is the same value as that of the major isoenzyme C of the persistent cauliflower (Lee et al., 1984). However, this differs from the optimum of 5.3 reported by Rosoff and Cruess (1949). Since their enzyme was a partially purified form of acetone powder, it is difficult to compare our results with their values.

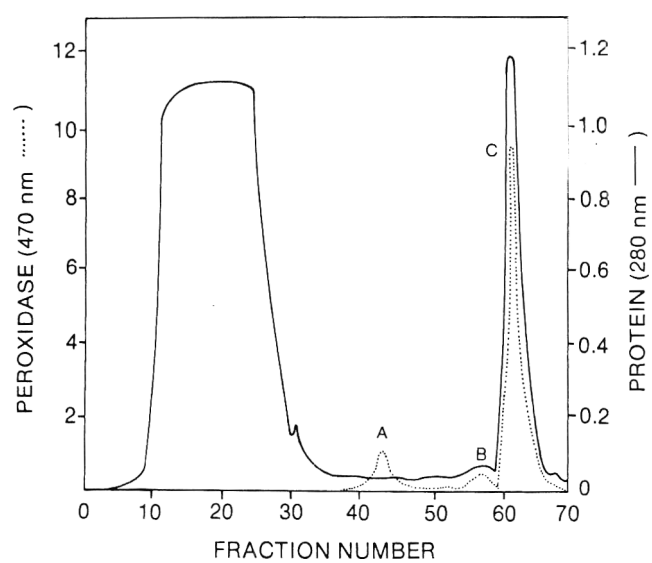

Fig. 1—Elution profile from a phenyl-Sepharose CL-4B column for the snowball cauliflower peroxidase and protein. Elution was made by stepwise decreasing gradients of ammonium sulfate-potassium phosphate buffer. Peroxidase activity was monitored by measuring absorbance at 470 nm of 50 μL sample containing 4 mL of each 10 mM guaiacol and H_2O_2.

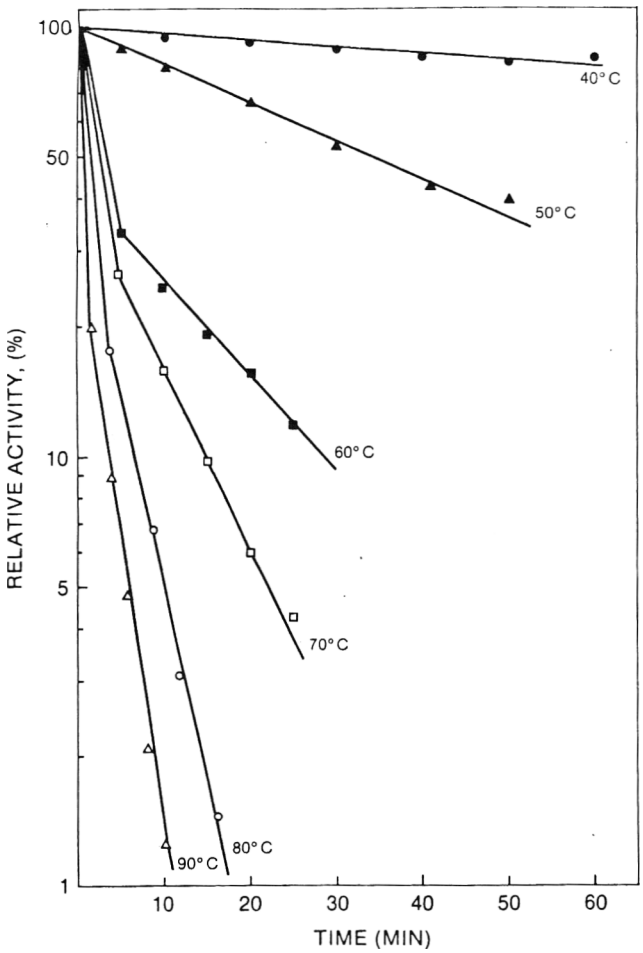

Fig. 2—Rate of heat inactivation of cauliflower peroxidase isoenzyme A.

Fig. 2 shows the residual percent activity of the isoenzyme A as a function of heating time at six different temperatures. The enzyme solutions treated at above 60°C showed breaking points at early stage of inactivation and then the rate of inactivation followed first order kinetics as did isoenzyme C. It appears that the isoenzyme A contained some of the heat labile component within the fraction. Isoenzyme C required only 3 min to destroy 50% of the activity (Lee et al., 1984), however, when isoenzyme A was heated at 50°C, it took more than 30 min to destroy approximately 50% of the activity; at 80°C approximately 5 min were required for 85% deactivation. When the logarithm of the first order rate constant for inactivation was plotted against the reciprocal of the absolute temperature (Arrhenius plot), the rate of activity loss showed a temperature dependence. The activation energy, Ea for the inactivation, was found to be 21.3 Kcal/mol, while that of isoenzyme C was Ea = 46.4 Kcal/mol from our previous study (Lee et al., 1984).

The complexity of peroxidase inactivation appears to be due to the presence of isoenzyme species. Yamamoto et al. (1962) reported that the isoenzymes of sweet corn consisted of heat-labile and heat resistant fractions. Similar results were also reported for horseradish by Ling and Lund (1978). Often the rate of thermal inactivation of composite peroxidases will not exhibit first order reaction kinetics due to the presence of peroxidase isoenzymes of different stability (Lu and Whitaker, 1974) and the present study of cauliflower peroxidase has proven the fact. The Ea value of 21.3 Kcal/mol on thermal inactivation from this study is very similar to that (Ea = 21 Kcal/mol) of heat resistant peroxidase isoenzyme from horseradish (Ling and Lund, 1978). The heat resistant fraction represented only a few percent of total enzyme activity, but because of its great heat resistance it could affect the quality of processed cauliflower during storage.

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A Research Note

Peroxidase Fractions from Asparagus of Varying Heat Stabilities

J. R. POWERS, M.J. COSTELLO, and H. K. LEUNG

ABSTRACT

Chromatofocusing was used to fractionate asparagus peroxidases. Basic isozymes of pI greater than 9.0 predominated throughout the spear. In addition, a more acidic peroxidase fraction is found in the asparagus spear tip. The basic and acidic fractions were similar in heat stability, but the acidic fraction reactivated readily after heating at 70°C.

INTRODUCTION

PEROXIDASE ACTIVITY is widely used as a blanching indicator in the processing of vegetables due to its relatively high heat resistance. Asparagus has been reported to contain several peroxidase isozymes as indicated by benzidine staining of electrophoretic gels (Haard et al., 1974). More recently, McLellan and Robinson (1983) separated peroxidase isozymes from cabbage and Brussel sprouts by isoelectric focusing and found the majority of activity to be due to isozymes with pI's greater than 8.0. Differences in heat stability of separated peroxidase isozymes have been reported in several systems including *Ficus*, *Linum*, and horseradish (Kon and Whitaker, 1965; Fields and Tyson, 1982; Lu and Whitaker, 1974). Naveh et al. (1982) reported that, based on heat inactivation data, whole corn contains two distinct peroxidase fractions. The present report examines similar differences in peroxidase fractions from asparagus.

MATERIALS & METHODS

Materials

Asparagus (*Asparagus officianalis* L., var. Martha Washington) was obtained from the Washington State University Department of Horticulture plots (Pullman, WA). Chromatofocusing materials, including column material PBE 94 and PB 96 elution buffer, were from Pharmacia (Piscataway, NJ). All other chemicals were of reagent grade, and distilled water was used throughout. Heating studies were done using a Brinkmann-Lauda Model T-2 water bath. Temperature of heated enzyme fractions was monitored using a copper-constantin thermocouple and an Esterline Angus Model D-2020 multipoint recording potentiometer. Spectrophotometric data were collected using a Beckman Model 35 UV/Vis Spectrophotometer equipped with a Haake FE circulating heater.

Methods

Asparagus was harvested by cutting spears 1-2 cm below the surface. Spears were cut to have approximately a 14 cm green portion and to be 1.0-1.6 cm in diameter at 12 cm from the tip. Immediately after cutting, spears were placed in ice. Within 1 hr of harvest, spears were divided into tip, mid and butt portions (0-5 cm, 5-10 cm and >10 cm respectively from tip). These portions were sliced into liquid nitrogen and the frozen material stored at −40°C until used for preparation of enzyme extracts. Crude peroxidase extracts were prepared as described by Haard et al. (1974). Peroxidase activities were assayed at 460 nm using 1mM H_2O_2 and 0.5 O-diansidine (final concentrations) buffered at pH 6 with 0.1M sodium maleate at 30°C.

Authors Powers, Costello, and Leung are affiliated with the Dept. of Food Science & Technology, Washington State Univ., Pullman, WA 99164-6330

Crude extracts were dialyzed against the chromatofocusing start buffer, 0.25M ethanolamine-acetic acid pH 10. The dialyzed extracts were applied to 10 x 0.5 cm PBE 94 chromatofocusing columns previously equilibrated with start buffer. After loading of enzyme extract, the column was eluted with 1:12 (v/v) PB 96, pH 6. Fractions were collected in 1.2 mL volumes. The pH of fractions was measured using an Orion 501 pH meter equipped with a Corning semi-microelectrode.

Heating studies were carried out on 0.2 mL aliquots of peroxidase preparations which had been dialyzed against sodium maleate (0.1M, pH 6) and sealed in 1 mL ampoules. Fractions were diluted to approximately equal protein concentration (1 mg/mL) prior to heating. Ampoules were removed from an ice bucket to the heating bath at time zero and were removed at specified intervals, transferred to a water-ice mixture to cool and assayed. The three minute heated fractions were allowed to reactivate at 25°C and assayed at intervals for peroxidase activity.

RESULTS & DISCUSSION

CHROMATOFOCUSING a crude peroxidase extract from the tip portion of asparagus resulted in the majority of the peroxidase activity eluting in the pH 10 range of the pH gradient. A smaller amount eluted by the 1M NaCl wash. Approximately 85% of the peroxidase activity was recovered from the columns. Butt and mid-spear extracts showed a similar pattern but the 1M NaCl wash resulted in elution of very little activity. These data indicate that a relatively basic group of peroxidases are dominant in asparagus spears. In addition, the tip portion of asparagus spears contains a relatively acidic group of isozymes. These data are in apparent agreement with Haard et al. (1974) in which, via benzidine/H_2O_2 staining of polyacrylamide gels run at pH 8.9, the major peroxidase isozymes were found to migrate with an R_f of 0.03-0.05. In addition, the tip portion of spears was found to contain a relatively strong activity at $R_f \sim$ 0.42 (presumably a more acidic isozyme).

Fractions were pooled from the tip extract chromatofocusing and designated "basic" and "salt", respectively, for the peroxidases eluted at pH 10 or eluted by 1M NaCl. In Fig. 1 are 70°C heat inactivation data for the "basic" and "salt" fractions. Both curves are hyperbolic indicating that the inactivation process is biphasic. When plotted as a first order process and treated by the method of successive residuals, these data suggest that each preparation contains two species based on heat stability. The more heat sensitive species were inactivated at the rate of 1.32 min^{-1} and 3.53^{-1} min for the "basic" and "salt" fractions, respectively. The more stable species represent ~40% of the "basic" activity while ~17% of the "salt" fractions activity was stable.

Aliquots of the 3 min 70°C heat inactivated fractions were allowed to reactivate at 25°C. As shown in Fig. 2, there was significant reactivation of the "salt" activity over a 1-hr period while the "basic" fraction reactivated much less. McLellan and Robinson (1981) have noted similar differences between ionically bound and soluble peroxidases from cabbage.

That there are oxidative enzymes in asparagus that reactivate after heat inactivation is suggested by the data of Drake et al. (1981). They reported that asparagus frozen after microwave blanching was significantly lower in ascor-

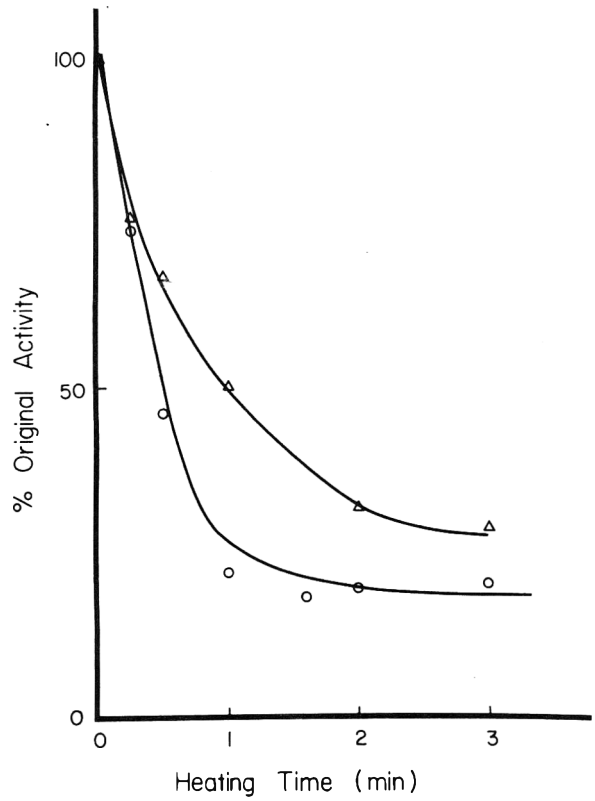

Fig. 1—Heat inactivation of "salt" (○) and "basic" (△) asparagus peroxidase fractions at 70°C and pH 6 (0.1M maleate). These fractions had activities at time zero of 2.5 and 6.5 $\triangle A_{460}$/min/mL.

bic acid and less acceptable to a sensory panel than asparagus blanched by more traditional methods.

The chromatofocusing technique described allows a rapid group fractionation of peroxidase isozymes from asparagus. Further work is underway to optimize this separation technique to further resolve the "basic" and "acidic" fractions into individual isozymes. Substrate specificities with respect to possible roles of the several peroxidase isozymes in off flavor development and lignification are also under investigation.

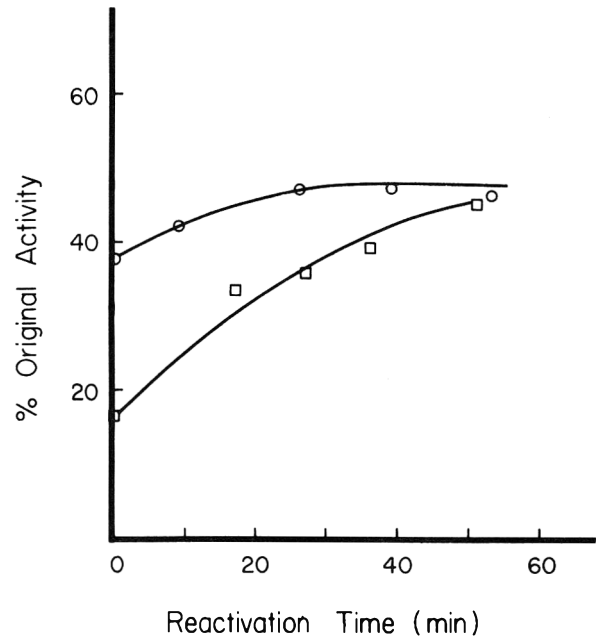

Fig. 2—Reactivation at 25°C and pH 6 (0.1M maleate) of "salt" (□) and "basic" (○) asparagus peroxidase after 70°C heat inactivation.

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A Research Note

Digitizer Aided Determination of Yield Stress in Semi-Liquid Foods

G. KALETUNC-GENCER and M. PELEG

ABSTRACT

The yield stress of semi-liquid foods could be conveniently determined from their apparent viscosity vs shear stress relationship. The data source of these plots were experimentally recorded flow curves which were digitized and processed using a graphics tablet and a computer. The applicability of the procedure was demonstrated in simulated flow curves with known yield stress and in published rheological data where the yield stress was independently determined.

INTRODUCTION

THE DETERMINATION of the yield stress in semi-liquid foods can be done by various direct and indirect methods. In general, the direct methods are based on a gradual and controlled increase of the stress until flow can be detected or by measuring the residual stress after a sheared specimen is let to relax (Van Wazer et al., 1963; Mizrahi and Berk, 1972; Balmaceda et al., 1973; Robinson-Lang and Rha, 1981). The yield stress determined by the second method, is that of the sheared fluid and therefore need not have the same value as that of the undisturbed material. The indirect methods are usually based on fitting the flow curve with a model that includes a yield stress term, and calculating its magnitude from statistical regression. The most popular models for such calculations are Herschel-Bulkley's model:

$$\tau = \tau_0 + k_1 \dot{\gamma}^n \qquad (1)$$

where τ is the shear stress, τ_0 the yield stress, $\dot{\gamma}$ the shear rate and k_1 and n constants and Casson's model:

$$\tau^{1/2} = k_2 + k_3 \dot{\gamma}^{1/2} \qquad (2)$$

where k_2 and k_3 are constants and the yield stress τ_0 is equal to k_2^2.

One difficulty with such indirect methods is that the calculated yield stress magnitude may depend on the selected model (e.g. Barbosa and Peleg, 1983). Similarly, direct extrapolation of the flow curve itself to zero shear rate may also introduce an element of arbitrariness to the yield stress determination.

According to Mewis (1980) and Bagley (1983) one of the most sensitive methods of yield stress determination is from a plot of the apparent viscosity vs the *shear stress* where the apparent viscosity (η_{APP}) is defined as the ratio between the shear stress and shear rate or:

$$\eta_{APP} = \frac{\tau}{\dot{\gamma}} \qquad (3)$$

Application of this method was recently reported by Mills and Kokini (1984) in Karaya gum dispersions. The method is based on the definition of a yield stress, which implies an infinite viscosity at zero shear rate. Therefore the existence of a yield stress, irrespective of any other rheological features, becomes evident by a "singularity" in the relationship between viscosity and shear stress. In practice, of course, the evidence will be in a fast acceleration of the curve ascent and the main question is whether this in actual data will be sufficient to determine the yield stress in an unambiguous manner. The objective of this work is to demonstrate the practicality of the method in yield stress evaluation in food systems, with special emphasis on the data processing by a digitizer and a computer.

Authors Kaletunc-Gencer and Peleg are affiliated with the Dept. of Food Engineering, Univ. of Massachusetts, Amherst, MA 01003.

MATERIALS & METHODS

PUBLISHED (Dzuy and Boger 1983), simulated (i.e. curves drawn using a known model), or experimentally determined flow curves of a commercial tomato paste and locust bean and xanthan gum solution (1:1 ratio 0.25% conc) (Rotovisco RV-3 Haake, Inc., Saddlebrook, NJ) were traced by the stylus of a graphics tablet (HIPAD, Houston Instrument Inc., Houston, TX) connected to an Apple II computer. Each data file so created was transformed into an apparent viscosity-shear stress file using Eq. (3) and the appropriate conversion factors for the units. The processed data were replotted, through a specially developed procedure (CALCOMP software) by a Cyber computer, as apparent viscosity vs. shear stress relationships.

RESULTS & DISCUSSION

BECAUSE THE DIGITIZING PROCESS is based on curve tracing by hand, it was necessary to estimate the effects of human errors on the reproducibility and accuracy of the procedure. Furthermore, since the method involves the detection of a "singularity" there is always a risk that the values obtained will have an error as a result of subjective judgement. It is, of course, crucial to determine whether this source of error is of practical significance or not. In order to answer these two questions the procedure was tested with simulated curves whose data were produced by Newtonian, Bingham's, power law pseudoplastic, Casson's and Herschel-Bulkley's flow equations. These simulated flow curves were traced by hand and the digitized data, so obtained were plotted in the form of apparent viscosity vs. shear stress relationships. Examples of such curves are shown in Fig. 1. This analysis was repeated by tracing the same curve various times and by using simulated curves with different yield stress and other flow constants. It has become clearly evident that the procedure was practically unaffected by human errors at the tracing stage. It was also clear that the existence of a yield stress could be identified unmistakably. Its magnitude was within an uncertainty range of less than 5 - 10%. This range was mainly determined not by human error but by the limitations arising from the calculation of the division products where the divisor magnitude starts to approach zero. Therefore, the estimated value of the yield stress appears to be about one or two "steps" (i.e., stress readings) from the last stress reading where the calculated magnitude of the corresponding viscosity is still reasonable and does not start to oscillate.

Yield stress of real systems

The proposed procedure was also tested with published data on titanium dioxide (TiO_2) suspensions (Dzuy and Boger, 1983). In this work the yield stress of the suspen-

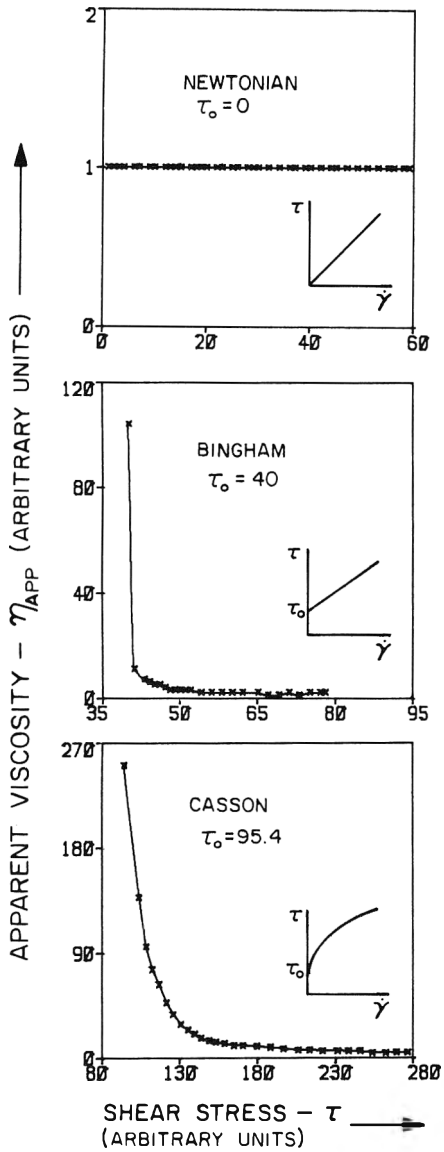

Fig. 1—Apparent viscosity vs shear stress relationship of simulated Newtonian, Bingham (τ_O = 40 arbitrary units) and Casson (τ_O = 95.4 arbitrary units) liquids (hand traced from the simulated flow curves).

sion was determined directly by different physical methods and was also calcualted from different models which yielded values between 106 - 128 N • m^{-2}. The magnitude of the yield stress of the TiO_2 suspension was practically the same regardless of the model chosen for its calculation (i.e. the Herschel-Bulkley's and Casson's equations yielded very close values which also corresponded to the values reached by the direct methods). Application of the digitizer aided determination method to the original data, also reported in the publication, yielded a value of 125 N • m^{-2}. In many foods, especially those whose rheology is governed by time related effects, such an agreement between the different methods may not be the case (e.g. Barbosa and Peleg, 1983). For such materials the proposed method has an advantage over the curve fitting methods since it is not based on any particular mathematical model whose validity must be established separately.

The procedure was also applied to commercial tomato paste and gums mixture in solution as shown in Fig. 2. The figure serves as an illustration of the technical convenience of the methods to determine the yield stress once the computer program has been set up. Because the magnitude of the yield stress of semi-fluid foods may depend on the sample shear history, the described procedure also offers a handy tool to study such dependencies as well as the effect of other factors such as temperature, pH and additives.

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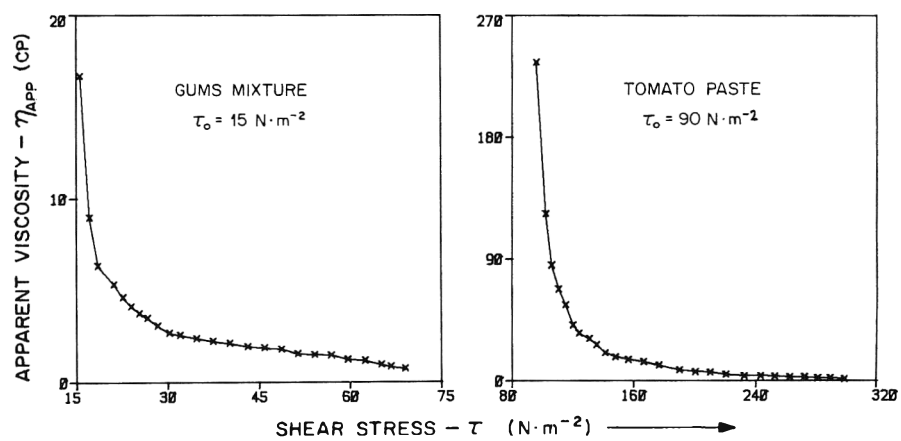

Fig. 2—Apparent viscosity vs shear stress relationship of a locust bean and xanthan gums mixture in solution (1:1 ratio 0.25% conc) — Estimated yield stress is 15 N • m^{-2}, and a commercial tomato paste — Estimated yield stress 90 N • m^{-2}.

A Research Note

Bonded Fused Silica Capillary Column GLC Determination of BHA and BHT in Chewing Gums

MICHAEL J. GREENBERG, JOSEPH HOHOLICK, RICHARD ROBINSON, KATHRYN KUBIS, JAMES GROCE, and LORRAINE WEBER

ABSTRACT

A gas chromatographic method was developed for the quantitation of BHA and BHT in toluene plus 2-propanol extracts of chewing gum samples. The method uses a thick film (1.0 micron) bonded fused silica open tubular capillary column which resolves antioxidants from flavor oil volatiles and gum base components as confirmed by mass spectral analysis. The chromatographic method is reproducible at 4.0 ppm and detects as little as 2.0 ppm. Regression analysis of antioxidant-biphenyl internal standard peak area ratios to antioxidant concentrations resulted in a highly linear equation with a correlation coefficient (R) of 0.99 for the 10 - 150 ppm range.

INTRODUCTION

MANY ANALYTICAL METHODS for the determination of 2,6-di-(tert-butyl)-4-methylphenol (BHT) and the 2 and 3-tert-butyl-4-hydroxyanisole isomers (BHA) in fats, oils, soaps, and cereals have used packed column GLC techniques such as those reported by Fry and Willis (1982), Isshiki et al. (1980), Min et al. (1982), Sedea and Toninelli (1981), Wyatt (1981), and AOAC Method 20.012 (1980).

The determination of BHA and BHT in chewing gum has received little attention in the literature. The only recent study conducted by Pellerin et al. (1982) using a reverse phase HPLC approach indicated interferences due to flavorings and other components in gum that may mask BHA.

Bonded fused silica open tubular (FSOT) capillary columns have been described by Shibamoto (1982) and Jennings (1981) to be superior to any existing GLC column category for volatile analysis in terms of resolving power, inertness, and reproducibility of peak integrations. The objective of this study was to develop a bonded FSOT column GLC method to determine BHA and BHT in chewing gum without interferences from flavor and gum base components.

MATERIALS & METHODS

Materials

Bubble gums of various flavorings and peppermint stick gum were formulated by the Wm. Wrigley Jr. Company (Chicago, IL). Biphenyl (99% pure) and BHT (99+% pure) were obtained from the Aldrich Chemical Company (Milwaukee, WI). BHA was obtained from ICN Biochemicals Company (Plainview, NY).

Analysis procedure

Chewing gum (10.0g) and 50.0 mL toluene are placed in a 4 oz glass bottle, capped, and shaken/rotated on a laboratory shaker/rotator for 16 hr. Fifty mL 20 μg/mL biphenyl internal standard in 2-propanol is added to the toluene extract (2-propanol precipitates the gum base polymer components) and the sample is shaken/rotated for 15 min. The solution is allowed to settle for 15 min, and a 40 mL aliquot of the solution is transferred to a centrifuge tube and centrifuged for 15 min. A 25 mL sample of the centrifuged solution is concentrated to 10 mL by distillation and the concentrate is further purified by passage through a Waters Sep-Pac Florisil cartridge prior to injection.

The authors are affiliated with the Research & Development Dept., Wm. Wrigley Jr. Company, 3535 S. Ashland Ave., Chicago, IL 60609.

Gas chromatography (GLC)

The GLC was carried out on a Perkin Elmer Sigma 115 equipped with a flame ionization detector and 60m X 0.25 mm (i.d.) FSOT bonded capillary column coated with 1.0 micron DB-1 (J and W Scientific, Inc., Rancho Cordova, CA). The column temperature was held at 65°C for 2 min, programmed linearly from 65°C to 135°C at 10°C/min and programmed from 135°C to 200°C at 1°C/min. The temperature was held at 200°C for 10 min. The injector and detector temperatures were 250°C and 300°C, respectively. The carrier gas was helium at a carrier velocity of 21 cm/sec. and split ratio of 106:1. Injection volumes were 1 μL. All injections were made splitless. The splitter was turned on 0.5 min after injection.

Gas chromatography-mass spectral (GLC-MS) analysis

A Finnigan 4500 GLC-MS - INCOS Data System equipped with the above DB-1 column directed into the ion source, source temperature of 150°C, and an ionizing potential of 70 eV were used. Mass spectral identifications were confirmed by computer-assisted spectra matching of authentic standards.

RESULTS & DISCUSSION

THE GAS CHROMATOGRAM of an extract of a peppermint flavored stick gum is presented in Fig. 1. The peppermint flavor volatiles and other gum components were baseline separated from the biphenyl internal standard and BHT in Fig. 1. Chromatographic analysis of flavor oils used in the gums indicated no detectable GLC peaks where the biphenyl internal standard, BHA and BHT eluted. Extracts of gum having no antioxidants had no interfering peaks where biphenyl, BHA and BHT eluted. GC/MS results indicate that the GLC peaks for each gum extract investigated had mass-to-charge ratios and relative abundances that agreed with the antioxidant standards.

A summary of BHA and BHT levels found in commercially available chewing gum is presented in Table 1. Fruit flavor and peppermint gum samples were 1 - 2 months in age and exhibited reproducibility on the order of 2 - 4 ppm with coefficient of variation values less than 4.0% for three replicate analyses per gum. Lemon and strawberry gum samples were five months of age and exhibited similar reproducibility as the one month samples, but had substantially lower BHA values. These lower BHA values are indicative of oxidation taking place during gum storage. A series of BHA and BHT standards with a concentration range of 10 - 150 ppm were chromatographed, and the antioxidant peak area (A_{BHA}, A_{BHT}) to internal standard peak area (A_{BiP}) correlated to the antioxidant concentration ([BHA], [BHT]) using regression analysis. Results are presented in Eq (1) and (2) where the terms in parentheses are the 95% confidence intervals, N is the number of data points, and R is the correlation coefficient. The equations are highly linear over the 10 - 150 ppm range, and are statistically significant at the 95% level of confidence: slopes are significantly different from zero and the intercepts statistically pass through the origin.

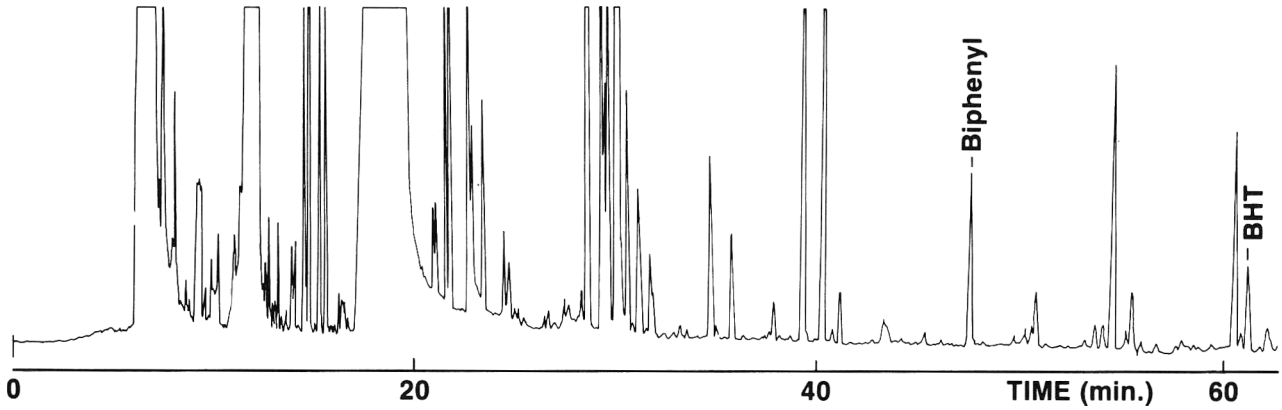

Fig. 1—Gas chromatogram of peppermint flavored gum extract.

Table 1—Reproducibility of method for BHA/BHT in chewing gum

Sample description	Anti-oxidant	X[a]	N[b]	S[c]	COV[d]
Fruit Flavored Bubble Gum —1	BHT	115	3	4.0	3.5
Fruit Flavored Bubble Gum —2	BHT	118	3	3.5	3.0
Fruit Flavored Bubble Gum —3	BHA	82	3	2.7	3.3
Fruit Flavored Bubble Gum —4	BHA	82	3	4.2	5.1
Lemon Flavored Bubble Gum	BHA	50	3	4.2	8.3
Strawberry Flavored Bubble Gum	BHA	59	3	6.6	11.1
Peppermint Stick Gum	BHT	53	3	2.1	3.9

[a] Mean antioxidant concentration in ppm
[b] Number of samples
[c] Standard deviation
[d] Coefficient of variation

$$[BHA] = 242.9 \pm (31.7)\frac{A_{BHA}}{A_{BiP}} - 5.9 \pm (7.5) \quad (1)$$

$$N = 5 \qquad R = 0.99$$

$$[BHT] = 169.9 \pm (9.3)\frac{A_{BHT}}{A_{BiP}} - 3.0 \pm (3.0) \quad (2)$$

$$N = 5 \qquad R = 0.99$$

Additional standards having concentrations of 2 and 5 ppm were chromatographed. The results show that the method can detect 5 ppm of BHA when the signal-to-noise ratio is around five and 2 ppm of BHT with a signal-to-noise ratio of ten.

The accuracy of the method was evaluated by adding known quantities of antioxidants. One experiment involved adding 123 ppm BHA and 122 ppm BHT to a gum sample. Amounts recovered were 130 ppm BHA (106% recovery) and 126.5 ppm BHT (104% recovery). A second experiment involved adding BHT to a gum sample having 96.3 ppm BHT at four levels (0, 30, 60, 100 ppm). The experimentally determined BHT levels $[BHT]_{exp}$ were correlated to the calculated BHT levels $[BHT]_{calc}$ using linear regression analysis. Results in Eq. (3) indicate that the equation was statistically significant at the 95% level of confidence and was highly linear. The slope indicated a high degree of recovery (96 ± 9%) and the intercept was statistically the same concentration value as the original sample of 96.3 ppm.

$$[BHT]_{exp} = 0.96 \pm (0.09)\,[BHT]_{calc} + 94.1 \pm (6.7) \quad (3)$$

$$N = 4 \qquad R = 0.99$$

In summary, BHA and BHT levels in chewing gum can be determined by subjecting toluene-2-propanol extracts to gas chromatography using a bonded thick film phase fused silica capillary column. Mass spectral data indicate that the method resolves these antioxidants from any flavor oil or gum base interferences. The method is reproducible as illustrated by the relatively low coefficient of variation data, and linear over the 10 - 150 ppm range.

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A Research Note

Amino Acid Content in Selected Breakfast Cereals

F. I. MEREDITH and W. O. CASTER

ABSTRACT

Protein and 17 amino acids were determined in 11 commercial breakfast cereals. Total amino acid concentration ranged from 5,408-19,872 mg/100g dry weight. Lysine was the limiting amino acid in all the breakfast cereals except Apple Jacks in which methionine was the limiting amino acid. The chemical score ranged from 11–45 which reflected the low protein quality in the breakfast cereals.

INTRODUCTION

BREAKFAST is considered by nutritionists to be the most important meal of the day. This is especially true for children who need this nourishment to perform effectively during the morning hours (Costley and Franta, 1977).

Breakfast cereals, which are a prominent and highly advertised food product, are an important food in the American diet. A search of the scientific literature revealed very little data on the amino acid content of breakfast cereals. The new Agriculture Handbook 8-8 (Douglass et al., 1982) on breakfast cereals also contains very little data on the amino acid content of breakfast cereals. Because of this lack of information and the importance of this food in the diet, an investigation was carried out to determine the protein and amino acid content in selected breakfast cereals.

Author Meredith is affiliated with the USDA-ARS, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30613. Author Caster is affiliated with the College of Home Economics, Univ. of Georgia, Athens, GA 30602.

MATERIALS & METHODS

ELEVEN COMMERCIAL BREAKFAST CEREALS (Bran Chex, Post 40% Bran Flakes, Cheerios, Quaker Oats Quick Grits, Kellogg's Corn Flakes, Rice Krispies, Apple Jacks, Kellogg's Sugar Frosted Flakes, Product 19, Heartland, and Special K) were purchased at local markets. The boxes of breakfast cereals were opened, dried in a freeze dryer, and the contents ground to pass a 40 mesh screen. Total nitrogen was determined on the dry cereals by AOAC (1980) Micro-Kjeldahl Method 47.021. Protein was calculated by multiplying the total nitrogen value by the protein factor given for each commercial breakfast cereal in Agriculture Handbook 8-8 (Douglass et al., 1982).

Duplicate acid hydrolyzates of each dry breakfast cereal were prepared for amino acid analysis (Meredith, 1982). Seventeen amino acids and ammonia were determined on a Durrum D500 automatic amino acid analyzer equipped with a single ion exchange column. Concentrations of the individual amino acids were obtained by measuring the color produced from the reaction of the amino acid and ninhydrin and all results were expressed on a dry weight basis. Tryptophan, which is destroyed during acid hydrolysis, was not determined as it requires a separate alkaline hydrolysis. The chemical score was calculated by the method of Sheffner (1967).

Table 1—Percent protein[a], ammonia concentration[b], and amino acid concentrations[b] in selected breakfast cereals

	Bran Chex	Post 40% Bran Flakes	Cheerios	Quaker Oats Quick Grits (dry)	Kellogg's Corn Flakes	Rice Krispies	Apple Jacks	Kellogg's Sugar Frosted Flakes	Product 19	Heartland	Special K
Protein factor	6.3	6.0	5.8	6.25	6.25	5.95	5.9	6.25	6.1	5.8	5.8
% Protein	11.78	10.98	14.28	10.0	7.93	6.84	5.37	4.81	8.30	12.24	20.18
Ammonia	375	414	947	633	309	499	237	219	333	418	1029
Amino acids											
Alanine	612	454	592	701	624	408	276	419	546	593	724
Aspartic acid	765	645	1069	535	443	644	375	303	510	967	1018
Arginine	657	424	800	325	162	257	287	105	302	861	942
Cystine	72	117	–	–	90	–	–	72	97	191	–
Glutamic acid	2281	2585	2838	1865	1677	1360	1356	1149	1748	2471	6262
Glycine	541	494	612	308	222	327	215	148	289	609	769
Proline	918	988	700	925	904	356	496	599	790	703	2262
Serine	495	462	562	412	380	367	262	253	379	564	972
Total non-essential AA[c]	6341	6169	7173	5071	4502	3719	3267	3120	4661	7250	12949
Histidine[d]	322	257	300	313	290	192	155	155	225	296	463
Isoleucine[e]	391	379	511	320	276	320	202	207	267	482	775
Leucine[e]	957	736	976	1249	1147	644	518	839	1012	930	1561
Lysine[e]	272	219	369	205	66	168	164	46	124	458	365
Methionine[e]	132	124	210	161	127	179	150	115	145	170	311
Phenylalanine[e]	527	407	715	441	480	437	339	336	497	664	1144
Threonine[e]	365	330	417	304	242	255	183	163	266	411	592
Tyrosine[e]	349	267	437	253	349	380	214	249	351	432	791
Valine[e]	522	500	734	485	365	441	279	250	387	628	921
Total essential AA[f]	3837	3219	4669	3731	3342	3016	2204	2288	3374	4280	6923
Total AA[c]	10178	9388	11842	8802	7844	6735	5471	5408	8035	11530	19872

[a] Total nitrogen X protein factor
[b] mg/100g dry weight
[c] Sum does not include ammonia
[d] Histidine essential for children
[e] Essential amino acids
[f] Sum of nonessential and essential amino acids does not include ammonia

RESULTS & DISCUSSION

THE PROTEIN CONCENTRATION found in the breakfast cereals studied ranged from 4.81% for Kellogg's Sugar Frosted Flakes to 20.18% for Special K (Table 1).

The ammonia concentration in Cheerios (947 mg/100g) and in Special K (1,029 mg/100g) was twice the level found in the remaining breakfast cereals (Table 1). The amino acid concentrations of aspartic acid and glutamic acid in Cheerios and Special K also were greater indicating the amides asparagine and glutamine were present in greater concentrations than in the other breakfast cereals. In each breakfast cereal, the nonessential amino acid present in the greatest concentration was glutamic acid. The sulfur amino acid cystine was determined in Bran Chex, Post 40% Bran Flakes, Kellogg's Corn Flakes, Kellogg's Sugar Frosted Flakes, Product 19, and Heartland. These cystine values, which were obtained by the use of an acid hydrolysis, are lower than would be obtained using performic acid oxidation and therefore should be considered as tentative. The greatest concentration of the total nonessential amino acids occurred in Special K (12,949 mg/100g) while the total nonessential amino acids in the other breakfast cereals ranged from 3,120 mg/100g (Sugar Frosted Flakes) to 7,250 mg/100g (Heartland).

As expected in foods processed from cereal grains, the breakfast cereals were low in the essential amino acids lysine and methionine (Table 1). The total essential amino acids for most of the breakfast cereals were similar except for Apple Jacks (2,240 mg/100g) and Kellogg's Sugar Frosted Flakes (2,288 mg/100g) which were the lowest and Special K (6,923 mg/100g) which was the highest.

Special K contained the greatest amount of total amino acids (19,872 mg/100g). This concentration was four times that of Kellogg's Sugar Frosted Flakes (5,408 mg/100g), the breakfast cereal with the lowest total amino acid concentration. Three of the breakfast cereals total amino acid concentrations were greater than 10,000 mg/100g (Bran Chex, Cheerios, and Heartland).

Samples of two of these breakfast cereals had been analyzed in a similar fashion some 6 yr earlier. In one case, the results were substantially identical (within SD $\leq$ 3%), while in the other, major discrepancies of certain amino acids were found (up to 30%) which suggested reformulation of the breakfast cereal without a change of the name. This, of course, would pose additional problems for those preparing and using handbook data.

The limiting amino acid in all of the breakfast cereals was lysine except for Apple Jacks which had methionine as the limiting amino acid. The chemical score for lysine in the breakfast cereals was Kellogg's Corn Flakes, 11; Kellogg's Sugar Frosted Flakes, 12; Product 19, 21; Special K, 21; Quaker Oats Quick Grits (dry), 26; Post 40% Bran Flakes, 27; Bran Chex, 30; Rice Krispies, 30; Cheerios, 33; and Heartland, 45. The chemical score for Apple Jacks (limiting amino acid methionine) was 34.

Not only are the protein chemical scores low for these human food products, but there are additional indications (Caster and Resurreccion, 1982) that direct feeding trials may prove beneficial. Most adults and children eat breakfast cereals, often as snacks, and most often in combination with milk. Nevertheless, an improvement of the protein quality would enhance the nutritional value.

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The research conducted by ARS-USDA on the commercial samples as reported in this manuscript was limited to analyses of their protein and amino acid content. The data are reported solely as factual information and are limited to the samples analyzed. No warranty or guarantee is made or implied that other samples of these products would have the same or similar composition. It is the policy of the USDA not to endorse commercial products tested in the research or commercial products used while conducting the research.

A Research Note

Incidence of Toxic *Alternaria* Species in Small Grains from the USA

VERNEAL R. BRUCE, MICHAEL E. STACK, and PHILIP B. MISLIVEC

ABSTRACT

A total of 230 small grain samples from the U.S.A. (148 wheat, 57 barley, 25 rye) were examined for the presence of species of *Alternaria* mold after surface disinfection with 5% sodium hypochlorite. One or more of nine different *Alternaria* species were detected in 184 of the samples. Of the eight species tested, all produced tenuazonic acid and seven produced alternariol and alternariol methyl ether. Production of these toxins by four of the species is reported for the first time. Although extracts from cultures of the eight species were toxic to brine shrimp, the above three toxins were not involved, suggesting that additional toxic metabolites were present.

INTRODUCTION

THE INDICENCE OF *Fusarium* Link species in wheat, barley, and rye from the USA is relatively low, but there were indications that the chief mold contaminants of these grains, both before and after surface disinfection with 5% sodium hypochlorite, were *Alternaria* Nees ex Fr. species (unpublished data). Others (Christensen, 1965; Christensen and Kaufmann, 1965 Semeniuk, 1954) also have reported the substantial occurrence of the genus *Alternaria* in small grains of the USA. However, except for occasional identifications of *A. tenuis* Nees (*A. alternata* Keissler), the individual *Alternaria* species encountered were not identified and there are 41 legitimate species of *Alternaria* according to Ellis (1971, 1976).

Isolates of the genus *Alternaria*, including some from overwintered Russian grain (Joffe, 1960, 1965), have been shown to be toxic to animals in laboratory studies. Christensen et al. (1968) reported that 53 of 60 isolates of *Alternaria* species were lethal to rats maintained on *Alternaria*-contaminated corn diets, and Doupnic and Sobers (1968) found that 31 of 96 isolates of *Alternaria* species caused deaths in chickens held on cracked corn diets contaminated with these isolates. Hamilton et al. (1969) reported that the interperitoneal injection of mice with *Alternaria* species extracts caused toxicity and sometimes death. Although no toxic metabolites were isolated and characterized in the above reports, subsequent studies (Harvan and Pero, 1976; Meronouk et al., 1972; Templeton, 1972) characterized a number of *Alternaria* mycotoxins, including tenuazonic acid (TA), alternariol (AOH), and alternariol methyl ether (AME).

To determine the predominant *Alternaria* species that occur in small grains from the USA, evaluate their ability to produce TA, AOH, and AME under laboratory conditions, and test the use of brine shrimp (Eppley, 1974) for detecting these mycotoxins, we conducted the following work.

MATERIALS & METHODS

Samples

A total of 230 small grain samples (148 wheat, 57 barley, 25 rye) from the 1979 crop were examined. Samples (ca. 250g) were collected by USDA grain inspectors from terminal elevators in various midwestern states. Detailed histories of individual samples were not available, other than designation of the terminal elevator from which they were collected. Upon receipt, samples were held at room temperature (22–25°C) until examined.

Determination of the presence of *Alternaria*

From each sample, 50 intact, visibly mold-free grains were surface-disinfected for 1 min in 5% sodium hypochlorite, rinsed three times in sterile water, and aseptically plated, five grains per plate, on potato dextrose agar (Difco) amended with 40 mg chlortetracycline HCl just before plates were poured, to inhibit bacteria. Plates were incubated 5–7 days at 22 ± 1°C before the presence of *Alternaria* species was determined.

Up to five isolates per sample of suspected *Alternaria* colonies were subcultured on malt extract agar (containing, per liter, 20g malt extract, 15g bacto agar, and 40 mg chlortetracycline HCl) and on standard nutrient agar (containing, per liter, 1.36g KH_2PO_4, 1.06g Na_2CO_3, 5.0g $MgSO_4$, 5.0g dextrose, 1.0g asparagine, 15g bacto agar, and 40 mg chlortetracycline HCl). Subcultures were incubated at 22 ± 1°C for 7–14 days, then identified according to Neergaard (1945) and Ellis (1971, 1976).

Mycotoxin determination

One hundred one identified isolates, involving eight of the nine *Alternaria* species detected, were qualitatively screened for the production of TA, AOH, and AME. Isolates were multi-spore inoculated into autoclaved (121°C, 15 min, 15 psi) 300 mL Erlenmeyer flasks containing 50g polished rice and 50 mL tap water, and incubated at 22–25 ± 2°C for 20 days. Contents of flasks were then extracted with 150 mL methanol, and production of the toxin(s) determined by thin-layer chromatography using Mallinckrodt 7 GF silica gel plates, a benzene:methanol:acetic acid (90:5:5) solvent system and TA, AOH, and AME reference standards. TA appears as a dark fluorescence quenching spot under short wave ultraviolet light. AOH and AME are blue fluorescent under long wave ultraviolet light. The crude extracts (0.5 mL of filtrate) and reference standards (100 μg) were also tested for toxicity to brine shrimp by the method of Eppley (1974).

RESULTS

ISOLATES of the genus *Alternaria* were detected in 114 of 148 (77%) surface-disinfected wheat samples. From these samples, 268 isolates were subcultured, purified, and speciated (Ellis, 1971, 1976; Neergaard, 1945). Eight different species were identified (Table 1): *A. alternata* (74.6% of the isolates); *A. longipes* (Ellis and Everh.) Mason (13.1%); *A. tenuissima* (Kunze ex Pers.) Wiltshire (4.5%); *A. brassicicola* (Schw.) Wiltshire (2.2%); *A. cheiranthi* Lib.) Bolle (2.2%); *A. raphani* Groves and Skolko (1.5%); *A. citri* Ellis and Pierce (1.1%); the *Alternaria* state of *Pleospora infectoria* Fuckel (0.7%). The genus was detected in all 57 surface-disinfected barley samples. Five species were detected (Table 1) from 136 isolates: *A. alternata* (85.3% of the isolates); *A. brassicicola* (5.1%); *A. tenuissima* (4.4%); *A. longipes* (3.7%); and *P. infectoria* (1.5%). Thirteen of the 25 surface-disinfected rye samples (52%) contained *Alternaria*. Thirty-nine isolates, representing six species, were identified (Table 1): *A. alternata* (71.8%) of the isolates; *P. infectoria* (10.3%); *A. longipes* (5.1%); *A. tenuissima* (5.1%); *A. brassicicola* (5.1%); and *A. brassicae* (Berk) Sacc (2.6%). *A. alternata* was the predominant species de-

Authors Bruce and Mislivec are with Division of Microbiology and Author Stack with Division of Chemistry & Physics, Food & Drug Administration, Washington, DC 20204.

Table 1—Incidence of *Alternaria* species in American small grains

Species	Isolation frequency (%) Wheat (268)[a]	Barley (136)	Rye (39)
A. alternata	74.6	85.3	71.8
A. longipes	13.1	3.7	5.1
A. tenuissima	4.5	4.4	5.1
A. brassicicola	2.2	5.1	5.1
A. cheiranthi	2.2	0.0	0.0
A. raphani	1.5	0.0	0.0
A. citri	1.1	0.0	0.0
P. infectoria[b]	0.7	1.5	10.3
A. brassicae	0.0	0.0	2.6

[a] The total number of *Alternaira* isolates speciated per grain type.
[b] The *Alternaria* state of *Pleospora infectoria*.

tected in this study (334 of the 443 isolates) (75.4%); however, with the exception of a report of the occurrence of *A. brassicae* in wheat (USDA, 1960) the low rates of occurrence of the eight other species (Table 1) have not been reported previously for American small grain.

Of the 268 *Alternaria* isolates identified, 101 (representing eight of the nine species detected in this study) were analyzed for the production of TA, AOH, and AME. The number of isolates per species reflected, in general, the relative prevalence of each in small grains. Sixty-one isolates were from wheat, 30 from barley, and 10 from rye.

On an isolate-by-isolate basis (Table 2), 42 produced all three toxins, 21 produced only TA, 13 produced only AOH and AME, one produced only AME, and 24 produced none. A total of 77 isolates (76.2%) produced at least one toxin. With the exception of the single isolate of *A. tenuissima*, which produced only AME, isolates which produced AME also produced AOH. In no case was only TA plus AOH or only TA plus AME detected. As analyzed by a high performance liquid chromatographic method which will be published elsewhere, the average yield from 18 cultures which produced TA was 2.2 mg TA per gram of rice. The average yields from 12 cultures which produced AOH and AME were 0.5 and 0.4 mg per gram of rice, respectively.

On the species-by-species basis, 46 of the 59 *A. alternata* isolates (77.9%) produced one or more of the toxins (Table 2), as did 14 of 16 (87.5%) of the *A. longipes* isolates, 7 of 9 (77.8%) of the *A. tenuissima* isolates, and 5 of 7 (71.4%) of the *A. brassicicola* isolates. The other four species, *P. infectoria, A. raphani,* and *A. cheiranthi,* produced all three toxins while *A. brassicae* produced only TA.

The use of brine shrimp as a bioassay method for the detection of TA, AOH, and AME was of little value. Although 76 of the 101 isolate extracts were toxic (toxicity ranging from 10% to 100% mortality), several of the toxic extracts contained none of the toxins, whereas extracts containing all three toxins had no toxic effect, nor were the TA, AOH, and AME reference standards toxic.

DISCUSSION

OUR FINDINGS INDICATE that the principal *Alternaria* species in small grains from the USA is, indeed, *A. alternata* (*A. tenuis*) but that additional *Alternaria* species are present in and on these foodstuffs, although at lower levels. However, since the grains were surface-disinfected before examination, it is possible that the additional species may occur in grains not disinfected at substantially higher rates.

The fact that seven of the eight additional *Alternaria* species have not been previously reported as American small grain contaminants may be partly due to the difficulties often encountered in speciating isolates of the genus *Alternaria*.

The results of this study indicate that a potential mycotoxin problem may exist in American small grains due to

Table 2—Production of TA, AOH, and AME by eight *Alternaria* species isolated from small grains

Species	No. of isolates	No. positive	No. of times toxin(s) detected All 3	TA only	AOH & AME only
A. alternata	59	46	20	16	10
A. longipes	16	14	13	1	0
A. tenuissima	9	7	4	1	1[a]
A. brassicicola	7	5	2	1	2
P. infectoria[b]	3	2	1	1	0
A. raphani	3	1	1	0	0
A. cheiranthi	3	1	1	0	0
A. brassicae	1	1	0	1	0
Total	101	77	42	21	13

[a] One isolate of *A. tenuissima* produced only AME.
[b] The *Alternaria* state of *Pleospora infectoria*.

the presence of *Alternaria* species as shown by the following evidence: (a) The species were detected after surface disinfection, indicating penetration, growth, and possible toxin production; and (b) 77 of the 101 isolates examined produced at least one of the three toxins sought. Thus, American small grains should be examined directly for the preformed presence of TA, AOH, and AME. This is the first report of the production of TA, AOH, and AME by the species *A. raphani, A. cheiranthi,* and *P. infectoria,* and of TA by *A. brassicae*.

The fact that 76 of the 101 isolates were toxic to brine shrimp, even though TA, AOH, and AME were nontoxic under the conditions used, suggests that additional known (altertoxins I and II, altenuene, altenisol, etc.) or unknown toxic *Alternaria* metabolites were present. Ongoing new studies have been initiated to assess the ability of isolates of various *Alternaria* species, from American small grains and from other American foods, to elaborate additional known and unknown *Alternaria* toxins.

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A Research Note

Factors Affecting Storage of Orange Concentrate

J. E. MARCY, T. R. GRAUMLICH, P. G. CRANDALL, and M. R. MARSHALL

ABSTRACT

Orange concentrate (OC) 66°Brix, was tested for effect of storage temperature and storage time on product quality. OC was stored at −12.2, −6.6, −1.1, and 4.4°C, and analyzed for °Brix, % acid, ascorbic acid, furfural, serum viscosity, apparent viscosity, browning, Hunter color values, and taste panel scores at monthly intervals for 1 yr. Significant ($p > 0.01$) decreases were found in ascorbic acid content and Hunter color value (Y) due to storage time, and temperature. Nonenzymatic browning increased and taste panel scores significantly decreased with storage temperature and time. Taste panelists were able to detect significant differences in flavor after 5 and 9 months at 4.4 and −1.1°C, respectively.

INTRODUCTION

BULK TANK STORAGE of orange concentrate (OC) offers several advantages including increased operational efficiencies and the ability to stabilize product quality throughout the processing year by blending concentrate from several bulk storage tanks. Storage temperature for bulk storage OC is very important to product quality because even a few days at 37°C are detrimental to OC quality (Curl et al., 1946; Curl, 1947; Kew, 1955).

The purpose of this experiment was to determine the effect of storage temperature, and storage time on OC quality.

MATERIALS & METHODS

Preparation of samples

Late season (June) 'Valencia' oranges (400 kg) were harvested and transported to the University of Florida Citrus Research & Education Center pilot plant (Lake Alfred, Fl). The juice was extracted with an FMC In-Line extractor (Lakeland, FL) and finished with an FMC Model 35 finisher, using a 5 mm screen size. Orange juice was concentrated to 66°Brix on a 230 kg/hr thermally accelerated short time (TASTE) evaporator as described by Miller et al. (1980). OC was collected in a nitrogen filled, stainless steel container maintained in a nitrogen atmosphere during handling and packaging. OC was filled into 177 mL metal cans lined with "C" enamel as described by Hendrix and Ghegan (1980). Cans were than stored at −17.7°, −12.2°, −6.6°, −1.1°, and 4.4°C.

Analysis methods

Duplicate samples from all treatments were used. Soluble solids and apparent viscosity were measured using concentrate, all other tests used concentrate that had been diluted with distilled water to 11.8°Brix.

Soluble solids content was measured as Brix by refractometer. Percent acidity was measured by titration with sodium hydroxide. Ascorbic acid was measured by titration (AOAC, 1980). Furfural was measured using the method of Dinsmore and Nagy (1974). Apparent viscosity was measured using a Brookfield Viscometer Model LVT (Brookfield Engineering Lab., Stoughton, MA) with spindle No. 4 at 6 rpm at 27°C. Serum viscosity was measured with a Brookfield Viscometer and a UL adapter at 60 rpm. Hunter color values were measured with a Hunter citrus colorimeter (Model D45D2 Hunter Citrus Colorimeter). Nonenzymatic browning was measured by absorbance at 420 nm according to the method of Meydav et al. (1977).

Taste panel

Samples were diluted to 11.8°Brix and served at 10°C to six trained panelists. A multiple comparison difference test and analysis was used (Larmond, 1967). Panelists were asked to score samples in comparison to a reference juice which has been stored at −17.7°C.

RESULTS & DISCUSSION

Brix, acid, furfural, viscosity

Of the nine product quality parameters tested, °Brix, % acid, furfural content, serum viscosity, and apparent viscosity showed no significant change during the test period (Table 1). Furfural content was below the quantitative range in all samples testsed with an estimated value of < 30 ppb.

Ascorbic acid

There was a significant decrease in ascorbic acid content (Table 1). Destruction of ascorbic acid reduces the nutritional value and provides reactive carbonyl groups which can be precursors to nonenzymatic browning (Joslyn, 1957; Huelin, 1953; Clegg, 1964). During the 12 month storage, 7 mg ascorbic acid/100 mL were lost at 4.4°C storage and at −12.2°C the OC lost 3.5 mg/100 mL. Crandall et al. (1983) reported after 1 yr the ascorbic acid content in 72°Brix OC was reduced by 4.1% at −6.7°C and 12% at 4.4°C.

Color

There was no change in absorbance in OC stored at −12.2°C but a slight change in the three other temperature treatments was found. Crandall et al. (1983) reported no

Table 1—Analysis of variance of orange concentrate stored at four temperatures for 12 months

		Source		
Variable		Storage temp	Storage time	Replicates
°Brix		—	—	—
% Acid		—	—	—
Ascorbic acid		**	**	—
Furfural		—	—	—
Serum viscosity		—	—	—
Apparent viscosity		—	—	—
Absorbance 420 nm		**	**	—
Hunter color	Citrus red	—	**	—
	Yellow	**	**	—
	Number	—	**	—
Taste panel		**	**	—

** = Significant effect ($p > 0.01$).
— = No significant effect.

The authors are affiliated with the Univ. of Florida, Institute of Food & Agricultural Sciences, Citrus Research & Education Center, Dept. of Food Science & Human Nutrition, 700 Experiment Station Road, Lake Alfred, FL 33850.

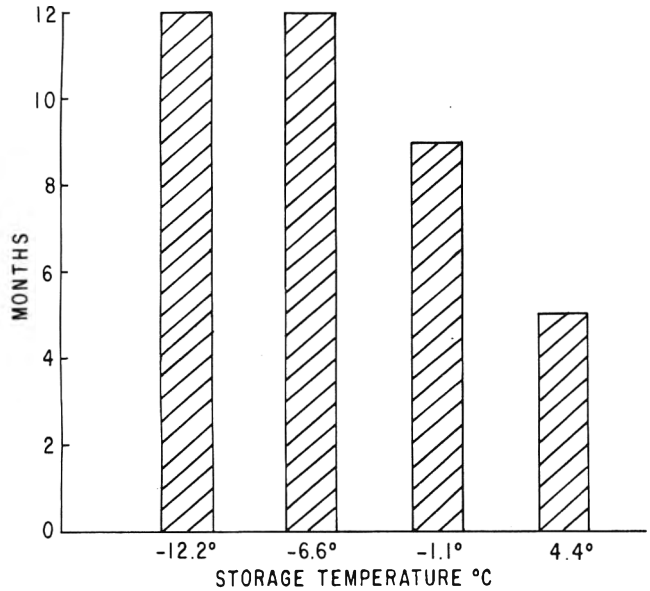

Fig. 1—Time before detectable ($p > 0.01$) flavor changes occur in 66° Brix orange concentrate stored at various temperatures.

color change in 72°Brix OC stored at 4.4°C. Citrus yellow (CY) is the Hunter color value affected by storage temperature (Table 1). Robertson and Reeves (1981) reported a high correlation between the bowning index (absorbance at 420 nm) and CIE tristimulus color values, but not enough to be useful for predictive purposes.

Taste panel

Fig. 1 shows the length of storage at each temperature before the panelists were able to distinguish ($p > 0.05$) storage treatments from the (−17.7°C) reference. Panelists were unable to detect a significant difference in OC stored at −12.2° and −6.6°C during the 12 month test. For OC stored at −1.1° and 4.4°C taste panel scores were significantly different at 9 months and 5 months respectively. Kanner et al. (1982) reported no statistical difference between 58°Brix OC stored at −18°C and concentrate stored at 5°, 12°, and 17°C for 17, 10, and 8 months, respectively. The large difference between these two estimates of flavor stability could be attributable to differences in expected OC quality, fruit quality, taste panelist, or taste panel procedures.

OC was stored in inert gas atmospheres at temperatures above those currently used in Florida with minimal changes in product quality. Of the quality parameters tested, the limiting factor is change in product flavor. The taste panel analysis used in this study was not designed to predict changes in consumer acceptance and therefore the difference in flavor may or may not affect product quality as perceived by the consumer.

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A Research Note

Influence of Water Activity on the Nonenzymatic Browning of Apple Juice Concentrate during Storage

J. L. TORIBIO, R. V. NUNES, and J. E. LOZANO

ABSTRACT

Highly concentrated clarified apple juice was kept in storage at 37°C over a period of 100 days. The soluble solids content ranged from 65-90.5° Brix. Color development was monitored as O.D. at 420 nm. A maximum nonenzymatic browning rate (NEB_r) was found to occur between water activities 0.53 and 0.55. It was assumed that (1) dilution of reactants was responsible for the browning rate reduction at high water contents while (2) viscosity inhibited the color formation at low water activities. Viscosity ranged from 48 to 1.3 x 10^6 cp and increased sharply when the commercial levels of concentration (70-72° Brix) were exceeded.

INTRODUCTION

REACTIONS BETWEEN sugars and amino acids in foodstuff take place over a wide range of water activity (Eichner and Karel, 1972; Labuza et al., 1970; Singh et al., 1983). A maximum browning reaction occurs in most foods at a certain value of a_w depending on the type of foodstuff.

The above reaction and the growth of microorganisms are the major limitations to stability during the storage of apple juice concentrate. At the standard levels of concentration, apple juices are stable against fermentation but the nonenzymatic browning reaction rate increases considerably up to at least 75°Brix (Toribio and Lozano, 1984).

Since some increase in storage life could be obtained by maintaining water activity level either above or below the point of maximum browning, it is desirable to determine the range of water activity at which this maximum occurs.

The present study attempts to follow changes in color during the storage of apple juice at very high concentrations.

MATERIALS & METHODS

GRANNY SMITH APPLES were manufactured into a single strength clarified juice following the general procedure reported in a previous work (Toribio and Lozano, 1984). Afterwards the juice was concentrated at 50°C under vacuum (35 mm Hg) in a Rotovapor Heidolph Mod. VVI. The samples were stored aseptically in glass vials at 37°C. The selected levels of soluble solids content were 65°, 70°, 75°, 80°, 85°, and 90° Brix. All concentrated samples were obtained by dilution of the 90° Brix sample.

Authors Toribio, Nunes, and Lozano are affiliated with PLAPIQUI (UNS-CONICET), 8000 Bahia Blanca, Argentina.

Physical analysis

Percent soluble solids were measured with a bench refractometer at 20°C. Color development was monitored as O.D. at 420 nm in samples pre-diluted to 12.5° Brix with distilled water. The absorbance was determined against water on a Perkin-Elmer Lambda 3 spectrophotometer using 1 cm cells. Viscosity was measured with a Brookfield Synchro-electric (Model RVT, Brookfield Eng. Laboratories Inc., Stoughton, MA) viscometer, with the guard in place, and a thermometer immersed in the juice. The beaker with the juice was placed in a thermostatically controlled water bath.

Accurate and high precision water activities were determined at 37 ± 0.1°C with an improved oil manometer, according to the vapor pressure manometric technique (VPM) as described by Nunes et al. (1982; 1984). Turbidity was also monitored by measuring O.D. at 625 nm. No significant changes were observed. Samples not immediately analyzed were stored in a freezer at −30°C until the analysis could be performed.

Chemical analysis

Total amino acids (AA), pH, total acidity (TA), total sugars (TS) and reducing sugars (RS) were estimated according to AOAC methods (AOAC, 1980); total phenolics (TP) in apple juice were determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965).

RESULTS & DISCUSSION

THE EXPERIMENTAL DATA obtained by measuring the color development (Absorbance at 420 nm) were correlated as previously with a least square nonlinear parameter algorithm (Toribio and Lozano, 1984). Thus a pseudo-first order kinetic was found to apply:

$$C = 1 - b \exp(-K t) \quad (1)$$

where C is the color as O.D. at 420 nm; K is the reaction constant; a and b are fitting parameters assumed to be dependent on the physical and chemical characteristics of the juice; and t the storage time.

The K, a and b values for the different soluble solids content and corresponding regression coefficients are listed in Table 1. Rates of nonenzymatic browning (NEB_r) expressed as changes in O.D. at 420 nm per day of storage were obtained through the first derivative of Eq. (1).

$$NEB_r = b K \exp(-K t) \quad (2)$$

Fig. 1 shows the NEB_r as influenced by the water activity over 100 days reaction. The NEB_r was further correlated with the water activity values (Table 1), yielding equations of the type:

Table 1—Physical and chemical analysis results and parameters of Eq. (1)

Soluble solids (°Brix)	a_w	Viscosity (centipoise)	Parameters Eq. (1) a	b	K	r^2	Chemical analysis[a]	
65	0.784	48	2.253	2.0533	4.98×10^{-3}	0.991	PH	3.51
70	0.746	120	2.2897	2.0897	5.367×10^{-3}	0.975	TA(g/l)	6
75	0.672	385	3.042	2.842	6.264×10^{-3}	0.964	RS(g/l)	89
81	0.558	1064	3.198	2.998	7.33×10^{-3}	0.982	TS(g/l)	104.4
84.5	0.478	433600	3.141	2.941	7.06×10^{-3}	0.979	TP(ppm)	659
90.5	0.212	1312000	3.813	3.613	4.51×10^{-3}	0.989	AA(meq/l)	7.8

[a] Juice rediluted to 12.5 °Brix with distilled water.

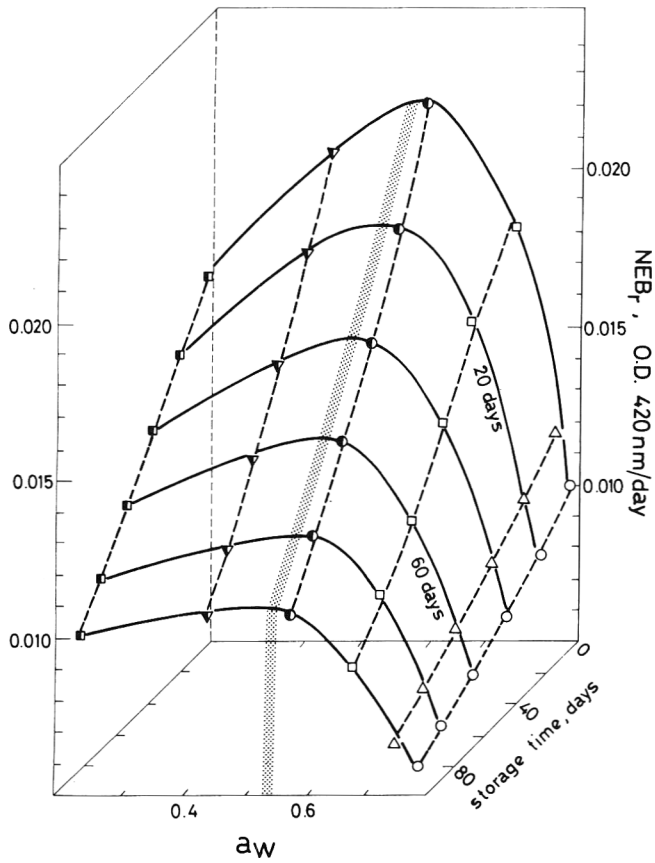

Fig. 1—Nonenzymatic browning rate (NEB_r) as a function of water activity (a_w) and time of storage (t): ○ 65°Brix; △ 70°Brix; □ 75°Brix; ○ 81°Brix; △ 84.5°Brix; □ 90.5°Brix. Full lines represent Eq. (3). Shaded area represents NEB_r maximum range.

$$NEB_r = A_1(t) + A_2(t)a_w + A_3(t)a_w^2 + A_4(t)a_w^3 + A_5(t)a_w^4 \quad (3)$$

where the fitting coefficients, A_n, are functions of the storage time only.

Equating the first derivative of Eq. (3) to zero at different times of reaction and solving the resulting cubic equations, a_w values at the maximum NEB_r were obtained. At 0, 20, 40, 60, 80, and 100 days of reaction, maximum browning rates were located at water activities 0.553, 0.553, 0.551, 0.544, 0.535, and 0.531, respectively.

The juice has a slower browning reaction rate at low water activities increasing up to the maximum point between a_w 0.53 and 0.55, as shown in Fig. 1. A further increase in a_w significantly reduces the color formation. It is assumed, in this case, that the increase in a_w tends to dilute the concentration of reactants, decreasing chemical reaction rate. Color inhibition by increasing the water content has been reported by Wolfrom et al. (1974); Labuza et al. (1970) Eichner (1975); and Eichner and Karel (1972). They also demonstrated that in high viscid systems the influence of the viscosity is "predominant over a wide range of water activities at low water contents." Looking at Table 1 it is possible to appreciate that clarified apple juice, which roughly increased 8 times its viscosity from 65° to 75°Brix became tremendously viscous at high solids content.

Another important observation drawn from this study is that browning reaction in apple juice cannot be markedly reduced by decreasing the water content. The NEB_r at any a_w value downward of the maximum range (0.53–0.55) appears to be faster than the NEB_r values corresponding to the commercial concentration levels (70–72°Brix).

In conclusion, in order to reduce the nonenzymatic browning reaction rate during the storage of clarified apple juice concentrate, water activity has to be maintained far from the maximum. This can be accomplished by following the concentration process either over or below 85°Brix. The former has no industrial interest because of the high viscosity and the low NEB_r decrease. The latter is better due to a high NEB_r decrease. It, therefore, follows that apple juice must be maintained at low concentration without compromising the biological stability of the product.

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A Research Note

Water Holding Capacity of Hemicelluloses from Fruits, Vegetables and Wheat Bran

W. D. HOLLOWAY and R. I. GREIG

ABSTRACT

The water-holding capacities of freeze-dried bean, cabbage, lettuce, onion, peach, pear, pumpkin, tomato, wheat bran and their hemicelluloses were determined by the centrifugation method. Cabbage had the greatest water-holding capacity at 35.8g of water per gram of dry food; bran the lowest at 5.2g water per gram. The water-holding capacity (WHC) of the hemicelluloses from fruits and vegetables varied considerably from 3.3 g/g in beans to 12.0 g/g from cabbage. Hemicelluloses extracted from wheat bran showed a marked increase in WHC from 5.2 g/g of water per gram of 'whole' bran to 22.8 g/g for hemicellulose. This decreased to 15.3g of water bound by hemicellulose that had been extracted after chlorite delignification. WHC of hemicelluloses from wheat bran and cabbage may account for their ability to increase stool weight.

INTRODUCTION

POPULATIONS with a low incidence of diverticular disease, appendicitis and benign and malignant tumors of the colon and rectum have large stool-weights and short intestinal transit times (Burkitt et al., 1974). A possible mechanism for this could be that a component of the diet, such as plant fiber, influences stool consistency (McConnell et al., 1974). Previous investigators have suggested that the WHC of feces is related to the pentose (hemicellulose) content of the diet (Cummings et al., 1978). This is in contrast to the WHC of intact fruits and vegetables which appear to be predominantly influenced by their pectin content (Kelsay, 1978).

This study was designed to determine the WHC of hemicelluloses isolated from some fruits, vegetables and wheat bran.

MATERIALS & METHODS

BEAN, CABBAGE, LETTUCE, onion, peach, pear, pumpkin, and tomato were freeze-dried. Samples of wheat bran and these freeze-dried foods were then subjected to sequential extraction to remove lipid, starch, water and oxalate soluble components. The hemicelluloses were then isolated from the residue, without prior delignification, using sodium hydroxide (100g liter^{-1}) under nitrogen. The sodium hydroxide soluble extracts were precipitated with 3 volumes ethanol, dialyzed, then freeze-dried. The dried material was ground using a laboratory grinder to a particle size between 150-250 microns. The alkali insoluble residue of wheat bran was delignified using chlorite, then re-extracted with sodium hydroxide. The composition of the alkali soluble extracts was determined by GLC and colorimetric analysis as has been previously described (Holloway, 1983). The water-holding capacities of the freeze-dried foods, hemicelluloses and microcrystalline cellulose were determined in duplicate by centrifuging (McConnell et al., 1974). The foods and extracts (0.1g) were put into tared 50 mL polypropylene centrifuge tubes to which 10 mL distilled-deionized water were then added. The tubes were capped before the contents were vigorously mixed. They were then held for 24 hr at 20°C before centrifuging at 13,200 x *g* for 1 hr (Sorvall SS-3, in swinging bucket rotor HB-4), the excess water decanted and the tubes inverted for 1 hr at 20°C. The tubes were then weighed, freeze-dried and re-weighed.

The reproducibility of the method was investigated by determining the water-holding capacity of ten samples of 'whole' freeze-dried pears and nondelignified, wheat bran hemicellulose.

RESULTS & DISCUSSION

THE COMPOSITION of the sodium hydroxide soluble polysaccharides is shown in Table 1. Their composition is variable; however, they are generally high in arabinose and xylose with varying amounts of galactose, glucose, uronic acid and protein.

The terms noncellulosic polysaccharides and pentoses have been used to describe the alkali soluble polysaccharides in foods that are often associated with cellulose. However, the term hemicellulose introduced by Schulze in 1891 (Rogers and Perkins, 1968) to describe carbohydrates extracted by alkali from woody plant tissue also appears to be appropriate to describe the polysaccharides extracted from plant foods.

The water-holding capacity (WHC) of the 'whole' foods and their hemicelluloses are shown in Table 2. The WHC of the foods ranged from 35.8g water bound per g dry cabbage to 5.2g water bound per g dry wheat bran. The results obtained in this study, using freeze-dried foods, are generally higher than that obtained for acetone-dried foods (McConnell et al., 1974). Possibly the greater re-hydration of freeze-dried foods is due to the morphological structure of the plant tissue being altered less than acetone dried material. The results for bran, 5.2g water per g dry solids, obtained in this study are close to that obtained for AACC Bran (Robertson and Eastwood, 1981a) at 5.8g water per g dry solids.

The WHC of the hemicelluloses varied considerably as shown in Table 2. The lowest hemicellulose WHC was 3.3g water/g dry solids in beans and tomato; onion, peach, lettuce and pumpkin from 4.3-5.4g water/g dry solids, respectively; pear gave the highest of the fruits at 10.1g water/g dry solids and the highest WHC of the vegetables was obtained from cabbage at 12.0g water/g dry solids. The greatest WHC of the hemicelluloses, 22.2g water/g, was obtained from wheat bran. However, WHC was lower in hemicellulose that had been extracted after delignification. On the basis of this it could be said that lignin may contribute to the WHC. A more likely mechanism for the decreased water-holding capacity may be due to depolymerization and chemical alteration of the polysaccharide induced by the delignification procedure (Glaudemans and Timell, 1957).

The determination of the reproducibility of the method used for 'whole' pears gave a mean value of 16.4g water per gram dry solids, a standard deviation of 0.8 and a coefficient of variation of 5.2%. Wheat bran hemicellulose (not delignified) gave a mean value of 22.2g water per gram dry solids, standard deviation of 1.4 and a coefficient of variation of 6.5%.

Different methods have been used to determine the water-holding capacity of foods such as centrifugation, filtration and suction pressure, and they have given different results (McConnell et al., 1974; Robertson et al., 1980;

Author Greig is affiliated with the Dept. of Food Technology, Massey University, Palmerston North, New Zealand. Author Holloway's present address is Applied Biochemistry Division, DSIR, Palmerston North, New Zealand.

Table 1—Composition of the isolated hemicelluloses expressed as a percentage of the individual and total analyzed components and correlation of water-holding capacity with composition

	Monosaccharides						Uronic			
	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	acid	Methoxyl	Acetyl	Protein
Beans	1.0	2.1	21.9	4.1	12.3	15.7	14.4	1.0	3.3	24.2
Cabbage	1.8	14.3	18.0	7.5	31.4	22.9	16.9	1.3	3.9	11.6
Lettuce	1.6	19.7	23.3	8.2	0	0	24.9	1.9	1.9	18.4
Onion	0.7	9.9	12.4	1.8	24.4	36.5	8.0	1.1	0	5.3
Peach	1.8	9.4	18.3	4.2	11.9	19.6	9.5	1.3	2.5	21.4
Pear	1.6	17.2	19.8	3.8	13.4	27.7	8.3	0.7	1.7	5.8
Pumpkin	0	0	14.2	15.5	32.7	7.2	12.5	2.6	0.8	14.5
Tomato	0	3.7	27.0	12.1	6.9	31.9	9.3	0.5	0.4	8.2
Wheat bran	0	31.9	60.1	0.3	12.2	4.0	0.7	0.1	0.1	0.1
Wheat bran (after chlorite)	0	29.4	61.8	0	0.8	5.7	1.2	0.1	0.5	0.5
Cellulose	0	0	0	0	0	100	0	0	0	0
Correlation of WHC	−0.278	0.847	0.802	−0.523	−0.074	−0.039	−0.583	−0.568	−0.231	−0.702

Table 2—Water-holding capacity (WHC) g water/g dry matter of whole foods and their hemicelluloses

	Water content of whole food	WHC of whole food	% yield of hemicellulose from the freeze-dried foods	WHC of hemicellulose
Beans	93.5	20.8	3.7	3.3
Cabbage	90.1	35.8	4.1	12.0
Lettuce	97.0	25.3	3.7	4.7
Onion	90.2	21.7	2.0	4.3
Peach	87.3	11.5	3.9	4.3
Pear	84.1	16.4	2.2	10.1
Pumpkin	91.4	13.2	3.6	5.4
Tomato	93.7	14.0	2.8	3.3
Wheat bran	14.0	5.2	7.9	22.2
Wheat bran (after chlorite)			3.8	15.3
Cellulose		3.3	0	0

Robertson and Eastwood, 1981b; Chen et al., 1984). The centrifugation method used in this study was found to be reproducible and is probably a measure of both bound and trapped water; bound water being a function of the chemical composition, trapped water relating to the morphological structure of the plant tissues. The differences in the WHC of the isolated hemicelluloses that no longer have any morphological structure, are due to differences in their chemical composition. Correlation of the water-holding capacity with the composition showed that there is a high positive correlation with the arabinose and xylose content (Table 1).

The laxative value of high fiber diets has been recognized for some time (Hippocrates 430 BC). However, it is not clear which component of fiber is the major contributing factor in the prevention of constipation. Williams and Olmstead (1936) showed that wheat bran, carrot and cabbage have a considerable influence on stool weight. The manner in which fiber controls the bulk of feces has not as yet been elucidated. However, colonic distension due to the presence of large amounts of nondigested material, enhanced by the water-holding capacity of biopolymers, appears to be involved (Monte, 1981).

While the pectin content of fruits and vegetables may well be the major cell wall constituent that influences the water-holding capacity of the intact food (Kelsay, 1978) the digestion of pectin before it reaches the large bowel would prevent it from acting as a laxative (Holloway et al., 1983). If a high WHC is a determining factor in laxation, the low WHC of intact wheat bran would not correlate with its high laxative effect. However, the high WHC of its hemicellulose which is relatively resistant to digestion in the human digestive tract (Holloway et al., 1980) suggests that at least in bran, and possibly cabbage, the physiological effect of reduced transit times, may be due to the physicochemical properties of the hemicellulose.

The hypothesis that hemicellulose is the major plant cell wall constituent that influences intestinal transit time, is also supported by the observation that on an edible weight basis wheat bran, 86.0% dry matter containing 34.2 g/100g of hemicellulose (Holloway et al., 1977), is an effective laxative whereas cabbage, which has 9.9% dry matter and a hemicellulose content of 0.1%, is less effective.

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A Research Note

Effect of Tumbling and Tumbling Temperature on Surface and Subsurface Contamination of *Lactobacillus Plantarum* and Residual Nitrite in Cured Pork Shoulder

H. W. OCKERMAN and K. KWIATEK

ABSTRACT

The effect of intermittent tumbling (up to 18 hr) was compared to a nontumbled treatment at processing temperatures of 3°C and 23°C on tissue surface inoculated with *Lactobacillus plantarum*. The surface and subsurface number of *L. plantarum* and the residual nitrite in boneless cured pork shoulder were determined. The *L. plantarum* levels were significantly increased ($P < 0.05$) by tumbling, increased by time (linear, $P < 0.01$) and by sample location with levels decreasing from nontumbled exudate to surface samples to internal samples. Residual nitrite in cured pork shoulder tumbled intermittently for 18 hr at 23°C was significantly lower ($P < 0.01$) than in nontumbled tissue. Nitrite level interactions for tumbling time x temperature were highly significant ($P < 0.01$).

INTRODUCTION

TUMBLING OR MASSAGING of meat is a relatively new process for improving the quality characteristics of cured meat (Mass, 1963; Rust and Olsen, 1973; Krause et al., 1978b; Cassidy et al., 1978). Theno et al. (1977) and Ockerman (1980) described both advantages and disadvantages of this new technology. Tumbling may involve continuous activity for short time periods (1-3 hr) or may involve an intermittent cycle for quite long periods; i.e., 10-15 min of every hour for 18-24 hr (Ockerman et al., 1978).

Cassidy et al. (1978) reported that tumbling leads to increased cell membrane disruption, disorganization of cell nuclei and decreasing clarity of striation. Tumbling combined with the reported disruption of the muscle sarcolemma has been found (Knipe et al., 1981) to force microorganisms into the internal regions of the tumbled meat, thus increasing the subsurface microbial numbers while apparently reducing the surface exudate, bacterial counts. Reduced microbial numbers in the exudate were observed after 15 and 18 hr of intermittent tumbling by Knipe et al. (1981), but in this study no effort was made to determine if the microorganisms were migrating into the disrupted tissue thus resulting in reduced numbers in the exudate. In an attempt to cure meat quickly, a temperature above refrigerated temperatures would be expected to increase the rate of cure migration but, perhaps, also decrease the shelf life (Moulton and Lewis, 1940). This may be of concern since hams used in tumbling and massaging are usually boneless and thus have been subjected to significant contamination during processing.

The objectives of this project were to determine the effect of tumbling at 3°C and 23°C on: (1) the number of surface and subsurface *Lactobacillus plantarum* after inoculation during tumbling and nontumbling cycles; and (2) the residual nitrite at various stages of tumbling.

MATERIALS & METHODS

SIXTEEN, 1.0-1.2 kg pieces of fresh boneless pork shoulder, closely trimmed of external fat and visible connective tissue were prepared using sterile techniques to keep the microbial load as low as possible for this experiment. Each piece was stitch pumped to 120% of green weight with curing solutions composed of the following ingredients by weight (w/w): 14.3% salt; 2.75% sugar; 0.29% sodium erythorbate; 0.0935% sodium nitrite; 2.75% sodium tripolyphosphate and 79.80% water.

These 16 samples allowed 4 replications in each treatment cell of tumbled or nontumbled (control) at each of two temperatures (3°C and 23°C). These temperatures were chosen to represent room temperature and meat holding refrigerated temperature. Each 1.0-1.2 kg piece of tissue was subdivided prior to analysis into 2 pieces which resulted in a total of 32 samples.

The pure culture of *L. plantarum* used in this study was obtained from The Dept. of Microbiology at The Ohio State Univ. The pure culture medium used for inocula preparation was ATP Broth (Difco) in which the pure culture *L. plantarum* was allowed to multiply for 48 hr at a temperature of 30°C. All samples were inoculated by placing 50 mL of the bacterial suspension on the surface of the meat pieces after they were stitch pumped with curing solution. The bacterial suspension contained approximately 5×10^8 viable cells per mL. Immediately after contamination, one-half of the muscle pieces were randomly assigned to the tumbling treatments and the other half to the nontumbling treatment (control). Both tumbled and nontumbled samples were placed in a cooler at 3°C or at room temperature at 23°C for the 18 hr treatment period. The tumbling process involved an intermittent cycle of 15 min on and 45 min off each hr for 18 hr.

Samples for investigation were taken at 6 stages: prior to brine injection (microbiological control) and at processing times of 0 (after brine injection and inoculation), 12, 15, 18 hr and after cooking (in a glass vessel in boiling water bath for 12 ± 3 min) to an internal temperature of 68°C. At each sampling period approximately a 0.5 cm slice was taken from the surface of the meat and another 0.5 cm slice from the subsurface area at the same location. A microbiological sample was taken at each sampling period from the exudate of the nontumbling treatment. The meat samples were homogenized with diluent (0.5% Bacto Peptone), dilutions made and the samples subsequently plated on ATP Agar and incubated four days at 25°C (Ockerman, 1982) for determination of *L. plantarum*.

The portion of the surface and subsurface samples remaining after the microbiological analysis was obtained was mixed in equal quantities for analysis of nitrite using the procedure described by Ockerman (1982).

Analysis of variance (Harvey, 1960) was used to determine the effect of tumbling, temperature, time (linear, quadratic, cubic) and their interaction on the log. of the microbial number and the nitrite level. An additional analysis of variance was used to compare values before and after cooking.

RESULTS & DISCUSSION

PORK TISSUE used in this study prior to brine injection with the curing solution and prior to inoculation with *L. plantarum* had a natural microflora level, as determined on ATP Agar, of Lactobacillus spp. on the surface of the meat of 4.41 ± 0.33 (log)/g and a subsurface level of 3.63 ± 0.14 (log)/g. Since the level of natural contamination by Lactobacillus spp. generally did not exceed 1% of the total contamination by *L. plantarum*, it was assumed that such a low level of natural contamination did not significantly influence the results.

Fig. 1 illustrates the log of *L. plantarum* of cured pork shoulder tissue at 3°C (upper) and 23°C (lower) treatment temperatures. The figure also shows the influence of tum-

Author Ockerman is affiliated with the Dept. of Animal Science, The Ohio State Univ., Columbus, OH 43210 and The Ohio Agricultural Research & Development Center, Wooster, OH 44691. Author Kwiatek is affiliated with the Dept. of Hygiene of Animal Products, Veterinary Research Institute, Pulawy, Poland.

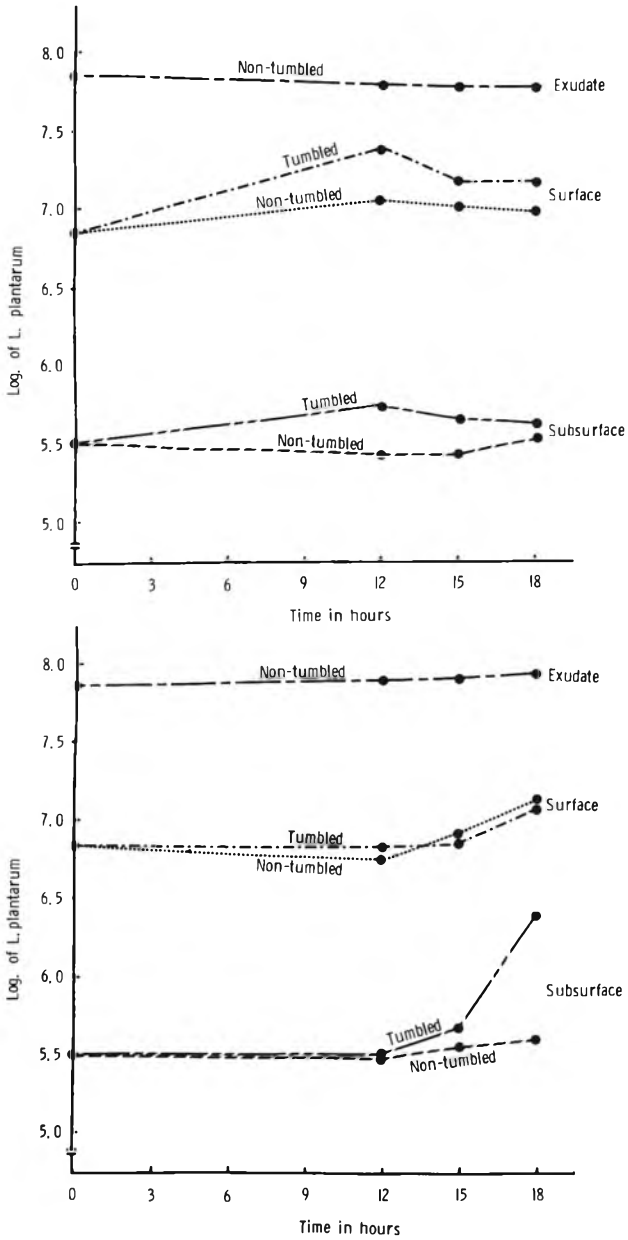

Fig. 1—Logs of surface, subsurface and nontumbling exudate for L. plantarum at 3°C (upper) and 23°C (lower).

bling and nontumbling as well as location such as surface, subsurface and nontumbling exudate on the microbial levels of cured pork during 18 hr of processing.

The analysis of variance indicated that tumbling was significant ($P < 0.05$) and that linear time and location were highly significant ($P < 0.01$) for microbial plate count for *L. plantarum*. Temperature was not significant and all of the interactions proved also to be nonsignificant at the 5% level. The major differences for microbial numbers in Fig. 1 are attributed to location, with numbers significantly decreasing from exudate to surface to internal samples in both 3°C and 23°C. Tumbling resulted in higher numbers of microorganisms in the internal tissue of product tumbled 18 hr at 23°C. This is as would be postulated with disruption of tissue as suggested by Cassidy et al. (1978) and with a more optimal growth temperature for *L. plantarum*. There is some nonsignificant suggestion that the 3°C tumbled surface tissue absorbed microorganisms from the exudate early in the tumbling cycle and that this level was reduced in the later stages of tumbling probably due to

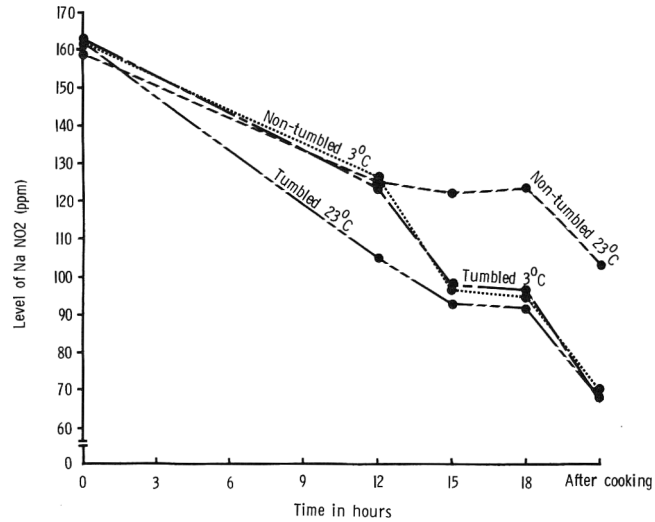

Fig. 2—Contents of sodium nitrite during tumbling and nontumbling process at 3°C and 23°C.

internal migration. This would agree with the observations of Knipe et al. (1981). The level of *L. plantarum* in the internal tumbled tissue at 3°C would also suggest greater microbial migration from the inoculum into the tissue at this temperature. The tumbled surface tissue had essentially the same number of *L. plantarum* microorganisms as the nontumbled surface tissue at 23°C suggesting that absorption of the exudate microorganisms had possibly occurred prior to the 12 hr sampling period and that migration toward the center of the sample had also occurred at a more rapid rate than at 3°C. The small increase observed on the surface tissues from 12 to 18 hr at 23°C probably was caused by the favorable growth temperature. Cooking reduced the number of *L. plantarum* to a nondetectable level in both the surface and subsurface tissue for all treatments.

Fig. 2 illustrates the level of nitrite in meat during tumbling and nontumbling processing at 3°C and 23°C. The analysis of variance indicated that tumbling time and tumbling temperature interactions were highly significant. After 12 hr of treatment, tumbling at 23°C resulted in lower nitrite levels than the other 3 treatments. This agrees with the reports of Mills et al. (1980), Ockerman and Organisciak (1978a, b) and Vartorella (1975), who stated that tumbling improved cure distributions, speeded cure migrations, and improved color development. The nontumbled tissue processed at 23°C had significantly higher residual nitrite than the other 3 treatments after 15 and 18 hr of processing and after processing and cooking. This can be explained partially by the nontumbling treatment and by the fact that increased temperature would promote the formation of nitrate from nitrite as described by Lee et al. (1978). This could explain the relatively lower nitrite levels during nontumbling at 3°C and the relatively higher levels of nitrite in nontumbled meat at a temperature of 23°C. The suggestion that tumbling at 23°C reduced nitrite level in the uncooked meat does not agree with the report of Krause et al. (1978a) that tumbling resulted in an increased residual nitrite. The cooking procedure resulted in a highly significant ($P < 0.01$) reduction in residual nitrite.

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A Research Note

Effects of Sodium Tripolyphosphate on Physical and Sensory Properties of Beef and Pork Roasts

LISA A. SMITH, SHARON L. SIMMONS, FLOYD K. McKEITH, PETER J. BECHTEL, and PAMELA L. BRADY

ABSTRACT

Six paired beef round and pork loin roasts were used to determine the effects of sodium tripolyphosphate (STPP) on fresh and reheated roasts. Roasts were pumped 10% of their weight with distilled water (control) or with distilled water containing 4.75% STPP. Roasts were cooked to internal temperatures of 70°C (beef) or 75°C (pork) and evaluated after 0, 1 or 3 days (roasts were reheated to cooked temperatures). This study indicates that phosphate in pork and beef roasts allowed them to be reheated after 1 and 3 days of refrigeration with minimal losses of juiciness, tenderness or flavor intensity. Phosphate injection reduced warmed-over flavor in reheated pork roasts but was not successful in decreasing the incidence of warmed-over flavors in reheated beef roasts.

INTRODUCTION

THE ADDITION OF PHOSPHATE to meat can improve many sensory traits. Savich and Jansen (1954) reported that the color of ground meat was stabilized for extended time periods when phosphate, propionate and ascorbic acid were utilized. Carpenter et al. (1961) found that beef color was improved with the addition of phosphate. Other investigators reported polyphosphates increase tenderization of both beef and pork (Kamstra and Saffle, 1959; Carpenter et al., 1961; Miller and Harrison, 1965). Hopkins and Zimont (1957) reported that both injecting phosphates and dipping in a phosphate solution resulted in tenderness improvements of beef steaks. Moisture retention and juiciness are also improved with phosphate addition (Sherman, 1961; Hellendoorn, 1962; Hamm, 1970; Shults et al., 1972). Mahon et al. (1970) reported moisture retention was highest when using alkaline phosphates such as sodium tripolyphosphate.

The effect of phosphate on flavor has been attributed to retention of proteins (Ellenger, 1972) and reduction of oxidative rancidity (Ramsey and Watts, 1963; Haymon et al., 1976; Keeton, 1983). Although the beneficial effects of phosphates have been realized in processed meats, previous USDA rulings prevented the use of phosphates in fresh intact muscle systems. A new ruling was introduced in 1982 (Federal Register Vol. 47, No. 49) which allows the use of some phosphates in precooked and frozen meats. This study was designed to evaluate the effects of phosphates on the palatability of fresh and reheated beef and pork roasts.

Authors Smith, Simmons, McKeith, Bechtel, and Brady are affiliated with the Depts. of Animal Science and Foods & Nutrition at the Univ. of Illinois. Address reprint requests to Dr. Floyd K. McKeith, 205 Meat Science Laboratory, 1503 South Maryland Drive, Univ. of Illinois, Urbana, IL 61801.

MATERIALS & METHODS

SIX PAIRED, BEEF inside round roasts (semimembranosus and adductor muscles) and six paired, boneless pork center loin roasts (longissimus muscle) were trimmed to 0.64 cm of external fat, frozen at −30°C, stored for approximately 4 wk and then thawed 48 hr at 1°C. One roast of each pair was designated as control and pumped an additional 10% of its weight with distilled deionized water. The other roast of each pair was pumped 10% of its weight with distilled deionized water containing 4.75% LEMOFOS™ (Stauffer Chemical Co., Washington, PA), resulting in 0.475% phosphate in the treated roasts. All roasts were allowed to equilibrate for 48 hr at 4°C, and then cooked in a preheated (163°C) South Bend convection oven to an internal temperature of 75°C (pork) or 70°C (beef) monitored with a Campbell CR 5 recording thermometer and Teflon-coated copper-constantan thermocouples. Cooked roasts were cut perpendicular to the long axis of the roast into three equal sections and assigned to one of three treatments: (1) served at time of cooking (day 0); (2) reheated (70°C, beef; 75°C, pork) after storage at 4°C for 24 hr (day 1); and (3) reheated (70°C, beef; 75°C, pork) after storage at 4°C for 72 hr (day 3). Roasts were wrapped in aluminum foil for storage and were reheated in the same manner as the initial cooking.

Sensory panel evaluations of warm (55°C) samples were performed utilizing a six-member experienced taste panel. Panelists consisted of faculty and staff at the University of Illinois. Prior to testing, panelists were familiarized with the ballot for this study through the evaluation of samples representing various levels of the test characteristics. Juiciness, flavor intensity, off-flavor intensity, warmed-over flavor, tenderness and acceptability were scored on an unstructured scale ranging from 0-13, where 0 = extremely tough, dry, bland, unacceptable or extreme off- or warmed-over flavor (flavor related to the storage of cooked uncured meat – Sato and Hegarty, 1971). Panelists were served six samples per session and

Table 1—Effect of polyphosphate on the sensory and chemical characteristics of pork

	0 Day Storage		1 Day Storage		3 Days Storage	
	Control	Phosphate	Control	Phosphate	Control	Phosphate
Juiciness[e]	5.68^{bc}	9.47^{a}	3.38^{c}	5.65^{bc}	4.35^{bc}	6.08^{b}
Flavor intensity[e]	8.13^{a}	6.85^{ab}	6.90^{ab}	7.22^{ab}	6.52^{b}	7.52^{ab}
Off-flavor intensity[e]	10.33^{a}	8.63^{a}	9.03^{a}	10.35^{a}	10.70^{a}	9.77^{a}
Warmed-over flavor[e]	12.02^{a}	12.13^{a}	8.70^{c}	10.57^{ab}	6.63^{d}	9.27^{bc}
Tenderness[e]	8.47^{bc}	11.63^{a}	6.40^{c}	9.42^{ab}	6.97^{bc}	9.52^{ab}
Acceptability[e]	8.22^{a}	7.70^{ab}	6.20^{bc}	7.70^{ab}	5.65^{c}	7.30^{abc}
Warner-Bratzler shear force (kg)	3.10^{ab}	1.52^{c}	4.55^{a}	2.76^{bc}	3.22^{ab}	1.78^{bc}
pH	5.68^{b}	6.19^{a}	5.66^{b}	6.04^{a}	5.74^{b}	5.96^{ab}
Free water (%)[f]	9.36^{b}	8.53^{c}	10.17^{a}	9.80^{ab}	9.82^{ab}	9.38^{b}
Cooking or reheating time (min)[g]	51.83^{c}	46.50^{c}	128.67^{ab}	113.83^{b}	131.67^{a}	117.17^{ab}

[a,b,c,d] Mean values in the same row bearing unlike superscripts differ significantly ($P < 0.05$).
[e] Scored on a scale with a range of 0–13 where 0 = extremely tough, dry, bland, unacceptable or extreme off- or warmed-over flavor.
[f] According to the method of Wierbicki and Deatherage (1958).
[g] Time required to cook fresh roasts or reheat stored roasts to 75°C.

Table 2—Effect of polyphosphate on the sensory and chemical characteristics of beef

	0 Day Storage		1 Day Storage		3 Days Storage	
	Control	Phosphate	Control	Phosphate	Control	Phosphate
Juiciness[e]	5.73[ab]	6.78[a]	4.92[b]	5.33[ab]	4.37[b]	4.88[b]
Flavor intensity[e]	8.95[a]	8.60[ab]	7.47[b]	7.72[b]	7.93[ab]	8.25[ab]
Off-flavor intensity[e]	10.80[a]	10.67[a]	9.25[a]	9.68[a]	10.88[a]	10.90[a]
Warmed-over flavor[e]	12.63[a]	12.43[a]	8.60[c]	9.82[b]	8.68[bc]	8.50[c]
Tenderness[e]	6.70[b]	9.12[a]	6.98[b]	7.30[ab]	7.58[ab]	8.42[ab]
Acceptability[e]	7.15[b]	8.95[a]	6.38[b]	7.30[b]	6.47[b]	6.90[b]
Warner-Bratzler shear force (kg)	3.46[ab]	3.11[ab]	3.86[a]	3.47[ab]	3.20[ab]	2.94[b]
pH	5.56[d]	5.93[bc]	5.46[d]	5.97[b]	5.79[c]	6.22[a]
Free water (%)[f]	10.52[ab]	10.22[ab]	10.37[ab]	9.94[b]	10.52[ab]	10.69[a]
Cooking or reheating time (min)[g]	41.50[c]	41.33[c]	83.33[ab]	76.83[b]	94.83[a]	91.17[ab]

[a,b,c,d] Mean values in the same row bearing unlike superscripts differ significantly ($P < 0.05$).
[e] Scored on a scale with a range of 0–13 where 0 = extremely tough, dry, bland, unacceptable or extreme off- or warmed-over flavor.
[f] According to the method of Wierbicki and Deatherage (1958).
[g] Time required to cook fresh roasts or reheat stored roasts to 70°C.

were provided with water in a room with partitioned booths and normal lighting.

Water-holding capacity was determined on cooked samples by the method described by Wierbicki and Deatherage (1958) and reported as % free water. Mean pH values were determined on duplicate homogenates (5g cooked meat and 25 mL distilled water). Warner-Bratzler shear force values were obtained using 1.27 cm cores which were removed from roasts after they were cooled to room temperature.

The data were analyzed using analysis of variance and mean separation (Duncan's multiple-range test) procedures (SAS, 1982).

RESULTS & DISCUSSION

THE TIME REQUIRED to reheat roasts on day 1 was significantly longer than the initial cooking time for control and injected pork and beef (Tables 1 and 2). Day 1 and 3 roasts were cooked from a lower initial temperature than day 0 roasts which could account for part of the longer cooking times.

Sensory panelists (Table 1) determined that phosphate-injected fresh cooked pork roasts (day 0) were juicier ($P < 0.05$) than control roasts, which agrees with the findings of Sherman (1961), Hellendoorn (1962), Hamm (1970) and Shults et al. (1972). Phosphate injected pork and beef roasts reheated on days 1 and 3 were not different in juiciness from the fresh cooked control roasts (Tables 1 and 2). Values for % free water supported the sensory panel evaluation of juiciness.

Flavor intensity of control beef roasts (Table 2) decreased significantly from day 0 to day 1 but were not different on day 3. Flavor intensity scores for control pork roasts (Table 1) were significantly lower on day 3 in comparison with day 0. This decrease was not observed for phosphate injected reheated beef or pork roasts ($P > 0.05$). The effect of phosphate on flavor retention supports the findings of Ellenger (1972). Off-flavor intensity scores for pork and beef roasts were not different ($P > 0.05$) for any treatment; however, panelists reported a metallic or soapy flavor in some of the phosphate injected roasts.

Scores for warmed-over flavor showed control pork roasts were lower in desirability ($P < 0.05$) after each reheating. Phosphate injected pork roasts at day 3 had more warmed-over flavor than day 0 roasts ($P < 0.05$), but had significantly less warmed-over flavor than the control roasts reheated on day 3. Phosphate injection in beef had little effect in controlling the development of warmed-over flavors on day 3.

Sensory evaluation values showed that fresh cooked, phosphate injected beef and pork roasts were more tender than fresh cooked control roasts ($P < 0.05$). These findings are in agreement with Kamstra and Saffle (1959) for pork, and Carpenter et al. (1961), Hopkins and Zimont (1957) and Miller and Harrison (1965) for beef. Tenderness values for pork roasts were improved ($P < 0.05$) with phosphate injection at day 1, and phosphate-injected roasts reheated on days 1 and 3 have similar scores for tenderness to fresh cooked control roasts ($P > 0.05$). Reheated, phosphate injected beef roasts were as tender as fresh cooked control roasts. In general, Warner-Bratzler shear values support sensory panel findings for tenderness.

Acceptability of control pork roasts declined from day 0 to day 3, while phosphate injected pork roasts did not differ significantly between days. Phosphate injected pork roasts at days 0, 1 and 3 were similar ($P > 0.05$) in overall acceptability to day 0 control roasts. Phosphate injected beef roasts at day 0 were more acceptable than control roasts at day 0, and phosphate injected roasts reheated on days 1 and 3 did not differ significantly from the day 0 control roasts. The pH values of phosphate injected roasts were higher on each day of evaluation than control roasts.

This study indicates that phosphate in pork and beef roasts allowed them to be reheated after 1 and 3 days of refrigeration with minimal losses of juiciness, tenderness or flavor intensity when compared to fresh cooked control roasts. In addition, phosphate injection reduced warmed-over flavors in pork roasts reheated on day 1 and day 3, but was not successful in decreasing the incidence of warmed-over flavors in reheated beef roasts.

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—Continued on page 1641

A Research Note

An Investigation into the Lipid Classes of Skipjack Tuna (*Katsuwonus pelamis*)

ADEBISI MOGEED BALOGUN and S.O. TALABI

ABSTRACT

The lipid classes of the muscle of Skipjack tuna (*Katsuwonus pelamis*) were investigated over a period of 10 months by thin-layer chromatography. Triglycerides, diglycerides, monoglycerides and phospholipids content varied between 48.36 ± 1.22% and 62.49 ± 3.40%; 20.04 ± 5.59 and 28.53 ± 4.50; 0.98 ± 0.20% and 4.84 ± 2.48% and 4.31 ± 0.96% and 9.26 ± 0.52%, respectively. Both triglycerides and diglycerides formed the major fractions (between 68 and 91%) of Skipjack muscle lipid. There was a good concentration of phospholipids. Free fatty acid levels were fairly low and varied between 0.62 ± 0.10% and 3.94 ± 0.31%. No levels of hydrocarbons were detected in lipid samples from May to September but fairly high levels of (14.97 ± 2.89% and 12.74 ± 2.68, respectively) were detected in November and January samples.

INTRODUCTION

RECENTLY, a survey was initiated to assess the availability and abundance of Skipjack tuna within Nigeria's Exclusive Economic Zone (EEZ) to enable the formulation of a rational policy on the exploitation and utilization of this resource. As a result, a number of utilization channels were therefore proposed. Apart from direct human consumption in the fresh form, other utilization possibilities were also proposed. Notable among these were: the utilization in the smoked or dried form, for canning and for mince production (to be used in the growing fast food industrial chains). However, since the utilization in the minced form will involve the comminution of the fish flesh which should be stored at low temperature (to prevent deterioration), it is pertinent to know the different lipid fractions of the flesh. It has been observed that stability and quality of frozen meat and fish depend essentially on the composition of constituent lipids and especially on the degree of unsaturation (Talabi, 1982; Greene, 1969; Igene et al., 1976). Sulzbacher and Gaddis (1968), Bratzler et al. (1977) have also concluded that autoxidation of the triglycerides, principally in the adipose tissue is responsible for the development of rancidity in new frozen meats. Others (Cadwell et al., 1960; Watts, 1962) believed that oxidative changes in tissue lipids during storage are primarily due to phospholipids. Whatever the contentions of the two schools of thought, it is clear that both the triglycerides and phospholipid fractions of tissue play significant roles in the oxidative changes associated with frozen meat. This study was therefore carried out to characterize the muscle of Skipjack tuna into its component fractions so as to determine the proportions of each of the triglycerides, phospholipids, and other lipids classes in the muscle lipid.

MATERIALS & METHODS

SKIPJACK TUNA SAMPLES used for this study were caught off the Nigerian Coastal waters by the "Pole and Line" methods. A Japanese boat (Fukuichi Maru No. 78) was used in the survey, which lasted for a period of about 10 months (between May, 1982, and January, 1983). At the end of each trip, samples were collected, washed and the muscles dissected after envisceration. The fish muscles were homogenized in a blender and used for the lipid class analysis.

Research reported herein was carried out at the Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria. Author Balogun is affiliated with the Dept. of Wildlife and Fisheries Management, Univ. of Ibadan, Ibadan, Nigeria. Author Talabi is affiliated with the Nigerian Institute for Oceanography and Marine Research, Victoria Islands, Lagos, Nigeria.

Lipid content

The lipid content was determined by method of Bligh and Dyer (1959) and the extracted lipid was used for the lipid class analysis.

Lipid class analysis

Class analysis of extracted lipid was determined by the method of Freeman and West (1966). The spots on thin-layer plates were scanned in a densitometer (supplied by the Helena Laboratories, U.S.A.) and the peaks were identified into the various lipid fractions by comparing with those of known standards. Peak area for each fraction was determined by triangulation. The amount of each component was calculated by expressing the peak area of each fraction as a percentage of the total peak areas.

RESULTS & DISCUSSION

THE LIPID CLASSES of tuna lipid are presented in Table 1. Diglycerides, triglycerides, phospholipid, and monoglycerides contents varied between 20.04 ± 5.59% and 28.53 ± 4.50%, 48.36 ± 1.22% and 62.49 ± 3.40%, 4.31 ± 0.90% and 9.26 ± 0.52% and 0.98 ± 0.20% and 4.84 ± 2.48%, respectively.

Free fatty acids content varied between 0.62 ± 0.10% and 3.94 ± 0.31%. Looking at the lipid class composition, triglycerides and diglyceride comprised the major fractions in the lipid of tuna muscle, forming between 68.40% and 91.02% of the total lipid fractions.

The triglyceride fraction of the tuna muscle lipid increased from 58.93 ± 3.15% in May to 62.49 ± 3.40% in August, and gradually decreased to 48.33 ± 1.22% in January. The diglyceride fraction showed little variation between May (25.48%) and September (26.91%). However, it decreased to 20.04% in November and 21.18% in January.

The variation observed in both the triglyceride and diglyceride fractions appeared to be related to the variations observed in lipid content. It was observed that the total lipid content on tuna muscle dropped from 2.57% in September to 1.88% and 1.90%, respectively, in November and January. This pattern of change was also observed in both diglyceride and the triglyceride fractions of the lipid.

The present result, which showed the triglyceride fraction to be in highest concentration, agrees with the earlier reports of Hardy and Mackie (1968) and Talabi (1971) that the depot lipids of fish are notably composed of triglycerides. Fairly high levels of diglycerides in tuna muscle lipid may probably be related to genetic factors as explained by Talabi (1982). The levels of free fatty acids may have resulted from the enzymatic hydrolysis of either triglycerides or phospholipid. As phospholipid levels were not constant throughout the period of survey, it is possible that the free fatty acids were produced from the enzymatic breakdown of phospholipids during low temperature storage of the fish. The variation observed in the monoglyceride content in tuna lipid may be related to changes in

Table 1—Lipid fractions of muscle of skipjack tuna (%)

Month	Lipid (%)	Phospholipid (%)	Monoglycerides (%)	Free fatty acids (%)	Cholesterol (%)	Triglycerides (%)	Diglycerides (%)	Cholesterol esters (%)	Hydrocarbons (%)
May	1.59	4.80 ± 1.50	0.98 ± 0.20	T[a]	7.81 ± 2.04	58.93 ± 3.15	25.48 ± 4.86	0.00	0.00
June	1.68	4.31 ± 0.96	4.72 ± 1.01	0.62 ± 0.10	6.62 ± 1.13	55.82 ± 3.60	28.53 ± 4.50	0.00	0.00
July	1.75	5.62 ± 1.36	2.33 ± 0.82	3.94 ± 0.31	T	57.68 ± 3.57	28.17 ± 4.83	1.76 ± 0.37	0.00
August	1.81	7.36 ± 2.77	2.74 ± 0.83	2.36 ± 0.52	T	62.49 ± 3.40	25.55 ± 2.50	0.00	0.00
September	2.57	8.95 ± 3.22	2.56 ± 1.33	3.51 ± 0.68	T	57.62 ± 0.28	26.91 ± 4.03	0.45 ± 0.07	0.00
November	1.88	9.26 ± 0.52	3.13 ± 1.06	1.95 ± 0.89	T	49.74 ± 1.04	20.04 ± 5.59	0.30 ± 0.10	14.97 ± 2.89
January	1.90	8.98 ± 2.03	4.84 ± 2.48	3.19 ± 1.74	T	48.36 ± 1.22	21.18 ± 4.25	0.72 ± 0.41	12.74 ± 2.68

[a] T = Occurred in trace amounts and they are not picked up by the densitometer.

the metabolic activity in Tuna fish as suggested for *Trachurus trachurus* by Talabi (1982).

Perhaps, one thing worthy of note about the lipid classes of tuna oil is the presence of fairly high levels of hydrocarbons in lipid samples for November and January. Hydrocarbons, such as squalene, have been reported to be natural components in some fish (Ackman, 1968). Pristane was also reported in sprat lipid by Hardy and Mackie (1968). However, Zsolray (1977) reported that pristane, like many other hydrocarbons, is not 'natural' in fish oils. As tuna is a migratory species, the hydrocarbons might have been formed from pollutants resulting from industrial activities contacted during migration.

The result of this study showed that the triglycerides constitute the fraction in highest proportion in tuna lipid and will therefore play a very important role in autoxidative changes of the muscle during low temperature storage. However, since stability and quality of frozen fish depend on the degree of unsaturation of the component lipid (Talabi, 1982), future work should concentrate on the component fatty acids of these various fractions.

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A Research Note

Role of Trimethylamine Oxide in the Freeze Denaturation of Fish Muscle—Is It Simply a Precursor of Formaldehyde?

G. RODGER and R. HASTINGS

ABSTRACTS

A possible alternative role for trimethylamine oxide (TMAO) in fish muscle "freeze denaturation" was tested. Since TMAO is known to stabilize proteins against conformational change, its enzymatic removal during frozen storage could cause protein destabilization and aggregation. However, addition of TMAO to muscle tissue did not influence the rate or extent of "freeze denaturation" as assessed by protein solubility measurements.

INTRODUCTION

DIFFERENT MARINE SPECIES have different ways of controlling osmotic pressure. Of particular interest to the physiologist are the elasmobranchs since they osmoregulate by maintaining a high concentration of urea in the blood (2 - 2.5%). To a protein chemist this is unusual since such concentrations of urea would normally result in enzyme inactivation, and in fact this was the basis of the study conducted by Yancey and Somero (1979). They found that urea at this concentration inactivated various enzyme systems in vitro, but, more importantly, that the presence of trimethylamine oxide (TMAO), also at the level found in elasmobranch blood, stabilized the enzyme structure against not only urea, but also heat denaturation.

Because TMAO is also found in several commercially important fish species, especially cod (*Gadus morhua*), and its decomposition during frozen storage to dimethylamine (DMA) and formaldehyde (FA) is considered the cause of texture toughening, this was an interesting finding. It led us to query whether the production of FA or the depletion of TMAO in the muscle causes protein aggregation. The latter effect could decrease the stability of the system, rendering it more susceptible to a "freeze denaturation" mechanism not involving formaldehyde cross-linking.

In the short study described in this paper we added TMAO to fish mince and observed the effect of its addition on two properties of the mince: (1) the solubility profile as a function of frozen storage time; and (2) the thermal transition properties of the muscle proteins.

MATERIALS & METHODS

Sample preparation

Fresh, skinned fillets from 2 days-on-ice cod (*Gadus morhua*) were obtained from a local fish merchant. The fish was minced using a Hobart mixer (model A∃ 200) with a 9 mm diameter mesh size. The mince was subsequently soaked for 20 hr in 3.3% TMAO (Sigma Chemical Co.) solution (fish:solution 5:1 w/w) to allow concentration equilibration. Since in vivo TMAO levels in cod muscle are of the order 330 mg% (Love, 1970), soaking in a tenfold concentration at the stated fish:solution ratio should slightly magnify any effects. Two "controls" were used in the study: (1) untreated mince; (2) mince which was soaked in distilled water for the same time, at the same fish:solution ratio as the experimental sample.

Authors Rodger and Hastings are affiliated with Unilever Research Laboratory, Greyhope Road, Aberdeen AB9 2JA, Scotland.

Freezing and storage

Aliquots of the treated and control mince were packed in polythene bags, blast frozen at −40°C, and stored at −10°C. Samples were measured at regular intervals for solubility.

Moisture analyses

Moisture contents were measured by difference, after drying the mince to constant weight at 180°C.

Solubility measurements

Five-gram samples of mince were homogenized in 195 mL 5% sodium chloride, 0.02M sodium bicarbonate pH 7.6, using a sealed unit, heavy duty Silverson homogenizer, set at full speed. After centrifugation of the homogenate at 10,000g for 30 min, the protein concentration in the supernatant was determined by the Biuret method, using Bovine Serum Albumin (Sigma Chemical Co.) as a standard. The results were calculated as mg soluble protein/g dry weight of sample.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on a Perkin Elmer DSC II. Samples were scanned at a heating rate of 10°K/min over the range 275 - 370°K at an instrument sensitivity of 0.2 mcal/sec. Peak transition temperatures were recorded as peak maxima (T_m) and the apparent transition heat, ΔH_{app}, was determined from the peak area and expressed in mcal/mg dry weight.

RESULTS & DISCUSSION

SOAKING COD MINCE in distilled water or TMAO solution resulted in an increase in moisture content, which necessitated a correction being made to the soluble protein results when a comparison was made with the untreated control. Fig. 1 shows the results of the solubility study corrected for increased water content, and indicates that neither addition of TMAO (or indeed water washing) affected the rate or extent of solubility loss during the frozen storage of fish muscle by an significant amount. This suggests that TMAO removal does not increase the susceptibility of muscle tissue to freeze denaturation.

Fig. 2, however, shows an interesting finding. The addition of TMAO to cod muscle "sharpens" the nature of the transitions, and also increases the transition temperatures of the two main protein species by 2 - 3°K. The latter observation is indicative of a stabilizing effect of TMAO against thermal denaturation; the "sharpening" of the peaks is possibly evidence that the proteins after treatment with TMAO undergo more cooperative unfolding. An additional point worth raising is whether this work gives any more insight into the mechanism of freeze denaturation. We have observed that TMAO stabilizes muscle proteins against heat, evidenced by the increases in transition temperatures, but has no apparent effect on the freeze denaturation process as measured by solubility studies.

Since thermal denaturation is an unfolding process, it would be reasonable to assume that TMAO stabilizes against conformational change. Since there is no effect on freeze denaturation, it is possible that this phenomenon is simply a side-to-side aggregation of actomyosin molecules with no conformational change, as argued by Connell (1968).

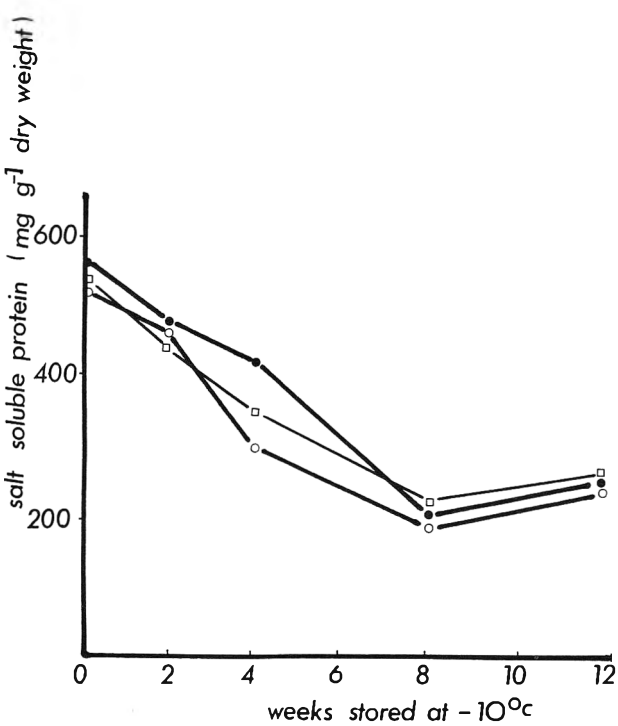

Fig. 1—Salt soluble protein in minced cod muscle stored at −10°C: ○—○ control; □—□ washed control; ●—● TMAO treated sample.

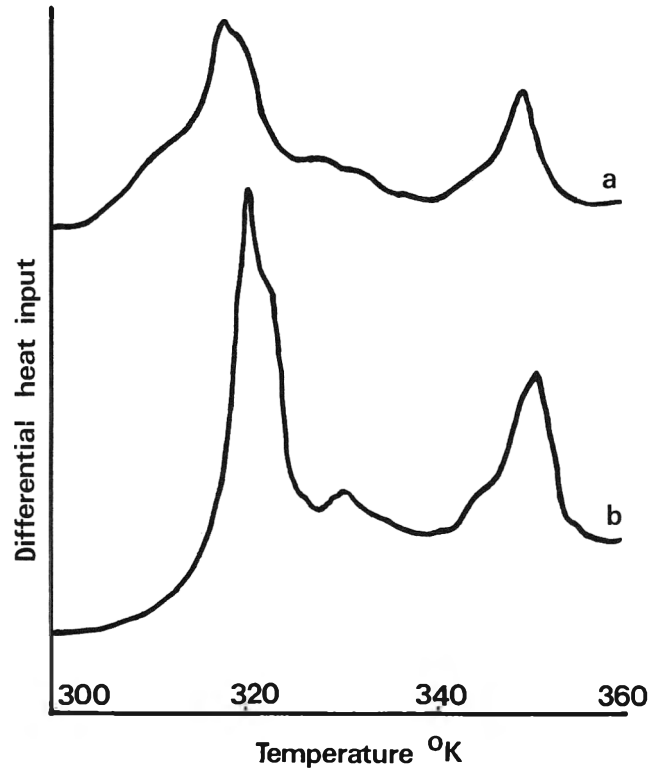

Fig. 2—Thermograms of minced cod muscle samples: (a) control mince; (b) TMAO-treated mince.

We would agree that the evidence presented contributes only slightly to the ongoing search for a full understanding of the mechanism of freeze denaturation but it is worth emphasizing that TMAO can influence some aspects of muscle protein behavior other than by being the precursor of formaldehyde.

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Institute of Food Technologists

The Institute of Food Technologists is an educational and scientific society of food professionals – technologists, scientists, engineers, educators, and executives – in the field of food technology. Food technologists apply science and engineering to the research, production, processing, packaging, distribution, preparation, evaluation, and utilization of foods. Individuals who are qualified by education, special training, or experience are extended an invitation to join in professional association with the select group of the food industry's scientific and technological personnel who are IFT members. Membership is worth many times its modest cost, reflecting positive benefits, stimulation, and opportunities for the individual in his/her business or profession.

OBJECTIVES

IFT has several major aims: to stimulate investigations into technological food problems; to present, discuss, and publish the results of such investigation; to raise the educational standards of food technologists; and to promote recognition of the scientific approach to food and the basic role of the food technologist in industry. All of these activities have the ultimate objective to provide the best possible foods for mankind.

ORGANIZATION AND PROGRESS

Organized July 1, 1939, at Cambridge, Mass., with a membership of less than 100, the Institute has grown to more than 21,000. It is worldwide in scope with more than 3,000 of its membership overseas.

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Professional Members. Any person who meets the following minimum requirements: (1) Bachelor's degree or higher from a college or university with a major in one or more of the sciences or branches of engineering associated with food technology; (2) Five years of professional experience in food technology, for which a master's degree may be presented as the equivalent of one year's experience; a doctor's degree, the equivalent to three year's experience.

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REGIONAL SECTIONS

Where 25 or more members live within commuting distance of a given geographic area, a regional section may be established. Presently, there are 50 regional sections.

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Where 50 or more members of the Institute have a common interest in a particular broad-based discipline of food technology, they may form a division. There are presently 12 divisions serving the areas of Biotechnology, Carbohydrates, Food Engineering, Food Packaging, Food Service, Microbiology, Muscle Foods, Nutrition, Quality Assurance, Refrigerated and Frozen Foods, Sensory Evaluation, and Toxicology and Safety Evaluation.

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When 25 or more members have a common interest in a rather narrow, product-oriented or similar special area, they may form a technology group to serve the needs of this specialized area. There are currently five STGs – Citrus Products, Dairy Products, Extension, Fruit and Vegetable Products, and Seafood Products.

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All Student Members of IFT are automatically members of the Student Association, which provides special services and activities for students. This Association, which is run by and for the students, also provides the organizational mechanism for giving students a voice in IFT affairs.

STUDENT CHAPTERS

An IFT Student Chapter certificate may be granted to a group of students enrolled in the food science and technology curriculum in a particular school who have organized to form a student club. There are 38 student chapters.

AFFILIATE ORGANIZATIONS

Affiliate certificates may be granted to food technology organizations outside the U.S.A. There are currently 15 chartered Affiliate Organizations.

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An Annual Meeting of the Institute provides a specially-organized technical program, awards program, and an exposition (FOOD EXPO) of equipment, services, processes, and ingredients. The program is designed to emphasize current trends and technological developments.

AWARDS

The Institute presents the following awards, usually annually:

Nicholas Appert Award. Purpose of this award (medal furnished by the Chicago Section, and $5,000 by IFT) is to honor a person for pre-eminence in the contributions to the field of food technology.

Babcock-Hart Award. Purpose of this award ($3,000 furnished by the Nutrition Foundation and a plaque by IFT) is to honor a person for contributions to food technology that have improved public health through some aspects of nutrition or more nutritious food.

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IFT Food Technology Industrial Achievement Award. Purpose of this award (plaque to company or companies involved) is to recognize and honor the developers of an outstanding new food process and/or product representing a significant advance in the application of food technology to food production, successfully applied in actual commercial operation.

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Fellows Awards. Any Professional Member who has been active for at least 10 years and who has outstanding contributions to the field of food science and technology is eligible to be elected a Fellow of the Institute.

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– Information Retrieval Services

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The International Food Information Service (IFIS) was created in 1968 to provide a number of services related to the retrieval of information from the literature of food science and technology.

IFIS's sponsors include the Commonwealth Agricultural Bureaux in England, the Gesellschaft für Information und Dokumentation in the Federal Republic of Germany, the Institute of Food Technologists in the United States, and the Centrum voor Landbouwpublikaties in Landbouwdocumentatie in the Netherlands.

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For further information contact:

Mr. Ernest Mann, Joint Managing Director
International Food Information Service
Lane End House, Shinfield
Reading RG2 9BB, United Kingdom
Telephone: 0044-734-88 32 67, Telex: 005 1-84 72 04
or
Dr. Udo Schutzsack, Joint Managing Director
International Food Information Service
Arabella Center, Lyoner-Strasse 44-48
6000 Frankfurt 71, West Germany
Telephone: (06 11) 66 87-338, Telex: 4 14 351 GIDFM

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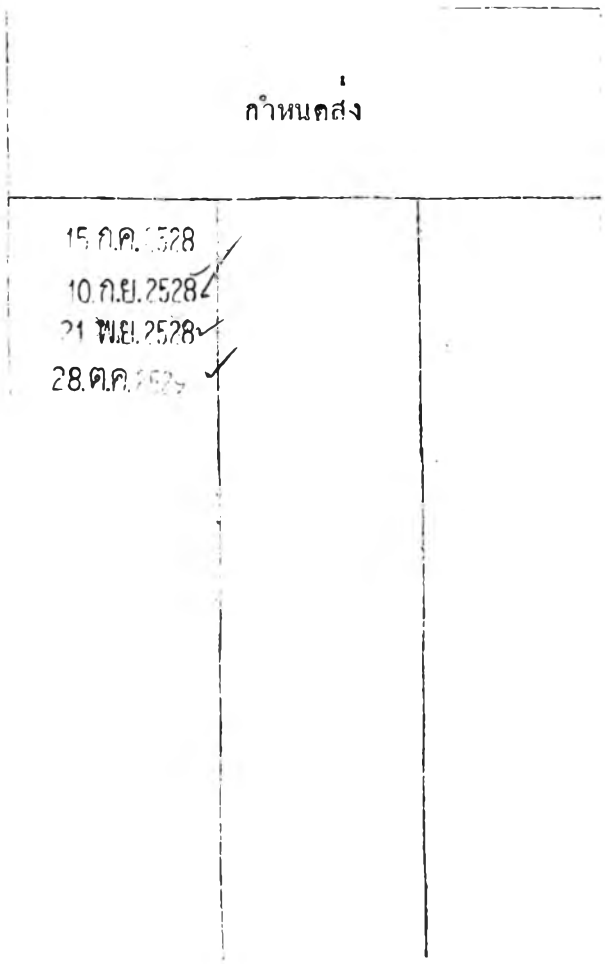
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INSTITUTE OF FOOD TECHNOLOGISTS
SUITE 2120
221 N. LA SALLE STREET
CHICAGO, ILLINOIS 60601